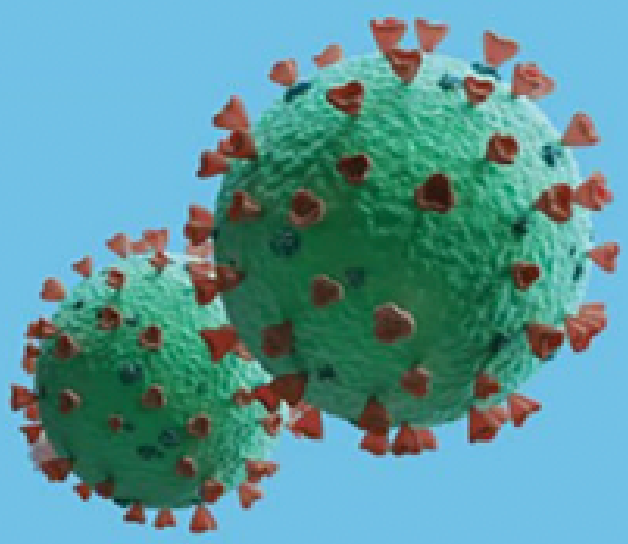
FEDERAZIONE RINASCIMENTO ITALIA

rinascimentoitalia.it

TOXICOLOGY OF COVID VACCINE PART 1

DR. LORETTA BOLGAN

Doctor of Pharmaceutical Chemistry and Technology
Ph.D. in Pharmaceutical Sciences
Scientific advisor



SUMMARY

THE ENHANCEMENT OF THE DISEASE 4

MOLECULAR MECHANISM OF ADE 8
ADE through enhanced infection 9
ADE through enhanced immune activation 9
ADE mediated by C1q 15
Non-canonical ADE 19
Mast cells and ADEs 20

VACCINE ANTIBODY-DEPENDENT ENHANCEMENT 29
ADE risk assessment for anti-SARS-Cov-2 vaccines 44

ANTIBODIES AND VIRAL INFECTION 48
Functions of membrane antibodies and soluble antibodies 48
The classes of antibodies 49
Immunoglobulin D 53

HEA STUDY 54
Serological tests to detect SARS-Cov-2 54
Antibody responses in COVID-19 patients 61
Cross-reactivity and heterologous immunity 63
Neutralization test 66

NEUTRALIZATION TESTING AND CONSTRUCTION OF PSEUDOTYPED VIRUSES 73
In vitro ADE testing 78
Criticisms and manipulations of preclinical studies on ADEs 79

THE CONFORMATIONS OF THE SPIKE AND ADE 80
Structure of protein S 80
Mechanism of action of protein S 85
The furin cleavage site of SARS-Cov-2 89
NRP-1 and SARS-Cov-2 95
The "open" and "closed" conformations of the Spike 100
ADE epitope and mRNA vaccines 103

SPIKE AND SYNCYTIAL FORMATION 109
Sincizi and Thrombosis 111
Sincizi and NeuroCovid 113
Sincizi and Tumors 115
Sincizi and lymphopenia by COVID-19 117
The potential role of syncytia in SARS-Cov-2 infection 118
Cell fusion and cardiac damage 121
Cell fusion and vaccines COVID-19 122

CONVALESCENT PLASMA 125
ADE and plasma from convalescents 131
Italian clinical studies of efficacy of plasma from convalescents 133

IN-DEPTH STUDY 135
THE NATURAL AND VACCINE ANTIBODY RESPONSE 135
Generation and characteristics of a neutralizing antibody 139
Neutralizing antibodies and SARS-Cov-2 144
NAbs induced by natural infections and their protective role 144
NAbs induced by vaccination 147
Comparison of natural and vaccine immunity 147
Factors influencing the production of NAb 149

NATURAL IgM 151

MONOCLONAL ANTIBODIES 173
Production of monoclonal antibodies 173
CHO cells as producers of anti-SARS-CoV-2 mAb 178
Mechanism of action and safety of monoclonal antibodies 180
Risks associated with the use of monoclonal antibodies against SARS-Cov-2 198

THE GLYCOBIOLOGY OF VIRAL INFECTIONS 203
Glycosylation and the immune system 223

Glycosylation and neurodegenerative diseases 249
Glycosylation and prion diseases 249
Other factors that induce amyloid protein aggregation 252
Prion diseases and spike of natural SARS-Cov-2 253
Prion diseases and SARS-Cov-2 vaccine spike 263

Association between blood type and COVID-19 285
Secretor status and ABH antigens (FUT2 gene) 296
Rh factor (Rhesus Factor) and susceptibility to SARS-Cov-2 297

THE ENHANCEMENT OF THE DISEASE

IN-DEPTH STUDY

COVID-19 THE VACCINE RESPIRATORY COMPLICATIONS - IMMUNOPATHOLOGY

Key terms in disease enhancement [1]

ADE

Antibody-dependent enhancement (ADE) can be mediated by the internalization in the cells of the immune system of a virus-antibody complex associated with the antibody's Fc receptor, thus leading to increased viral replication and cytokine release.

ERD

Enhanced respiratory disease (ERD) manifests with more severe clinical symptoms after infection with respiratory viruses, such as respiratory syncytial virus and influenza virus, due to previous immune responses. ERD usually presents with a peribronchiolar monocytic infiltrate with an excess of eosinophils. ERD can occur during infection with homotypic or heterotypic serotype virus after vaccination, natural infection, or transfer of maternal passive immunity.

GO

Vaccine-associated disease enhancement (VADE) partially overlaps with ADE and ERD. In contrast to ERD, VADE concerns only the vaccine-associated reaction and, more importantly, is not limited to respiratory injury. For example, heterotypic serotype Dengue virus infection can cause more severe Dengue hemorrhagic fever in vaccinated individuals. This phenomenon is related to VADE but does not include ERD. VADE can be attributed to T helper cell type 2-dependent and antibody-dependent mechanisms.

All viruses initiate infection by adhering to host cells through the interaction between viral proteins and receptor/co-receptor molecules on target cells.
As discussed in detail in previous papers, (COVID-19 Immunopathology and Pulmonary Complications Immunopathology) the humoral host response is responsible for the generation of antibodies specific to surface proteins that inhibit this phase of the infection cycle, resulting in the neutralization of the virus; however, in some cases, these antibodies may paradoxically promote the infectious process as part of a phenomenon better known as antibody-dependent enhancement (ADE). [2]

[1] Su S, Du L, Jiang S.
Learning from the past: development of safe and effective COVID-19 vaccines.
Nat Rev Microbiol. 2021;19(3):211-219. doi:10.1038/s41579-020-00462-y
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566580/

[2] Sánchez-Zuno GA, Matuz-Flores MG, González-Estevez G, et al.
A review: Antibody-dependent enhancement in COVID-19: The not so friendly side of antibodies. Int J Immunopathol Pharmacol. 2021;35:20587384211050199. doi:10.1177/20587384211050199
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8512237/

Takada A, Kawaoka Y.
Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications.
Rev Med Virol. 2003 Nov-Dec;13(6):387-98. doi: 10.1002/rmv.405.
https://pubmed.ncbi.nlm.nih.gov/14625886/

Ajmeriya S, Kumar A, Karmakar S, Rana S, Singh H.
Neutralizing Antibodies and Antibody-Dependent Enhancement in COVID-19: A Perspective [published online ahead of print, 2022 Feb 4].

J Indian Inst Sci. 2022;1-17. doi:10.1007/s41745-021-00268-8
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8814804/

Wang S, Wang J, Yu X, et al.
Antibody-dependent enhancement (ADE) of SARS-CoV-2 pseudoviral infection requires FcγRIIB and virus-antibody complex with bivalent interaction.
Commun Biol. 2022;5(1):262. Published 2022 Mar 24. doi:10.1038/s42003-022-03207-0
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8948278/

Boonnak K, Dambach KM, Donofrio GC, Tassaneetrithep B, Marovich MA.
Cell type specificity and host genetic polymorphisms influence antibody-dependent enhancement of dengue virus infection.
J Virol. 2011;85(4):1671-1683. doi:10.1128/JVI.00220-10
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3028884/

Ricke DO.
Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies.
Front Immunol. 2021;12:640093. Published 2021 Feb 24. doi:10.3389/fimmu.2021.640093
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7943455/

Maemura T, Kuroda M, Armbrust T, Yamayoshi S, Halfmann PJ, Kawaoka Y.
Antibody-Dependent Enhancement of SARS-CoV-2 Infection Is Mediated by the IgG Receptors FcγRIIA and FcγRIIIA but Does Not Contribute to Aberrant Cytokine Production by Macrophages.
mBio. 2021 Oct 26;12(5):e0198721. doi: 10.1128/mBio.01987-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8546849/

Cloutier M, Nandi M, Ihsan AU, Chamard HA, Ilangumaran S, Ramanathan S.
ADE and hyperinflammation in SARS-CoV2 infection- comparison with dengue hemorrhagic fever and feline infectious peritonitis.
Cytokine. 2020;136:155256. doi:10.1016/j.cyto.2020.155256
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7439999/

Coish JM, MacNeil AJ.
Out of the frying pan and into the fire? Due diligence warranted for ADE in COVID-19.
Microbes Infect. 2020;22(9):405-406. doi:10.1016/j.micinf.2020.06.006
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7311339/

Shukla AK, Misra S.
Antibody-dependent enhancement of virus infection and disease: implications in COVID-19.
J Basic Clin Physiol Pharmacol. 2022 Jan 7;33(1):13-16. doi: 10.1515/jbcpp-2021-0264.
https://www.degruyter.com/document/doi/10.1515/jbcpp-2021-0264/html

Liu L, Wei Q, Lin Q, et al.
Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection.
JCI Insight. 2019;4(4):e123158. Published 2019 Feb 21. doi:10.1172/jci.insight.123158
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478436/

Peron JPS, Nakaya H.
Susceptibility of the Elderly to SARS-CoV-2 Infection: ACE-2 Overexpression, Shedding, and Antibody-dependent Enhancement (ADE).
Clinics (Sao Paulo). 2020;75:e1912. doi: 10.6061/clinics/2020/e1912.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7213670/

Okuya K, Hattori T, Saito T, et al.
Multiple Routes of Antibody-Dependent Enhancement of SARS-CoV-2 Infection.
Microbiol Spectr. 2022;10(2):e0155321. doi:10.1128/spectrum.01553-21
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9045191/

Farshadpour F, Taherkhani R.
Antibody-Dependent Enhancement and the Critical Pattern of COVID-19: Possibilities and Considerations.
Med Princ Pract. 2021;30(5):422-429. doi:10.1159/000516693
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8339023/

Clark NM, Janaka SK, Hartman W, et al.
Anti-SARS-CoV-2 IgG and IgA antibodies in COVID-19 convalescent plasma do not facilitate antibody-dependent enhancement of viral infection.
Preprint. bioRxiv. 2021;2021.09.14.460394. Published 2021 Sep 14. doi:10.1101/2021.09.14.460394
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8452094/

Zheng J, Wang Y, Li K, Meyerholz DK, Allamargot C, Perlman S.
Severe Acute Respiratory Syndrome Coronavirus 2-Induced Immune Activation and Death of Monocyte-Derived Human Macrophages and Dendritic Cells. J Infect Dis. 2021;223(5):785-795. doi:10.1093/infdis/jiaa753
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7799009/
Bohländer F, Riehl D, Weißmüller S, Gutscher M, Schüttrumpf J, Faust S.
Immunomodulation: Immunoglobulin Preparations Suppress Hyperinflammation in a COVID-19 Model via FcγRIIA and FcαRI.

In summary, this mechanism involves the endocytosis of virus-antibody immune complexes in cells through the interaction of the Fc region of the antibody with cellular Fc receptors (FcRs).
FcγRI (CD64) binds with high affinity to IgG in monomeric form, while FcγRII (CD32) and FcγRIII (CD16) do so with low affinity and are activated by immune complexes. [3] Although ADE is mainly mediated by IgG antibodies, IgM along with complement and IgA antibodies have also been described as capable of mediating ADE. [4]
The phenomenon of ADE is an event that occurs for some viruses, where preexisting nonneutralizing or sub-neutralizing antibodies to viral surface proteins that were generated during a previous infection can intensify the inflammatory process during a secondary infection with any virus related to the original antigen, favoring its entry into the cell.[5]

Front Immunol. 2021;12:700429. Published 2021 Jun 10. doi:10.3389/fimmu.2021.700429
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8223875/

Bastug, A., Bodur, H.
SARS-CoV-2 Infection and Antibody-Dependent Enhancement. In: Nayak, J., Naik, B., Abraham, A. (eds) Understanding COVID-19: The Role of Computational Intelligence.
Studies in Computational Intelligence, (2022) vol 963. Springer, Cham. https://doi.org/10.1007/978-3-030-74761-9_5
https://link.springer.com/chapter/10.1007/978-3-030-74761-9_5

Antoine Danchin, Oriane Pagani-Azizi, Gabriel Turinici, Ghozlane Yahiaoui
COVID-19 adaptive humoral immunity models: weakly neutralizing versus antibody-disease enhancement scenarios
medRxiv 2020.10.21.20216713; doi:https://doi.org/10.1101/2020.10.21.20216713
https://www.medrxiv.org/content/10.1101/2020.10.21.20216713v2.full.pdf

Bahnan W, et al
Spike-Dependent Opsonization Indicates Both Dose-Dependent Inhibition of Phagocytosis and That Non-Neutralizing Antibodies Can Confer Protection to SARS-CoV-2.
Front Immunol. 2022 Jan 14;12:808932. doi: 10.3389/fimmu.2021.808932.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8796240/

Bachmann MF, Mohsen MO, Zha L, Vogel M, Speiser DE.
SARS-CoV-2 structural features may explain limited neutralizing-antibody responses.
NPJ Vaccines. 2021 Jan 4;6(1):2. doi: 10.1038/s41541-020-00264-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7782831/

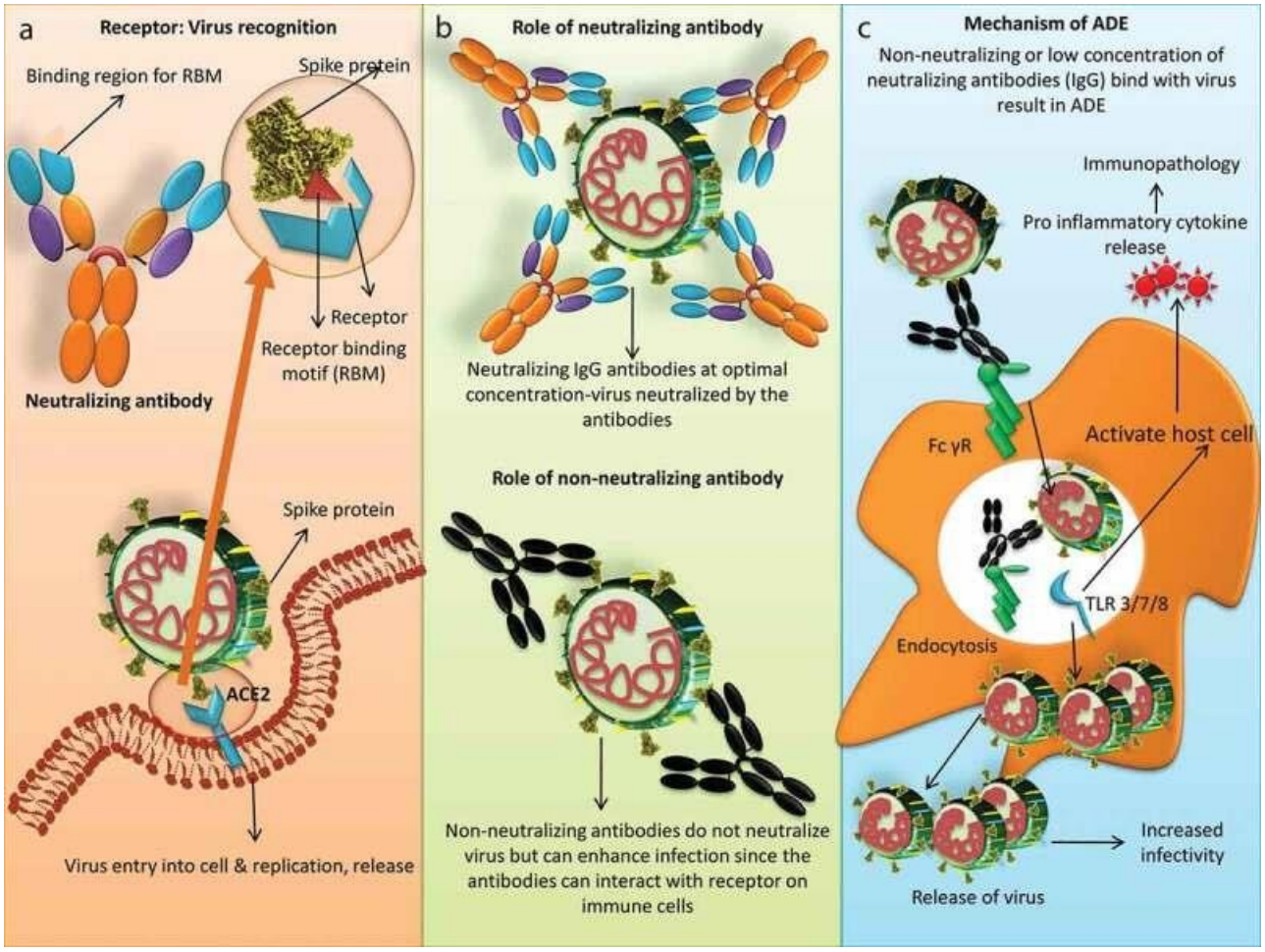
Li D, et al
In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies.
Cell. 2021 Aug 5;184(16):4203-4219.e32. doi: 10.1016/j.cell.2021.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232969/

Jane M. Orient
Negative Evidence: Antibody-Dependent Enhancement
Journal of American Physicians and Surgeons Volume 27 Number 1 Spring 2022
https://jpands.org/vol27no1/orient.pdf

[3] Cloutier M, Nandi M, Ihsan AU, Chamard HA, Ilangumaran S, Ramanathan S.
ADE and hyperinflammation in SARS-CoV2 infection- comparison with dengue hemorrhagic fever and feline infectious peritonitis.
Cytokine. 2020;136:155256. doi:10.1016/j.cyto.2020.155256
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7439999/

[4] Kulkarni R.
Antibody-dependent enhancement of viral infections. In: Bramhachari PV. (ed) Dynamics of Immune Activation in Viral Diseases. Singapore: Springer Singapore. (2020) pp. 9-41. ISBN 9789811510441.

[5] Wen J, Cheng Y, Ling R, et al.
Antibody-dependent enhancement of coronavirus.
Int J Infect Dis. 2020;100:483-489. doi:10.1016/j.ijid.2020.09.015
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7483033/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7484565/
Role of neutralizing and nonneutralizing antibodies in viral infection and mechanism of ADE in SARS-CoV-2. **(a)** Spike protein of SARS- CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) receptor and initiates replication. ACE2 recognizes the receptor binding motif on the spike protein, and the same receptor binding motif (RBM) is recognized by antibodies. **(b**) Neutralizing antibodies at the optimal concentration neutralize the virus while nonneutralizing antibodies can increase infection. **(c)** Mechanism of ADE for SARS-CoV-2. The virus-antibody complex (neutralizing or nonneutralizing) binds to the Fcγ receptor on surface immune cells such as monocytes or macrophages leading to virus entry without the use of the ACE2 receptor. This leads to increased virus replication and release. Virus-antibody binding to FcγR can also induce a proinflammatory response. Viral RNA in endosomes signals through Toll-like receptor 3 (TLR3), TLR7 or TLR8 activating in the host cell the release of proinflammatory cytokines leading to immunopathology

ADE was first described in 1964 by Hawkes, who demonstrated increased infectivity *in vitro* of various arboviruses such as Japanese encephalitis virus, West Nile virus, Murray Valley encephalitis virus, and Getah virus. [6]

Previous reports already hypothesized that pre-existing nonneutralizing antibodies were also responsible for increased infection with various human and animal viruses, including dengue virus (DENV), Zika virus (ZIKV), Ebola, human immunodeficiency virus (HIV) Aleutian mink disease parvovirus, Coxsackie B virus, equine infectious anemia, feline infectious peritonitis, monkey hemorrhagic fever, goat arthritis, porcine reproductive and respiratory syndrome (PRRSV), and African swine fever.[7]

[6] Karthik K, Senthilkumar TMA, Udhayavel S, Raj GD.
Role of antibody-dependent enhancement (ADE) in the virulence of SARS-CoV-2 and its mitigation strategies for the development of vaccines and immunotherapies to counter COVID-19.
Hum Vaccin Immunother. 2020;16(12):3055-3060. doi:10.1080/21645515.2020.1796425
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7484565/

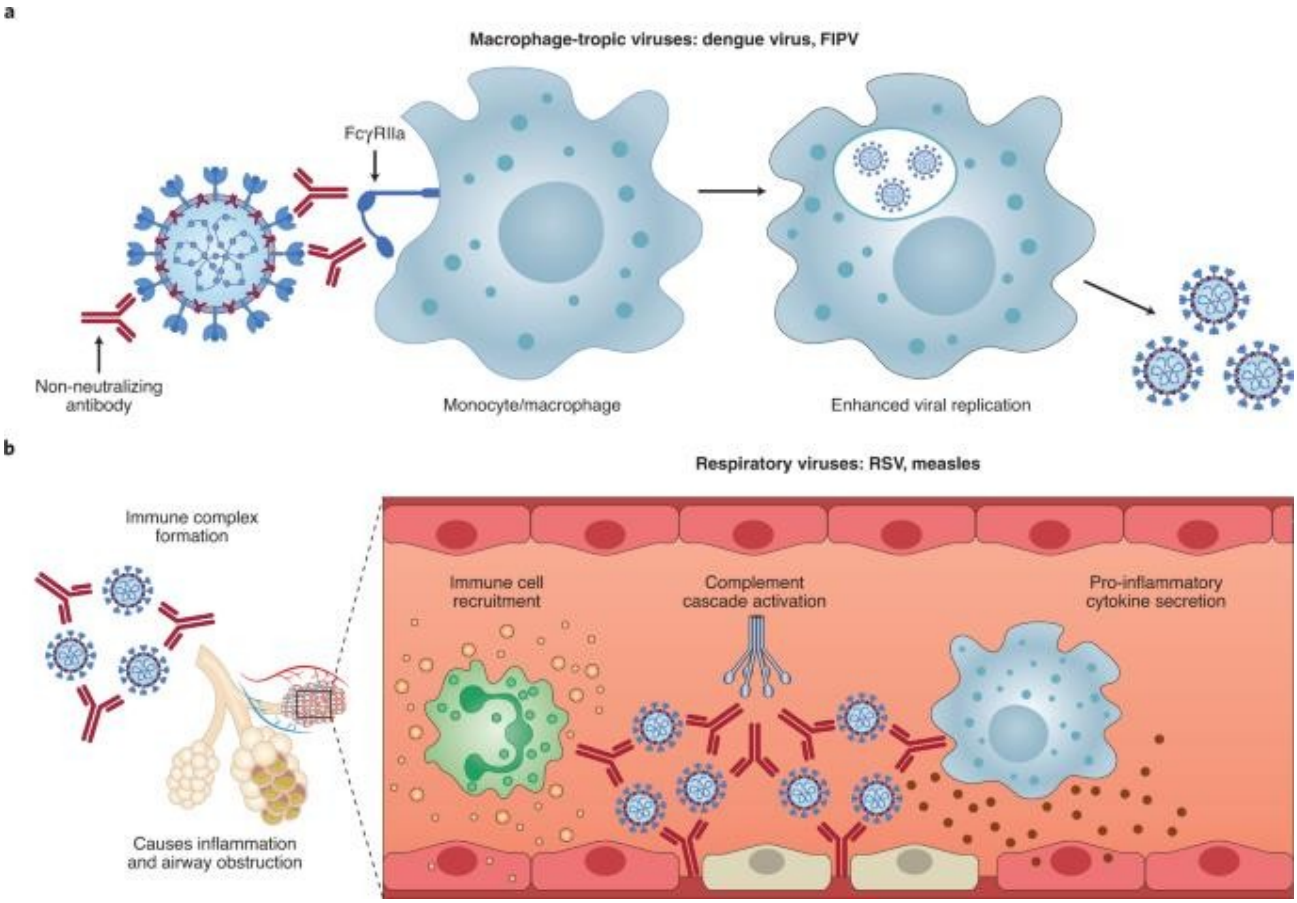
[7] Khandia R, Munjal A, Dhama K, et al.
Modulation of Dengue/Zika Virus Pathogenicity by Antibody-Dependent Enhancement and Strategies to Protect Against Enhancement in Zika Virus Infection.

MOLECULAR MECHANISM OF ADE

ADE has been documented to occur through two distinct mechanisms:

1- Through increased virus uptake in phagocytic cells expressing the Fc receptor gamma IIa (FcγRIIa) with increased viral infection and replication
2- through the formation of immune complexes that cause increased inflammation and immunopathology.

Although these mechanisms are not mutually exclusive, their classification has been proposed to understand the biological process at the molecular level. [8]



Front Immunol. 2018;9:597. Published 2018 Apr 23. doi:10.3389/fimmu.2018.00597
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5925603/

[8] Lee WS, Wheatley AK, Kent SJ, DeKosky BJ.
Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies.
Nat Microbiol. 2020 Oct;5(10):1185-1191. doi: 10.1038/s41564-020-00789-5.
https://pubmed.ncbi.nlm.nih.gov/32908214/

Hussman JP.
Severe Clinical Worsening in COVID-19 and Potential Mechanisms of Immune-Enhanced Disease.
Front Med (Lausanne). 2021;8:637642. Published 2021 Jun 22. doi:10.3389/fmed.2021.637642
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8258105/

Xu L, Ma Z, Li Y, Pang Z, Xiao S.
Antibody dependent enhancement: Unavoidable problems in vaccine development.
Adv Immunol. 2021;151:99-133. doi:10.1016/bs.ai.2021.08.003
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8438590/

https://www.nature.com/articles/s41564-020-00789-5
(a) For macrophage-tropic viruses such as dengue virus and FIPV, nonneutralizing or sub-neutralizing antibodies cause increased viral infection of monocytes or macrophages via FcγRIIa-mediated endocytosis, resulting in more severe disease. **B)** For nonmacrophage respiratory viruses such as RSV and measles, nonneutralizing antibodies can form immune complexes with viral antigens within airway tissues, resulting in secretion of pro-inflammatory cytokines, recruitment of immune cells, and activation of the complement cascade within lung tissue. The resulting inflammation can lead to airway obstruction and in severe cases can cause acute respiratory distress syndrome. COVID-19 immunopathology studies are still ongoing, and the latest available data suggest that infection of human macrophages by SARS-CoV-2 is unproductive. Existing evidence suggests that immune complex formation, complement deposition, and local immune activation present the most likely ADE mechanisms in COVID-19 immunopathology.

ADE through enhanced infection

As mentioned above, FcRs are mainly expressed by immune cells and are receptors for the Fc portion of an antibody.
In enhanced infection-mediated ADE, nonneutralizing or subneutralizing antibodies bind to the viral surface, forming an immune complex that is internalized by Fc receptor-bearing cells, including monocytes/macrophages and dendritic cells, and induce activation of the signal cascade for FcγR-mediated phagocytosis, which consequently results in increased viral load and disease severity. [9]
Importantly, Fc receptor activation triggers a signaling cascade that also induces IFN-stimulated gene (ISG) expression with potent antiviral effects, and viruses must suppress these antiviral responses in target cells for ADE to occur, with mechanisms that will be detailed later. [10]

ADE via enhanced immune activation

The second, recently described and less studied mechanism by which ADE can occur is well represented by pathogens that cause respiratory infections. Under these conditions, Fc-mediated antibody effector functions are able to initiate a strong immune cascade that results in severe lung pathology. [8]
This mechanism can also be induced when virus-antibody-C1q complexes bind to the C1q receptor present on cells and the classical complement pathway is triggered, leading to C3 activation and subsequent endocytosis. [11]

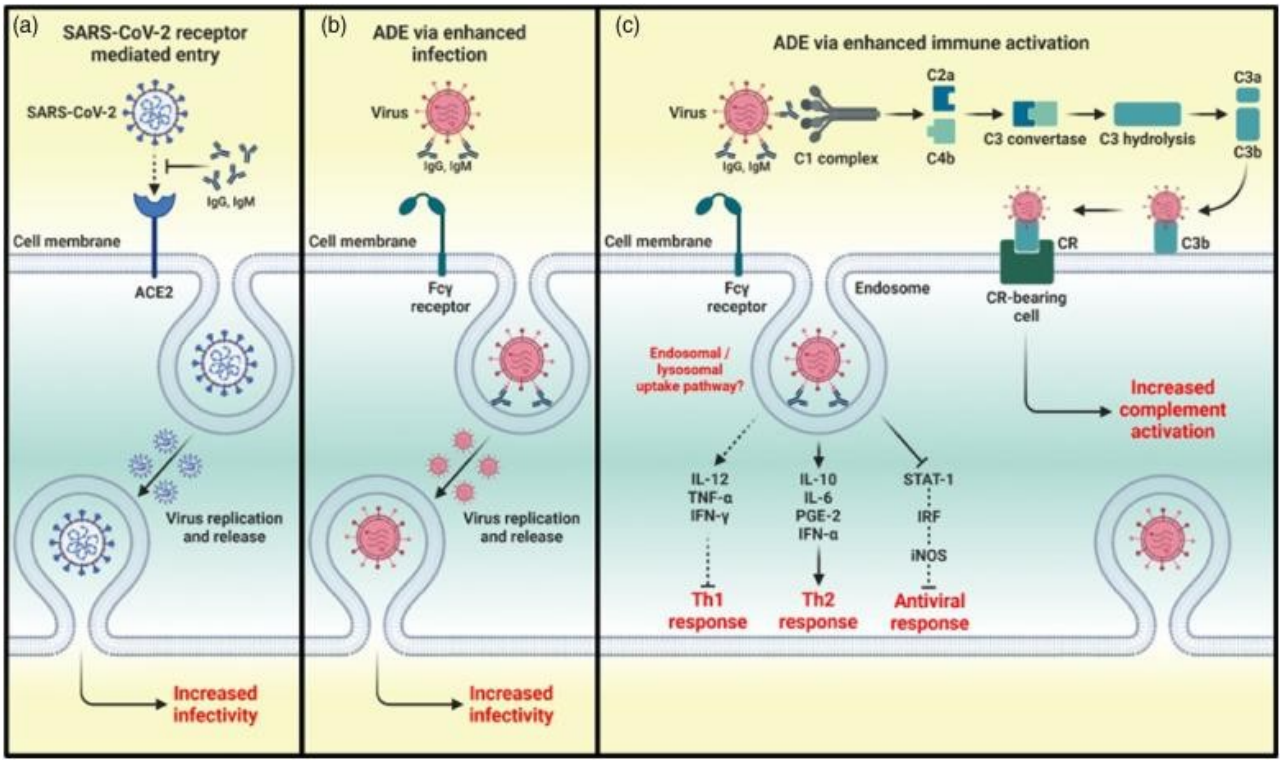
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8512237/
ADE phenomenon. **(a)** The conventional mechanism of SARS-CoV 2 infection consists of the binding of its S protein to the cellular ACE2 receptor. After SARS-CoV-2 binds to the receptor, a conformational change occurs in the S protein required for fusion of the viral envelope with the cell membrane for subsequent endocytosis. Subsequently, SARS-CoV-2 releases its genetic material into the host cell. RNA from the viral genome is then translated into proteins required for subsequent virion assembly in the RE and Golgi. These virions are then transported through vesicles to the outside of the cell by exocytosis. The ADE phenomenon can be classified into two different mechanisms: ADE through enhanced infection and ADE through enhanced immune activation. **(b)** In ADE through enhancement of infection, antibodies of a nonneutralizing or sub-neutralizing nature cause viral infection through FcγRIIa-mediated endocytosis, resulting in a more severe disease phenotype. **(c)** In ADE through enhanced immune activation, nonneutralizing antibodies can form immune complexes with viral antigens within airway tissues, resulting in secretion of pro-inflammatory cytokines, recruitment of immune cells, and activation of the complement cascade within lung tissue. ADE, antibody-dependent enhancement; ACE2, angiotensin-converting enzyme 2; CR, complement receptor; ER, endoplasmic reticulum;

[9] Gan ES, Ting DH, Chan KR.
The mechanistic role of antibodies to dengue virus in protection and disease pathogenesis.
Expert Rev Anti Infect Ther. 2017 Feb;15(2):111-119. doi: 10.1080/14787210.2017.1254550.
https://pubmed.ncbi.nlm.nih.gov/27796143/

[10] Langerak T, Mumtaz N, Tolk VI, et al.
The possible role of cross-reactive dengue virus antibodies in Zika virus pathogenesis.
PLoS Pathog. 2019;15(4):e1007640. doi:10.1371/journal.ppat.1007640
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6472811/

[11] Comas-Garcia M.
Packaging of Genomic RNA in Positive-Sense Single-Stranded RNA Viruses: A Complex Story.
Viruses. 2019;11(3):253. Published 2019 Mar 13. doi:10.3390/v11030253
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6466141/

FcγRIIa, Fc γ receptor IIa; IFN-a, interferon a; IL, interleukin; IRF, interferon regulatory factors; iNOS, inducible nitric oxide synthase; PGE2, prostaglandin E2; RNA, ribonucleic acid; TNF-a, tumor necrosis factor.



Also for coronaviruses and viruses of the SARS family, when ADE develops, virus-specific IgG antibodies form unstable complexes with the virus, and after binding to FcγRII receptors expressed by some immune cells[12] facilitate infection of the cells themselves[13] . In particular, it has been shown for SARS-CoV-1 that virus-specific S-protein antibodies can facilitate virus entry into B cells[14] and into

[12] Iwasaki A, Yang Y.
The potential danger of suboptimal antibody responses in COVID-19.
Nat Rev Immunol. 2020;20(6):339-341. doi:10.1038/s41577-020-0321-6
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7187142/

[13] Tirado SM, Yoon KJ.
Antibody-dependent enhancement of virus infection and disease.
Viral Immunol. 2003;16(1):69-86. doi: 10.1089/088282403763635465.
https://pubmed.ncbi.nlm.nih.gov/12725690/

Yip MS, Leung NH, Cheung CY, et al.
Antibody-dependent infection of human macrophages by severe acute respiratory syndrome coronavirus.
Virol J. 2014;11:82. Published 2014 May 6. doi:10.1186/1743-422X-11-82
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4018502/

Li L, Wo J, Shao J, et al.
SARS-coronavirus replicates in mononuclear cells of peripheral blood (PBMCs) from SARS patients.
J Clin Virol. 2003;28(3):239-244. doi:10.1016/s1386-6532(03)00195-1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7128964/

Yilla M, Harcourt BH, Hickman CJ, et al.
SARS-coronavirus replication in human peripheral monocytes/macrophages.
Virus Res. 2005;107(1):93-101. doi:10.1016/j.virusres.2004.09.004
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114182/

[14] Kam YW, Kien F, Roberts A, et al.
Antibodies against trimeric S glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcgammaRII-dependent entry into B cells in vitro.
Vaccine. 2007;25(4):729-740. doi:10.1016/j.vaccine.2006.08.011

macrophages[15] , while SARS-Cov-2 can infect primary human CD4 T cells [+16] and other CD32 cells[+] (which include monocytes, macrophages, some types of B cells and dendritic cells). [17]
According to some researchers, monocytes and macrophages play a key role in the acute inflammatory process that occurs in some COVID-19 patients[18] and concern about the potential involvement of ADE in the pathogenesis of COVID-19 has been highlighted in various researches[19] .
It is plausible that the initiation of SARS-CoV-2 replication in immune cells is a key step in the development of the disease and its evolution from mild to severe form.[20]

---

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7115629/

[15] Yip MS, Leung HL, Li PH, Cheung CY, Dutry I, Li D, Daëron M, Bruzzone R, Peiris JS, Jaume M.
Antibody-dependent enhancement of SARS coronavirus infection and its role in the pathogenesis of SARS.
Hong Kong Med J. 2016 Jun;22(3 Suppl 4):25-31. https://www.hkmj.org/system/files/hkm1603sp4p25.pdf

[16] Banerjee A, Nasir JA, Budylowski P, et al.
Isolation, Sequence, Infectivity, and Replication Kinetics of Severe Acute Respiratory Syndrome Coronavirus 2.
Emerg Infect Dis. 2020;26(9):2054-2063. doi:10.3201/eid2609.201495
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7454076/

[17] Pontelli MC, Castro IA, Martins RB, et al.
Infection of human lymphomononuclear cells by SARS-CoV-2.
bioRxiv; 2020. DOI: 10.1101/2020.07.28.225912.
https://europepmc.org/article/ppr/ppr193957

Junqueira C, et al
FcγR-mediated SARS-CoV-2 infection of monocytes activates inflammation.
Nature. 2022 Apr 6. doi: 10.1038/s41586-022-04702-4.
https://www.nature.com/articles/s41586-022-04702-4

Sefik E, et al
Inflammasome activation in infected macrophages drives COVID-19 pathology.
bioRxiv [Preprint]. 2022 Apr 1:2021.09.27.461948. doi: 10.1101/2021.09.27.461948. Update in: Nature. 2022 Apr 28
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8491846/
https://www.biorxiv.org/content/10.1101/2021.09.27.461948v2.full.pdf

Junqueira C, Crespo Ã, Ranjbar S, et al.
SARS-CoV-2 infects blood monocytes to activate NLRP3 and AIM2 inflammasomes, pyroptosis and cytokine release. Preprint. Res Sq. 2021;rs.3.rs-153628. Published 2021 Aug 11. doi:10.21203/rs.3.rs-153628/v1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8366805/

Mallapaty S.
What triggers severe COVID? Infected immune cells hold clues.
Nature. 2022 Apr;604(7905):231. doi: 10.1038/d41586-022-00965-z.
https://www.nature.com/articles/d41586-022-00965-z

[18] Merad M, Martin JC.
Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages.
Nat Rev Immunol. 2020 Jun;20(6):355-362. doi: 10.1038/s41577-020-0331-4. Epub 2020 May 6. Erratum in: Nat Rev Immunol. 2020 Jun 2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7201395/

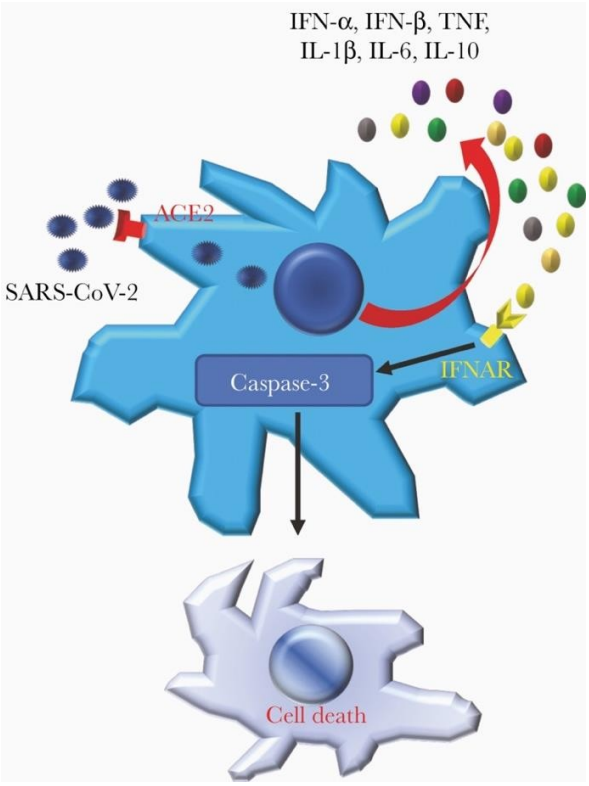
[19] Tetro JA.
Is COVID-19 receiving ADE from other coronaviruses?
Microbes Infect. 2020 Mar;22(2):72-73. doi: 10.1016/j.micinf.2020.02.006.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102551/

Iwasaki A, Yang Y.
The potential danger of suboptimal antibody responses in COVID-19.
Nat Rev Immunol. 2020 Jun;20(6):339-341. doi: 10.1038/s41577-020-0321-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7187142/

[20] Pontelli MC, et al
SARS-CoV-2 productively infects primary human immune system cells in vitro and in COVID-19 patients.
J Mol Cell Biol. 2022 Apr 22:mjac021. doi: 10.1093/jmcb/mjac021.
https://academic.oup.com/jmcb/advance-article/doi/10.1093/jmcb/mjac021/6572370

Nicole L. et al
SARS-CoV-2 infection of circulating immune cells is not responsible for virus dissemination in severe COVID-19 patients
bioRxiv 2021.01.19.427282; doi: https://doi.org/10.1101/2021.01.19.427282

ADE may explain the observed dysregulation of the immune system, including mass apoptosis of the immune cells, as well as the development of cytokine storm in some patients.
The replication of SARS-Cov-2 in the immune cell can be abortive, that is, without the production of viable (replication-capable) virions. [21] In this case, weak low titer or nonproductive infection of immune cells is not antibody-mediated and therefore not associated with ADE, but depends on ACE2-protein spike binding, which equally leads to mass death of immune cells, the inflammatory cascade, and finally the cytokine storm.[22]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7799009/
In this study, using human circulating monocyte-derived macrophages (MDM) and monocyte-derived dendritic cells (MDDC), the authors demonstrated that SARS-CoV-2 abortively infected both cell types in an ACE2-dependent manner. The abortive infection induced the expression of higher levels of cytokines and chemokines (interferon [IFN]-α, TNF, IL-6, IL-8, IL-10 and CXCL-10), leading to IFN type I-mediated cell death. Compared with SARS- CoV, SARS-CoV-2 induced high levels of most cytokines and chemokines and more cell death. IFNAR: anti alpha/beta interferon receptor antibody

In monocytes or macrophages bearing the Fc receptor ($CD32^+$), the virus internalized in a stable complex with antibodies, cannot escape and is usually destroyed. Elimination of the virus promotes host recovery, as shown on the left side of the following figure. In contrast, in the case of ADE, the virus breaks free from the less stable complex with the antibody and initiates the replicative cycle within the immune cell, as shown on the right side of the figure.[23]

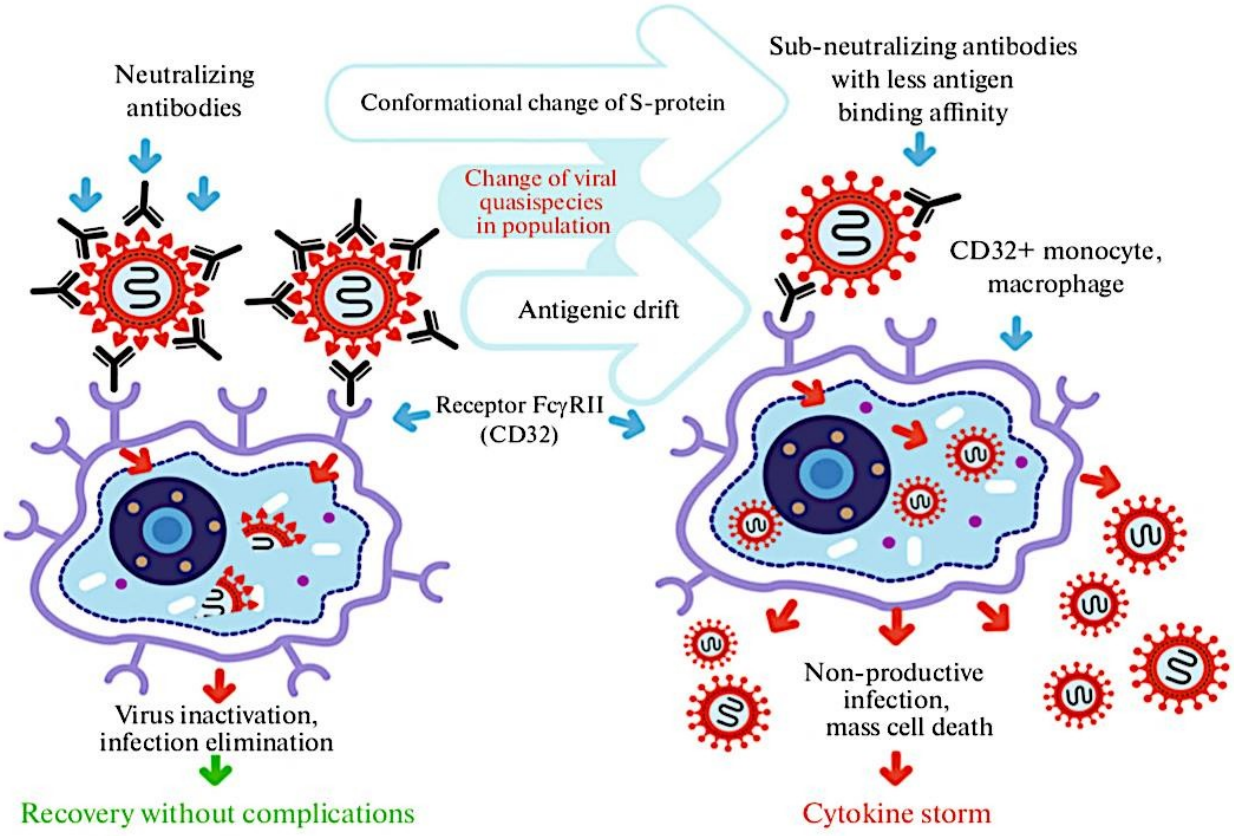
https://www.biorxiv.org/content/10.1101/2021.01.19.427282v1.full.pdf

Junqueira, C., Crespo, Â., Ranjbar, S. et al.
FcγR-mediated SARS-CoV-2 infection of monocytes activates inflammation.
Nature (2022). https://doi.org/10.1038/s41586-022-04702-4
https://www.nature.com/articles/s41586-022-04702-4

[21] Boumaza A, Gay L, Mezouar S, Bestion E, Diallo AB, Michel M, Desnues B, Raoult D, La Scola B, Halfon P, Vitte J, Olive D, Mege JL.
Monocytes and macrophages, targets of SARS-CoV-2: the clue for Covid-19 immunoparalysis.
J Infect Dis. 2021 Jan 25:jiab044. doi: 10.1093/infdis/jiab044.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7928817/pdf/jiab044.pdf

[22] Zheng J, Wang Y, Li K, Meyerholz DK, Allamargot C, Perlman S.
Severe Acute Respiratory Syndrome Coronavirus 2-Induced Immune Activation and Death of Monocyte-Derived Human Macrophages and Dendritic Cells.
J Infect Dis. 2021 Mar 3;223(5):785-795. doi: 10.1093/infdis/jiaa753.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7799009/

[23] Zaichuk TA, Nechipurenko YD, Adzhubey AA, Onikienko SB, Chereshnev VA, Zainutdinov SS, Kochneva GV, Netesov SV, Matveeva OV.
The Challenges of Vaccine Development against Betacoronaviruses: Antibody Dependent Enhancement and Sendai Virus as a Possible Vaccine Vector. Mol Biol. 2020 Sep 4:1-15. doi: 10.1134/S0026893320060151.



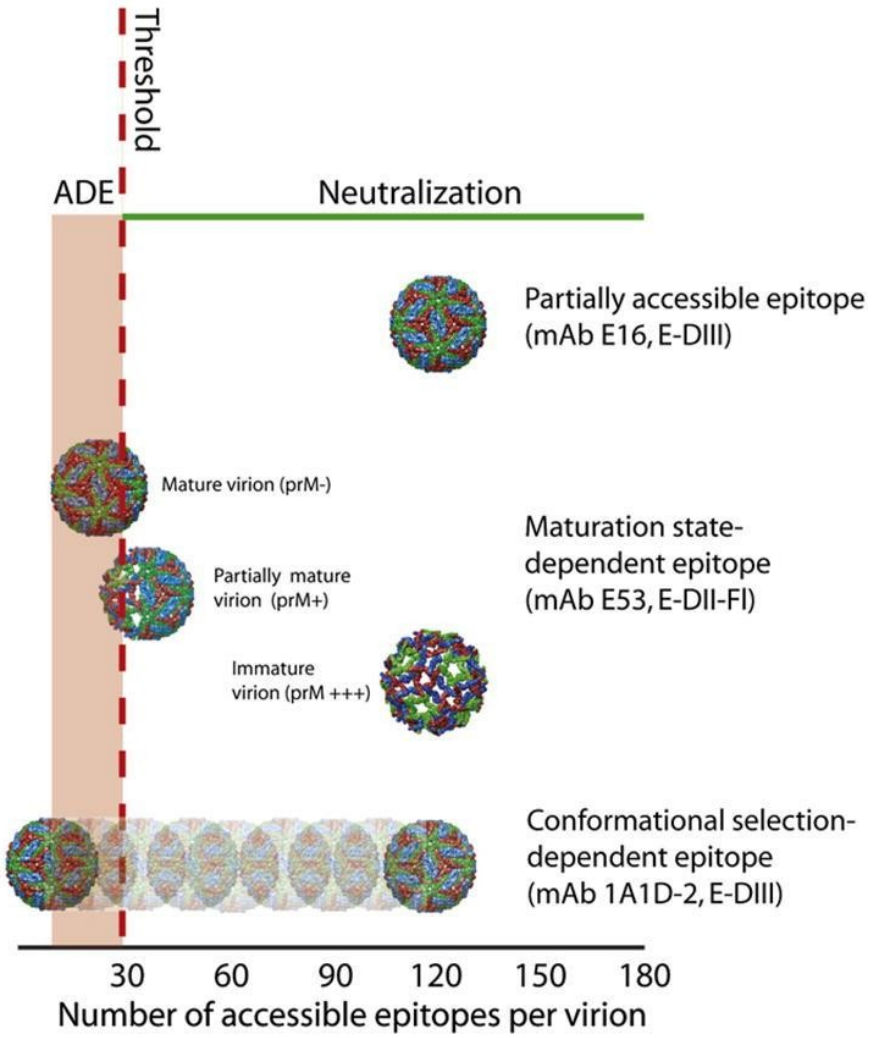
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7473411/
Diagram of antibody-dependent enhancement of infection (ADE) for SARS-CoV-1. On **the left**, a scenario of the correct immune response is shown, when specific neutralizing and protective antibodies contribute to the elimination of the virus from the body. According to this scenario, viruses are phagocytosed as stable antigen-antibody complexes and destroyed by macrophages or other immune cells. **On the right is** an immunopathological scenario that occurs when the antigen of the virus changes and, because of this change, IgG antibodies form imperfect complexes with the virus. The unstable antibody-virus complex binds to the FñγRII receptor of immune cells and is taken up by these cells. Also, inside the cell, the virus leaves the endosome, which is already devoid of the antibody, and begins the replicative cycle.

In the "multiple-event" neutralization model (*multiple-hit hypothesis*), virus blockade is related to the number of antibodies coating the virion, which is influenced by the concentration and affinity of the antibody.
For a given antibody concentration and specific binding domain, the stoichiometry of antibody recruitment to a virion depends on the strength of the antibody-antigen interaction.
According to this theory, ADE is induced when the stoichiometry is below the neutralization threshold. Therefore, higher affinity antibodies can reach the threshold at a lower concentration and mediate a better protective response.[24]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7473411/

Nechipurenko YD, Anashkina AA, Matveeva OV.
Change of Antigenic Determinants of SARS-CoV-2 Virus S-Protein as a Possible Cause of Antibody-Dependent Enhancement of Virus Infection and Cytokine Storm.
Biophysics (Oxf). 2020;65(4):703-709. doi:10.1134/S0006350920040119
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7569100/

[24] Pierson TC, Fremont DH, Kuhn RJ, Diamond MS.
Structural insights into the mechanisms of antibody-mediated neutralization of flavivirus infection: implications for vaccine development.
Cell Host Microbe. 2008;4(3):229-238. doi:10.1016/j.chom.2008.08.004
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2678546/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2678546/
A model of stoichiometric requirements for flavivirus neutralization
Neutralization of flavivirus infection is a multiple-effect phenomenon that requires engagement of the virion by the antibody with a stoichiometry that exceeds a threshold (modeled as 30 mAbs based on studies with WNV E-DIII-LR-specific mAbs; red dashed line). [25] Whether a single antibody can dock to the virion with sufficient stoichiometry to exceed this threshold depends on its affinity for viral antigens and the total number of accessible epitopes on the average virion (shown schematically on the x-axis). Epitopes that are differentially accessible on virions depending on the extent of virion maturation or the structural dynamics of the viral particle add to the complexity of the model (shown schematically for maturation-dependent epitopes and those binding selective conformations, respectively). Neutralization by highly accessible determinants can be achieved by engaging in relatively low occupancy, whereas antibodies binding cryptic determinants must bind a larger fraction of them. Not all epitopes appear to exist in the average virion at levels that exceed this threshold. Engagement of the virion with a stoichiometry below this threshold may support antibody-dependent enhancement of infection.

[25] Pierson TC, Xu Q, Nelson S, Oliphant T, Nybakken GE, Fremont DH, Diamond MS.
The stoichiometry of antibody-mediated neutralization and enhancement of West Nile virus infection.
Cell Host Microbe. 2007 Apr 19;1(2):135-45. doi: 10.1016/j.chom.2007.03.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2656919/.

ADE mediated by C1q

In the recent article by Okuya et al, "*Multiple Routes of Antibody-Dependent Enhancement of SARS-CoV-2 Infection,*"[26] C1q-mediated ADE was explored in depth.
Specifically, 89 serum samples collected from acute and convalescent COVID-19 patients were tested, and 62.9% were positive for SARS-CoV-2-specific IgG. FcγR- and/or C1q-mediated ADE was detected in 50% of IgG-positive sera, while most of them showed neutralizing activity in the absence of FcγR and C1q.
Importantly, ADE antibodies were found in 41.4% of acute patients with COVID-19 (median 6; interquartile range [IQR] 2.25-9 days after onset), suggesting the possibility of ADE in promoting virus replication even in the acute phase of primary SARS-CoV-2 infection; moreover, C1q-mediated ADE tended to be more prevalent than FcγR-mediated ADE in patients with mild and moderate symptoms, compared with sera in the convalescent phase.
Because C1q binds more efficiently to IgM and polymeric IgG-like IgM than to monomeric IgG antibodies[27] , it was believed that some anti-S-specific IgM antibodies may contribute to disease exacerbation *in vivo,* and in this study, not only IgG antibodies but also IgM antibodies were found in some of the acute patients with COVID-19.
Previous studies showed that the epitopes recognized by ADE antibodies were generally distinct from those for neutralizing antibodies, although some ADE antibodies show neutralizing activity at high concentrations[28] .
Interestingly, ADE antibodies against MERS-CoV, which can recognize a particular epitope on the trimeric S receptor binding site, stabilized the receptor binding site, and this triggered a conformational change of MERS-CoV S that led to increased infection[29] .

---

[26] Okuya K, Hattori T, Saito T, Takadate Y, Sasaki M, Furuyama W, Marzi A, Ohiro Y, Konno S, Hattori T, Takada A.
Multiple Routes of Antibody-Dependent Enhancement of SARS-CoV-2 Infection.
Microbiol Spectr. 2022 Apr 27;10(2):e0155321. doi: 10.1128/spectrum.01553-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9045191/

[27] Smith RI, Coloma MJ, Morrison SL.
Addition of a mu-tailpiece to IgG results in polymeric antibodies with enhanced effector functions including complement-mediated cytolysis by IgG4. J Immunol. 1995 Mar 1;154(5):2226-36.
https://pubmed.ncbi.nlm.nih.gov/7868896/

Gadjeva MG, Rouseva MM, Zlatarova AS, Reid KB, Kishore U, Kojouharova MS.
Interaction of human C1q with IgG and IgM: revisited. Biochemistry. 2008 Dec 9;47(49):13093-102. doi: 10.1021/bi801131h.
https://pubmed.ncbi.nlm.nih.gov/19006321/

[28] Kuzmina NA, Younan P, Gilchuk P, Santos RI, Flyak AI, Ilinykh PA, Huang K, Lubaki NM, Ramanathan P, Crowe JE Jr, Bukreyev A.
Antibody-Dependent Enhancement of Ebola Virus Infection by Human Antibodies Isolated from Survivors.
Cell Rep. 2018 Aug 14;24(7):1802-1815.e5. doi: 10.1016/j.celrep.2018.07.035.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6697154/.

Yamanaka A, Kotaki T, Konishi E.
A mouse monoclonal antibody against dengue virus type 1 Mochizuki strain targeting envelope protein domain II and displaying strongly neutralizing but not enhancing activity.
J Virol. 2013 Dec;87(23):12828-37. doi: 10.1128/JVI.01874-13.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3838163/

Takada A, Ebihara H, Feldmann H, Geisbert TW, Kawaoka Y.
Epitopes required for antibody-dependent enhancement of Ebola virus infection.
J Infect Dis. 2007 Nov 15;196 Suppl 2:S347-56. doi: 10.1086/520581.
https://pubmed.ncbi.nlm.nih.gov/17940970/

Zhou Y, et al
Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor associates with distinct epitopes on the RBD.
Cell Rep. 2021 Feb 2;34(5):108699. doi: 10.1016/j.celrep.2021.108699.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7802522/

[29] Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, Shi Z, Zhou Y, Du L, Li F.
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
J Virol. 2020 Feb 14;94(5):e02015-19. doi: 10.1128/JVI.02015-19.

A more recent study has shown that certain antibodies that recognize the N-terminal domain of SARS- CoV-2 S induce a conformational change of the receptor binding domain and enhance viral infectivity independently of the FcγR- and C1q-mediated ADE pathways[30] .
However, the authors believe that such ADE antibodies are not prevalent in natural SARS-CoV- 2 infection because none of the sera tested in the study had increased viral infectivity in the absence of FcγR and C1q.

Methodology for the study of ADE:

To study FcγR-mediated ADE, the authors stabilized FcγRIIa-expressing Vero E6 cells (Vero E6/FcγRIIa cells) and confirmed that an EBOV-specific ADE antibody (ZGP12/1.1)[31] effectively increased the infectivity of pseudotyped VSV with EBOV glycoprotein (GP) (VSV-EBOV) in this cell line (**Fig. A**).
Similarly, C1q-mediated ADE was confirmed using ZGP12/1.1 cells and Vero E6 cells lacking FcγR[31] (**Fig. B**). This antibody showed neither neutralization nor ADE activity in the absence of FcγR or C1q (**Fig. C**).
Using these assay conditions, the authors investigated FcγR- and C1q-mediated ADE activities in serum samples collected for this study. Vero E6/FcγRIIa cells were infected with VSV-SARS- Cov-2[32] mixed with serially diluted serum samples (1:40 to 1:40960) for FcγR-mediated ADE assay. For C1q-mediated ADE assay, Vero E6 cells were infected with virus mixed with serially diluted serum samples in the presence of C1q.
As an ADE-negative control test (i.e., neutralization condition), Vero E6 cells were infected with virus mixed with serum dilutions in the absence of C1q.

https://journals.asm.org/doi/10.1128/spectrum.01553-21
ADE mechanisms and assay validation for FcγR- and C1q-mediated ADE using VSV-EBOV and a monoclonal antibody specific for EBOV glycoprotein. Infectious titers of VSV-EBOV mixed with indicated concentrations of monoclonal antibody ZGP12/1.1 were measured in **(A)** Vero E6/FcγRIIa cells, **(B)** Vero E6 cells in the presence of C1q, and **(C)** Vero E6 cells in the absence of C1q. The relative number of infected cells was calculated by setting the number of GFP-positive cells in the absence of the antibody to 100%. The dots and error bars indicate the means and standard deviations of the triplicate wells, respectively. The right panels show the patterns of the respective conditions.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

[30] Liu Y, et al
An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies.
Cell. 2021 Jun 24;184(13):3452-3466.e18. doi: 10.1016/j.cell.2021.05.032.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8142859/

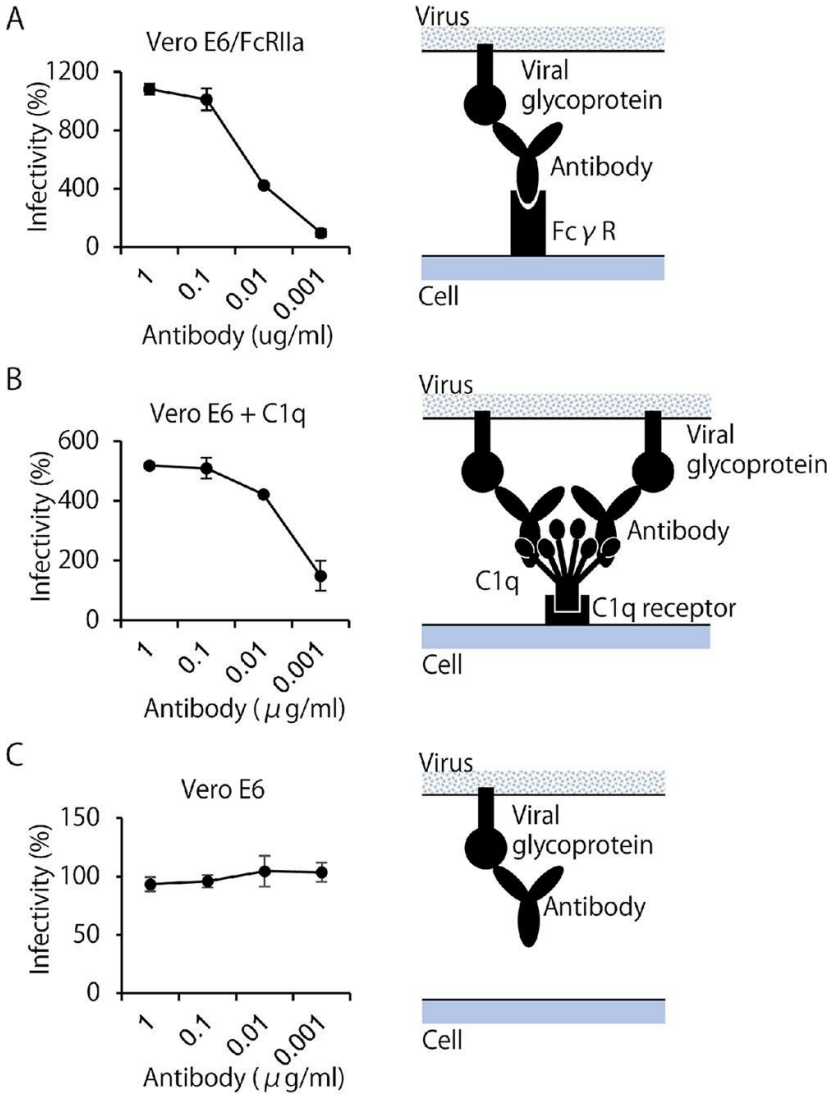
[31] Takada A, Ebihara H, Feldmann H, Geisbert TW, Kawaoka Y.
Epitopes required for antibody-dependent enhancement of Ebola virus infection.
J Infect Dis. 2007 Nov 15;196 Suppl 2:S347-56. doi: 10.1086/520581.
https://pubmed.ncbi.nlm.nih.gov/17940970/

[32] Case JB, et al
Neutralizing Antibody and Soluble ACE2 Inhibition of a Replication-Competent VSV-SARS-CoV-2 and a Clinical Isolate of SARS-CoV-2.
Cell Host Microbe. 2020 Sep 9;28(3):475-485.e5. doi: 10.1016/j.chom.2020.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332453/.

Weisblum Y, et al
Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants.
Elife. 2020 Oct 28;9:e61312. doi: 10.7554/eLife.61312.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7723407/

Millet JK, Tang T, Nathan L, Jaimes JA, Hsu HL, Daniel S, Whittaker GR.
Production of Pseudotyped Particles to Study Highly Pathogenic Coronaviruses in a Biosafety Level 2 Setting.
J Vis Exp. 2019 Mar 1;(145):10.3791/59010. doi: 10.3791/59010.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6677141/

Condor Capcha JM, Lambert G, Dykxhoorn DM, Salerno AG, Hare JM, Whitt MA, Pahwa S, Jayaweera DT, Shehadeh LA.
Generation of SARS-CoV-2 Spike Pseudotyped Virus for Viral Entry and Neutralization Assays: A 1-Week Protocol.
Front Cardiovasc Med. 2021 Jan 15;7:618651. doi: 10.3389/fcvm.2020.618651.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7843445/



Previous studies on other viruses have indicated that ADE is often observed at subneutralizing concentrations of ADE antibodies[33] . The data suggest that neutralizing activity is dominant when overall levels of SARS-CoV-2-specific antibodies are high, and the risk of ADE may appear at a time when neutralizing antibodies are reduced below the level of detection.
A possible case of ADE was studied for this purpose in a patient with reinfection by variant SARS- CoV-2[34] who manifested a high viral load and more complicated disease than the first infection.

[33] Takada A, Kawaoka Y.
Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications.
Rev Med Virol. 2003 Nov-Dec;13(6):387-98. doi: 10.1002/rmv.405.
https://pubmed.ncbi.nlm.nih.gov/14625886/

Yamanaka A, Kotaki T, Konishi E.
A mouse monoclonal antibody against dengue virus type 1 Mochizuki strain targeting envelope protein domain II and displaying strongly neutralizing but not enhancing activity.
J Virol. 2013 Dec;87(23):12828-37. doi: 10.1128/JVI.01874-13.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3838163/

[34] Tillett RL, et al
Genomic evidence for reinfection with SARS-CoV-2: a case study.
Lancet Infect Dis. 2021 Jan;21(1):52-58. doi: 10.1016/S1473-3099(20)30764-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7550103/

Although these studies suggest a positive correlation between disease severity and IgG response, supporting the production of ADE antibodies[35] , it can also be speculated that it is simply due to the difference in the extent of viral infection and the subsequent immune response, without being directly associated with the presence of ADE antibodies. Indeed, it has been found that elevated early antibody responses correlate with increased disease severity for both SARS[36] and COVID-19[37] . Thus, it is important to distinguish between these different mechanisms of disease enhancement in order to proceed with appropriate treatment.

Li et al[38] recently studied the in *vitro* and *in vivo* functions of antibodies that potentiate and neutralize SARS-Cov-2 infection. Specifically, they isolated neutralizing antibodies (NAbs) against

[35] Gerhards C, Thiaucourt M, Kittel M, Becker C, Ast V, Hetjens M, Neumaier M, Haselmann V.
Longitudinal assessment of anti-SARS-CoV-2 antibody dynamics and clinical features following convalescence from a COVID-19 infection.
Int J Infect Dis. 2021 Jun;107:221-227. doi: 10.1016/j.ijid.2021.04.080.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8080496/

Fill Malfertheiner S, Brandstetter S, Roth S, Harner S, Buntrock-Döpke H, Toncheva AA, Borchers N, Gruber R, Ambrosch A, Kabesch M, Häusler S.
Immune response to SARS-CoV-2 in health care workers following a COVID-19 outbreak: A prospective longitudinal study.
J Clin Virol. 2020 Sep;130:104575. doi: 10.1016/j.jcv.2020.104575.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7406471/

Crawford KHD, et al
Dynamics of Neutralizing Antibody Titers in the Months After Severe Acute Respiratory Syndrome Coronavirus 2 Infection.
J Infect Dis. 2021 Feb 3;223(2):197-205. doi: 10.1093/infdis/jiaa618.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7543487/

Park JH, Cha MJ, Choi H, Kim MC, Chung JW, Lee KS, Jeong DG, Baek MS, Kim WY, Lim Y, Yoon SW, Choi SH.
Relationship between SARS-CoV-2 antibody titer and the severity of COVID-19.
J Microbiol Immunol Infect. 2022 May 5:S1684-1182(22)00059-7. doi: 10.1016/j.jmii.2022.04.005.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9069977/

[36] Lee N, Chan PK, Ip M, Wong E, Ho J, Ho C, Cockram CS, Hui DS.
Anti-SARS-CoV IgG response in relation to disease severity of severe acute respiratory syndrome.
J Clin Virol. 2006 Feb;35(2):179-84. doi: 10.1016/j.jcv.2005.07.005.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108264/

[37] Liu X, Wang J, Xu X, Liao G, Chen Y, Hu CH.
Patterns of IgG and IgM antibody response in COVID-19 patients.
Emerg Microbes Infect. 2020 Dec;9(1):1269-1274. doi: 10.1080/22221751.2020.1773324.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7448841/

Chen W, Zhang J, Qin X, Wang W, Xu M, Wang LF, Xu C, Tang S, Liu P, Zhang L, Liu X, Zhang Y, Yi C, Hu Z, Yi Y.
SARS-CoV-2 neutralizing antibody levels are correlated with severity of COVID-19 pneumonia.
Biomed Pharmacother. 2020 Oct;130:110629. doi: 10.1016/j.biopha.2020.110629.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7425713/

Young BE, Onet al Singapore 2019 Novel Coronavirus Outbreak Research team.
Viral dynamics and immune correlates of COVID-19 disease severity.
Clin Infect Dis. 2020 Aug 28:ciaa1280. doi: 10.1093/cid/ciaa1280.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7499509/

Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg A, Nadkarni G, Glicksberg BS, Houldsworth J, Cordon-Cardo C.
SARS-CoV-2 viral load predicts COVID-19 mortality.
Lancet Respir Med. 2020 Sep;8(9):e70. doi: 10.1016/S2213-2600(20)30354-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836878/

Luo YR, Chakraborty I, Yun C, Wu AHB, Lynch KL.
Kinetics of SARS-CoV-2 Antibody Avidity Maturation and Association with Disease Severity
[published online ahead of print, 2020 Sep 14]. Clin Infect Dis. 2020;ciaa1389. doi:10.1093/cid/ciaa1389
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7543300/pdf/ciaa1389.pdf

Fajnzylber J, et al
Massachusetts Consortium for Pathogen Readiness. SARS-CoV-2 viral load is associated with increased disease severity and mortality.
Nat Commun. 2020 Oct 30;11(1):5493. doi: 10.1038/s41467-020-19057-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7603483/

[38] Li D, et al
In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies.

receptor binding domain (RBD) or the N-terminal domain (NTD) of the viral spike from individuals with acute or convalescent SARS-CoV-2 or a history of SARS-CoV infection.
Some RBD NAbs induced Fc-γ receptor (FcγR)-mediated enhancement of viral infection in *vitro,* while five nonneutralizing NTD antibodies mediated FcγR-independent enhancement of infection *in vitro.* Three of 46 monkeys infused with enhancing antibodies had higher lung inflammation scores than controls, and one monkey presented alveolar edema and elevated inflammatory cytokines from bronchoalveolar lavage.
In summary, although antibody-enhanced infection *in vitro* is not predictive for enhanced infection *in vivo*, increased lung inflammation was found in macaques infused with SARS-CoV-2 antibodies confirming an ADE mechanism.

Non-canonical ADE

Recently, Liu et al[39] found that antibodies against a specific site on the NTD (N-terminal domain) of the SARS-CoV-2 spike protein directly increase the binding of ACE2 to the spike protein, thereby increasing SARS-CoV-2 infectivity.
Although the ADE induced by enhancing antibodies is relatively inferior to the Fc receptor-mediated ADE observed in other viruses such as Dengue virus, Fc receptors are not involved in this new type of ADE.
Therefore, enhancer antibodies could be involved in SARS-CoV-2 infection for a wide range of cells that do not express Fc receptors. This suggests that enhanced ACE2 binding capacity to the spike protein, although small, plays an important role in SARS-CoV-2 infection.
The RBD (Receptor-Binding Domain) of the spike protein is quite flexible, and ACE2 binds preferentially to the open conformation of the RBD[40] , as will be discussed in more detail below, suggesting a vital role in the infectivity of SARS-CoV-2[41] .
Using a specific antibody, the authors found that the enhancing antibodies induced the open conformation of RBD upon binding to NTD.
Interestingly, variants B.1.1.7 (alpha variant) and B.1.135, which will be discussed in more detail in the variant section, contain the H69-V70 deletion and the D215G mutation, respectively, which are very close to the enhancer antibody binding site. This suggests that mutations around the epitopes of the enhancer site are able to influence the infectivity of SARS-CoV-2.

Cell. 2021 Aug 5;184(16):4203-4219.e32. doi: 10.1016/j.cell.2021.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232969/

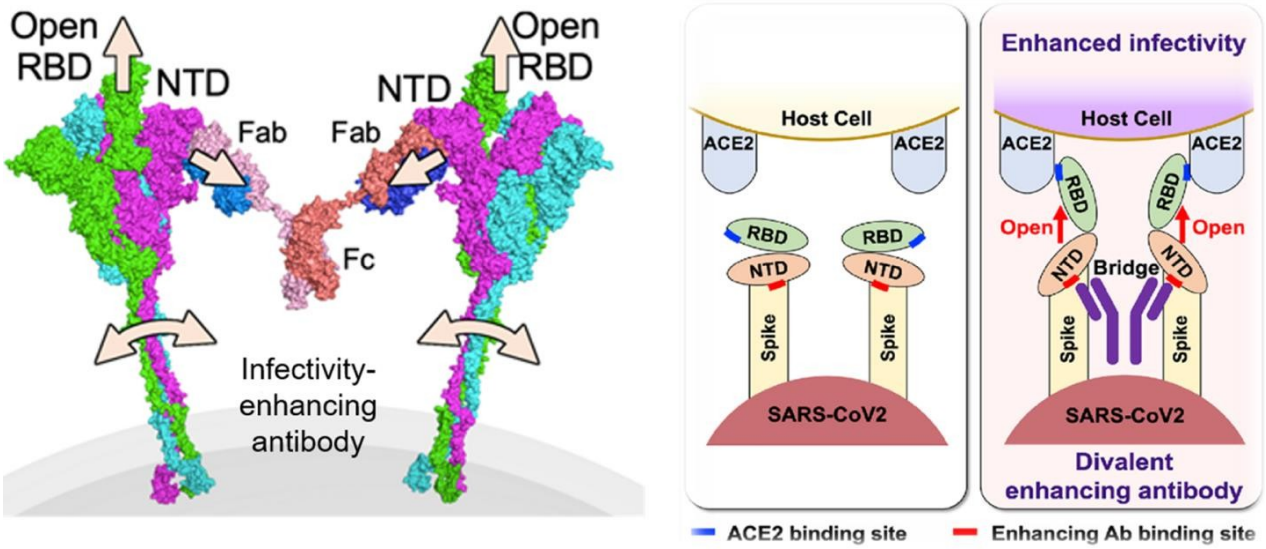
[39] Liu Y, Soh WT, Kishikawa JI, et al.
An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies.
Cell. 2021;184(13):3452-3466.e18. doi:10.1016/j.cell.2021.05.032
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8142859/

Yafei Liu, Yukoh Nakazaki, Hisashi Arase,
Infectivity-enhancing antibodies against SARS-CoV-2,
Translational and Regulatory Sciences, 2022, Volume 4, Issue 1, Pages 1-4, https://doi.org/10.33611/trs.2021-021,
https://www.jstage.jst.go.jp/article/trs/4/1/4_2021-021/_article/-char/en

[40] Henderson R, Edwards RJ, Mansouri K, et al.
Controlling the SARS-CoV-2 spike glycoprotein conformation.
Nat Struct Mol Biol. 2020;27(10):925-933. doi:10.1038/s41594-020-0479-4
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8581954/

Lu M, Uchil PD, Li W, et al.
Real-Time Conformational Dynamics of SARS-CoV-2 Spikes on Virus Particles.
Cell Host Microbe. 2020;28(6):880-891.e8. doi:10.1016/j.chom.2020.11.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7664471/

[41] Yurkovetskiy L, Wang X, Pascal KE, et al.
Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant.
Cell. 2020;183(3):739-751.e8. doi:10.1016/j.cell.2020.09.032
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7492024/



https://www.jstage.jst.go.jp/article/trs/4/1/4_2021-021/_article
Mechanism of action of anti-infective antibodies. Although Fab fragments of the infectivity-enhancing antibody bind to the spike protein like conventional antibodies, no infectivity-enhancing effect is observed. On the other hand, because F(ab´)$_2$ fragments show an infectivity enhancing effect, a completely new function was revealed. Here, antibody-cross-linked NTDs induce open RBDs by extraction, resulting in increased infectivity (modified from Liu et al., Cell 2021). NTD, N-terminal domain; RBD, receptor binding domain; ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ab, antibody.

Mast cells and ADEs

Mast cells are tissue-resident cells containing mediators that can regulate both innate and adaptive immune responses[42] .
Mast cell enrichment at environmental interfaces allows these cells to be among the first to respond during pathogen invasion, along with dendritic cells and epithelial cells[43] .
In addition, they are typically located near blood vessels, lymphatics, and nerve endings, allowing them to have long-range effects on the host's response to pathogens[44] , and thus are critical for immune surveillance, provoking an immediate reaction to invading pathogens and initiating an appropriate innate and adaptive immune response.[45]

[42] Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CM, Tsai M.
Mast cells as "tunable" effector and immunoregulatory cells: recent advances.
Annu Rev Immunol. 2005;23:749-86. doi: 10.1146/annurev.immunol.21.120601.141025.
https://pubmed.ncbi.nlm.nih.gov/15771585/

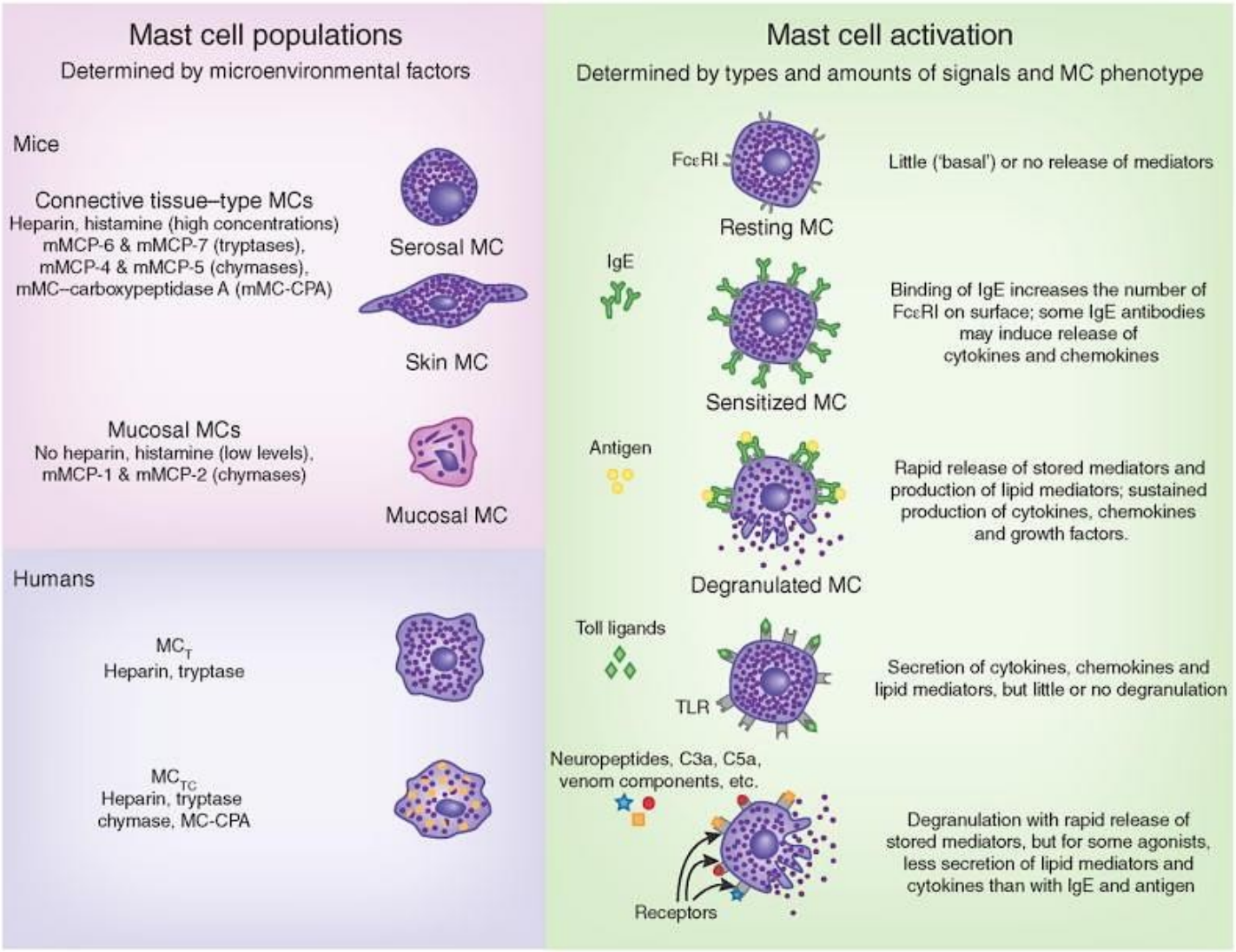
[43] Abraham SN, St John AL.
Mast cell-orchestrated immunity to pathogens.
Nat Rev Immunol. 2010 Jun;10(6):440-52. doi: 10.1038/nri2782.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4469150/

[44] Marshall JS, Jawdat DM.
Mast cells in innate immunity.
J Allergy Clin Immunol. 2004 Jul;114(1):21-7. doi: 10.1016/j.jaci.2004.04.045.
https://pubmed.ncbi.nlm.nih.gov/15241339/

Katsoulis-Dimitriou K, Kotrba J, Voss M, Dudeck J, Dudeck A.
Mast Cell Functions Linking Innate Sensing to Adaptive Immunity.
Cells. 2020 Nov 25;9(12):2538. doi: 10.3390/cells9122538.
https://www.mdpi.com/2073-4409/9/12/2538/htm

[45] Graham AC, Temple RM, Obar JJ.
Mast cells and influenza a virus: association with allergic responses and beyond.
Front Immunol. 2015;6:238. Published 2015 May 18. doi:10.3389/fimmu.2015.00238
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4435071/

Mast cells express a wide range of cell surface receptors that mediate innate response and cytosolic receptors that mediate appropriate immune responses to infectious agents.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3412172/

Mast cell populations and functional activation pattern. Mast cells (MCs) in mice or humans can be subclassified (**left**) into populations defined by anatomical location and/or mediator content (such as proteoglycans (heparin to chondroitin sulfates) or proteases (tryptase, chymase, or MC-CPA)). In IgE-associated immune responses to allergens or parasites (**top right**), activation of mast cells through cross-linking of IgE bound to high-affinity receptors for IgE (FcεRI) on the cell surface by bi- or multivalent antigens results in rapid exocytosis of cytoplasmic granules (degranulation) and production of lipid mediators (such as leukotrienes and prostaglandins) and more sustained secretion of many cytokines, chemokines, and growth factors. Although many of these mediators have proinflammatory effects, others may have effects that suppress inflammation or promote tissue remodeling or repair. Non-IgE-dependent signals (**bottom right**) can elicit different patterns of mediator release in mast cell populations that express appropriate receptors for such ligands. Microenvironmental factors can influence the phenotype of mast cells developing under basal conditions at different anatomical sites (**left**), including those phenotypic features that allow mast cells to respond to various ligands (such as the expression pattern of receptors for those ligands) or to produce different mediators (**right**). TLRs are examples of the numerous pattern recognition receptors expressed by various mast cell populations. MCT, mast cells containing mainly tryptase; MCTC, mast cells containing both tryptase and chymase; C3a and C5a, anaphylatoxins of the complement system.

Galli SJ, Borregaard N, Wynn TA.
Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils.
Nat Immunol. 2011 Oct 19;12(11):1035-44. doi: 10.1038/ni.2109.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3412172/

In particular, mast cells express various Fc receptors including FcεRI, FcγRI and FcγRIII[46] , and are also able to respond through a wide variety of pattern recognition receptors (PRRs), including toll-like receptors (TLRs), nod-like (NLR), retinoic acid-inducible gene 1 (RLR), and C-type lectin receptors (CLR), each of which plays an essential role in innate immunity by detecting conserved molecular patterns expressed by pathogens[47] .
Mast cells can also be activated through recruitment of complement receptors[48] , CD48,[49] and integrins[50] . Finally, they can respond to pathogens indirectly through the IL-33 signaling pathway[51] .

---

[46] Beaven MA, Metzger H.
Signal transduction by Fc receptors: the Fc epsilon RI case.
Immunol Today. 1993 May;14(5):222-6. doi: 10.1016/0167-5699(93)90167-j.
https://pubmed.ncbi.nlm.nih.gov/8517921/

[47] Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, Iwasaki A, Flavell RA.
Recognition of single-stranded RNA viruses by Toll-like receptor 7.
Proc Natl Acad Sci U S A. 2004 Apr 13;101(15):5598-603. doi: 10.1073/pnas.0400937101. Epub 2004 Mar 19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC397437/

Diebold SS, Kaisho T, Hemmi H, Akira S, Reis and Sousa C.
Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA.
Science. 2004 Mar 5;303(5663):1529-31. doi: 10.1126/science.1093616. Epub 2004 Feb 19.
https://pubmed.ncbi.nlm.nih.gov/14976261/

Pichlmair A, Schulz O, Tan CP, Näslund TI, Liljeström P, Weber F, Reis, and Sousa
C. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-
phosphates.
Science. 2006 Nov 10;314(5801):997-1001. doi: 10.1126/science.1132998. Epub 2006 Oct 12.
https://pubmed.ncbi.nlm.nih.gov/17038589/

Kato H, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, Tsujimura T, Takeda K, Fujita T, Takeuchi O, Akira S.
Cell type-specific involvement of RIG-I in antiviral response.
Immunity. 2005 Jul;23(1):19-28. doi: 10.1016/j.immuni.2005.04.010
https://www.cell.com/action/showPdf?pii=S1074-7613%2805%2900142-1

Seth RB, Sun L, Ea CK, Chen ZJ.
Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3.
Cell. 2005 Sep 9;122(5):669-82. doi: 10.1016/j.cell.2005.08.012.
https://www.cell.com/action/showPdf?pii=S0092-8674%2805%2900816-0

Kato H, et al
Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses.
Nature. 2006 May 4;441(7089):101-5. doi: 10.1038/nature04734. Epub 2006 Apr 9.
https://pubmed.ncbi.nlm.nih.gov/16625202/

[48] Marshall JS, Jawdat DM.
Mast cells in innate immunity.
J Allergy Clin Immunol. 2004 Jul;114(1):21-7. doi: 10.1016/j.jaci.2004.04.045.
https://pubmed.ncbi.nlm.nih.gov/15241339/

[49] Rocha-de-Souza CM, Berent-Maoz B, Mankuta D, Moses AE, Levi-Schaffer F.
Human mast cell activation by Staphylococcus aureus: interleukin-8 and tumor necrosis factor alpha release and the role of Toll-like receptor 2 and CD48 molecules.
Infect Immun. 2008 Oct;76(10):4489-97. doi: 10.1128/IAI.00270-08. Epub 2008 Jul 21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2546849/

Malaviya R, Ikeda T, Ross E, Abraham SN.
Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha.
Nature. 1996 May 2;381(6577):77-80. doi: 10.1038/381077a0.
https://pubmed.ncbi.nlm.nih.gov/8609993/

[50] Edelson BT, Li Z, Pappan LK, Zutter MM.
Mast cell-mediated inflammatory responses require the alpha 2 beta 1 integrin.
Blood. 2004 Mar 15;103(6):2214-20. doi: 10.1182/blood-2003-08-2978. Epub 2003 Nov 26.
https://www.sciencedirect.com/science/article/pii/S0006497120500165?via%3Dihub

[51] Boyce JA.
Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation.
Immunol Rev. 2007 Jun;217:168-85. doi: 10.1111/j.1600-065X.2007.00512.x.

Therefore, mast cells are able to respond to a wide range of pathogen-derived or pathogen-induced stimuli, but they do not respond uniformly to all stimuli[52] .
For example, signaling through TLR4s leads to a strong pro-inflammatory cytokine response but limited mast cell degranulation.
In contrast, signaling through TLR2s induces both an inflammatory cytokine response and mast cell degranulation[53] .
Mast cell activation is therefore an important regulator of appropriate immune response, and aberrant or prolonged activation can result in tissue immunopathology[54] , as occurs in complications of acute SARS-Cov-2 infection and post-COVID sequelae. [55]

---

https://pubmed.ncbi.nlm.nih.gov/17498059/

[52] Rao KN, Brown MA.
Mast cells: multifaceted immune cells with diverse roles in health and disease.
Ann N Y Acad Sci. 2008 Nov;1143:83-104. doi: 10.1196/annals.1443.023.
https://pubmed.ncbi.nlm.nih.gov/19076346/

Elieh Ali Komi D, Wöhrl S, Bielory L.
Mast Cell Biology at Molecular Level: a Comprehensive Review.
Clin Rev Allergy Immunol. 2020 Jun;58(3):342-365. doi: 10.1007/s12016-019-08769-2.
https://pubmed.ncbi.nlm.nih.gov/31828527/

Valent P, et al
Mast cells as a unique hematopoietic lineage and cell system: From Paul Ehrlich's visions to precision medicine concepts. T heranostics. 2020 Aug 29;10(23):10743-10768. doi: 10.7150/thno.46719. PMID: 32929378; PMCID: PMC7482799.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7482799/

West PW, Bulfone-Paus S.
Mast cell tissue heterogeneity and specificity of immune cell recruitment.
Front Immunol. 2022 Jul 29;13:932090. doi: 10.3389/fimmu.2022.932090.
https://pubmed.ncbi.nlm.nih.gov/35967445/

[53] Supajatura V, Ushio H, Nakao A, Okumura K, Ra C, Ogawa H.
Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4.
J Immunol. 2001 Aug 15;167(4):2250-6. doi: 10.4049/jimmunol.167.4.2250
https://www.jimmunol.org/content/167/4/2250.long

[54] Noto CN, Hoft SG, DiPaolo RJ.
Mast Cells as Important Regulators in Autoimmunity and Cancer Development.
Front Cell Dev Biol. 2021 Oct 12;9:752350. doi: 10.3389/fcell.2021.752350.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8546116/

Justiz Vaillant AA, Vashisht R, Zito PM.
Immediate Hypersensitivity Reactions. 2022 Jul 8. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK513315/.

Toyoshima S, Okayama Y.
Neuro-allergology: Mast cell-nerve cross-talk.
Allergol Int. 2022 Jul;71(3):288-293. doi: 10.1016/j.alit.2022.04.002.
https://doi.org/10.1016/j.alit.2022.04.002

[55] Schaller T, Märkl B, Claus R, Sholl L, Hornick JL, Giannetti MP, Schweizer L, Mann M, Castells M. Mast cells in lung damage of COVID-19 autopsies: A descriptive study.
Allergy. 2022 Jul;77(7):2237-2239. doi: 10.1111/all.15293.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9111237/

Bot I.
The Mast Cell: A Novel Actor in Cardiac Microvessel Dysfunction.
Arterioscler Thromb Vasc Biol. 2021 Apr;41(4):1337-1338. doi: 10.1161/ATVBAHA.121.316043
https://www.ahajournals.org/doi/10.1161/ATVBAHA.121.316043

Szukiewicz D, Wojdasiewicz P, Watroba M, Szewczyk G.
Mast Cell Activation Syndrome in COVID-19 and Female Reproductive Function: Theoretical Background vs. Accumulating Clinical Evidence.
J Immunol Res. 2022 Jun 22;2022:9534163. doi: 10.1155/2022/9534163.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9242765/

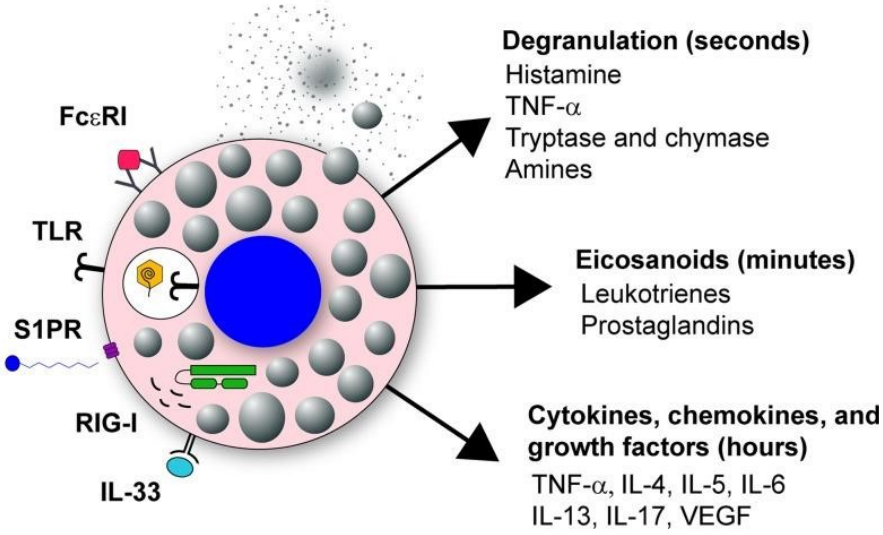
Arun S, Storan A, Myers B.
Mast cell activation syndrome and the link with long COVID.

Mast cells have two distinct phases of activation: immediate degranulation, resulting in the release of pre-synthesized mediators, and delayed secretion of *de novo* synthesized secondary mediators. [56]

Delayed secretion of *de novo* secondary effector molecules can be further divided into two classes:

- prostaglandins and eicosanoids released within minutes of activation,
- cytokines, chemokines, and growth factors that are released within hours of activation.

Together, these mast cell secretions can increase epithelial and endothelial cell permeability and activation state, which together with chemotactic molecules cause increased recruitment of inflammatory cells into infected tissues.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4435071/

Mast cell activation in response to viral infection.
Mast cells are classically known for their response to polyvalent IgE cross-linking at the FcεR1 receptor, which is important in protective immunity against helminth infection and pathologically associated with allergic disease.
However, mast cells are also important tissue sentinel cells to initiate the inflammatory response to pathogens. Mast cells can recognize and respond to viruses through several different receptors. These receptors include TLR signaling, such as TLR3 detection of dsRNA, binding of sphingosine-1-phosphate (S1P) to its receptor S1PR, and RIG-I recognition of uncapped vRNA. The involvement of these receptors causes mast cell activation leading to immediate degranulation, de novo synthesis of eicosanoids within minutes of activation, and de novo synthesis of numerous cytokines, chemokines, and growth factors within hours of activation.

Br J Hosp Med (Lond). 2022 Jul 2;83(7):1-10. doi: 10.12968/hmed.2022.0123.
https://pubmed.ncbi.nlm.nih.gov/35938771/

Wechsler JB, Butuci M, Wong A, Kamboj AP, Youngblood BA.
Mast cell activation is associated with post-acute COVID-19 syndrome.
Allergy. 2022 Apr;77(4):1288-1291. doi: 10.1111/all.15188.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9299596/

[56] Abraham SN, St John AL.
Mast cell-orchestrated immunity to pathogens.
Nat Rev Immunol. 2010 Jun;10(6):440-52. doi: 10.1038/nri2782.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4469150/

Sayed BA, Christy A, Quirion MR, Brown MA.
The master switch: the role of mast cells in autoimmunity and tolerance.
Annu Rev Immunol. 2008;26:705-39. doi: 10.1146/annurev.immunol.26.021607.090320.
https://pubmed.ncbi.nlm.nih.gov/18370925/

Metz M, Maurer M.
Mast cells--key effector cells in immune responses.
Trends Immunol. 2007 May;28(5):234-41. doi: 10.1016/j.en.2007.03.003. Epub 2007 Apr 2.
https://pubmed.ncbi.nlm.nih.gov/17400512/

Albert-Bayo M, Paracuellos I, González-Castro AM, Rodríguez-Urrutia A, Rodríguez-Lagunas MJ, Alonso-Cotoner C, Santos J, Vicario M.
Intestinal Mucosal Mast Cells: Key Modulators of Barrier Function and Homeostasis.
Cells. 2019 Feb 8;8(2):135. doi: 10.3390/cells8020135.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6407111/

ADE and multisystem inflammatory syndrome

Antibody-mediated mast cell activation can occur following various infections and vaccines[57] , and has been most carefully studied as an immunopathological mechanism for MISC-C (multisystem inflammatory syndrome in children) and MISC-A (multisystem inflammatory syndrome in adults) as a complication of COVID-19[58] and SARS-Cov-2 vaccines[59] .

---

[57] Ricke DO.
Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies.
Front Immunol. 2021;12:640093. Published 2021 Feb 24. doi:10.3389/fimmu.2021.640093
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7943455/

Tkaczyk C, Okayama Y, Woolhiser MR, Hagaman DD, Gilfillan AM, Metcalfe DD.
Activation of human mast cells through the high-affinity IgG receptor.
Mol Immunol. 2002 Sep;38(16-18):1289-93. doi: 10.1016/s0161-5890(02)00077-9.
https://pubmed.ncbi.nlm.nih.gov/12217397/

Tkaczyk C, Okayama Y, Woolhiser MR, Hagaman DD, Gilfillan AM, Metcalfe DD.
Activation of human mast cells through the high-affinity IgG receptor.
Mol Immunol. 2002 Sep;38(16-18):1289-93. doi: 10.1016/s0161-5890(02)00077-9.
https://pubmed.ncbi.nlm.nih.gov/12217397/

van Erp EA, van Kasteren PB, Guichelaar T, Ahout IML, de Haan CAM, Luytjes W, Ferwerda G, Wicht O.
In Vitro Enhancement of Respiratory Syncytial Virus Infection by Maternal Antibodies Does Not Explain Disease Severity in Infants.
J Virol. 2017 Oct 13;91(21):e00851-17. doi: 10.1128/JVI.00851-17.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5640862/

Smatti MK, Al Thani AA, Yassine HM.
Viral-Induced Enhanced Disease Illness.
Front Microbiol. 2018 Dec 5;9:2991. doi: 10.3389/fmicb.2018.02991.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6290032/

Jares Baglivo S, Polack FP.
The long road to protect infants against severe RSV lower respiratory tract illness.
F1000Res. 2019 May 2;8:F1000 Faculty Rev-610. doi: 10.12688/f1000research.18749.1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6498742/

Winarski KL, Tang J, Klenow L, Lee J, Coyle EM, Manischewitz J, Turner HL, Takeda K, Ward AB, Golding H, Khurana S.
Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics.
Proc Natl Acad Sci U S A. 2019 Jul 23;116(30):15194-15199. doi: 10.1073/pnas.1821317116.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6660725/

Mamontov A, Losev I, Korzhevskii D, Guselnikova V, Polevshchikov A, Desheva Y.
Study of Antibody-Dependent Reactions of Mast Cells In Vitro and in a Model of Severe Influenza Infection in Mice.
Front Immunol. 2021 Jul 14;12:689436. doi: 10.3389/fimmu.2021.689436.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8317171/

Desheva Y, Mamontov A, Petkova N, Karev V, Nazarov P.
Mast cell degranulation and histamine release during A/H5N1 influenza infection in influenza-sensitized mice.
Life Sci. 2020 Oct 1;258:118230. doi: 10.1016/j.lfs.2020.118230.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7413848/

[58] Vogel TP, et al
Multisystem inflammatory syndrome in children and adults (MIS-C/A): Case definition & guidelines for data collection, analysis, and presentation of immunization safety data.
Vaccine. 2021 May 21;39(22):3037-3049. doi: 10.1016/j.vaccine.2021.01.054.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7904456/

Waseem M, Shariff MA, Tay ET, Mortel D, Savadkar S, Lee H, Kondamudi N, Liang T.
Multisystem Inflammatory Syndrome in Children.
J Emerg Med. 2022 Jan;62(1):28-37. doi: 10.1016/j.jemermed.2021.07.070.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8445772/

Theoharides TC, Conti P.
COVID-19 and Multisystem Inflammatory Syndrome, or is it Mast Cell Activation Syndrome?
J Biol Regul Homeost Agents. 2020 Sep-Oct,;34(5):1633-1636. doi: 10.23812/20-EDIT3.
https://pubmed.ncbi.nlm.nih.gov/33023287/

[59] Nune A, Iyengar KP, Goddard C, Ahmed AE.

In particular, MIS-C appears to be a clinical syndrome that shares aspects with other inflammatory conditions, in which large amounts of cytokines cause dysfunction of several organs, including Kawasaki disease, sepsis, macrophage activation syndrome, and secondary HLH. [60]
Its action on the vascular bed is very important, as it causes hypotension and leakage of fluid and immune system cells into the lung, heart, and other organs.[61]
Noteworthy is cardiac involvement with myocardial dysfunction, pericarditis, valvular dysfunction, or coronary anomalies[62] .

Multisystem inflammatory syndrome in an adult following the SARS-CoV-2 vaccine (MIS-V).
BMJ Case Rep. 2021 Jul 29;14(7):e243888. doi: 10.1136/bcr-2021-243888.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8323360/

Wangu Z, Swartz H, Doherty M.
Multisystem inflammatory syndrome in children (MIS-C) possibly secondary to COVID-19 mRNA vaccination.
BMJ Case Rep. 2022 Mar 30;15(3):e247176. doi: 10.1136/bcr-2021-247176.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8968554/

Stappers S, Ceuleers B, Van Brusselen D, Willems P, de Tavernier B, Verlinden A.
A case of multisystem inflammatory syndrome (MIS-A) in an adult woman 18 days after COVID-19 vaccination.
Acta Clin Belg. 2022 Aug;77(4):772-777. doi: 10.1080/17843286.2021.1977899.
https://pubmed.ncbi.nlm.nih.gov/34511054/

Jain E, Donowitz JR, Aarons E, Marshall BC, Miller MP.
Multisystem Inflammatory Syndrome in Children after SARS-CoV-2 Vaccination.
Emerg Infect Dis. 2022 May;28(5):990-993. doi: 10.3201/eid2805.212418.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9045439/

[60] Simon Junior H, Sakano TMS, Rodrigues RM, Eisencraft AP, Carvalho VEL, Schvartsman C, Reis AGADC.
Multisystem inflammatory syndrome associated with COVID-19 from the pediatric emergency physician's point of view.
J Pediatr (Rio J). 2021 Mar-Apr;97(2):140-159. doi: 10.1016/j.jped.2020.08.004
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7486073/

[61] Mahase E.
Covid-19: concerns grow over emerging inflammatory syndrome in children.
BMJ. 2020 Apr 28;369:m1710. doi: 10.1136/bmj.m1710.
https://pubmed.ncbi.nlm.nih.gov/32345602/

[62] Wu EY, Campbell MJ.
Cardiac Manifestations of Multisystem Inflammatory Syndrome in Children (MIS-C) Following COVID-19.
Curr Cardiol Rep. 2021 Oct 1;23(11):168. doi: 10.1007/s11886-021-01602-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8486157/

Sperotto F, Friedman KG, Son MBF, VanderPluym CJ, Newburger JW, Dionne A.
Cardiac manifestations in SARS-CoV-2-associated multisystem inflammatory syndrome in children: a comprehensive review and proposed clinical approach.
Eur J Pediatr. 2021 Feb;180(2):307-322. doi: 10.1007/s00431-020-03766-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7429125/

Fialho I, Mateus C, Martins-Dos-Santos G, Pita J, Cabanelas N, Baptista SB, Roque D.
Recurrent Kounis syndrome-a life-threatening event after COVID-19 vaccine administration.
J Cardiol Cases. 2022 Jun;25(6):400-403. doi: 10.1016/j.jccase.2022.01.014.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8818380/

Symptoms of MIS-C	Complications of MIS-C
• Fever	• Myocarditis
• Headache or mental status change	• Coronary artery aneurysm
• Conjunctivitis	• Hypotension and hypoperfusion
• Oral mucosa changes	• Serositis
• Sore throat	• Acute respiratory distress syndrome and respiratory failure
• Cough	• Acute kidney injury
• Abdominal pain	• Hepatic failure
• Vomiting or diarrhea	
• Rash	
• Lymphadenitis	
• Swollen extremities	

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8445772/

A proposed model to explain the multisystem inflammatory syndrome in infants in infants with maternal antibodies against SARS-CoV-2 involves activation and degranulation of mast cells by antibodies against SARS-CoV-2 bound to the FcεR1 receptor, with increased histamine levels.[63]
Binding of the SARS-CoV-2 nucleocapsid protein to the $_{PTGS2}$ promoter could induce the release of prostaglandin $_{E2}$ ($_{PGE2}$) from hyperactive mast cells as an alternative mechanism leading to increased histamine levels in older children and adults. [64]

[63] Graciano-Machuca O, Villegas-Rivera G, López-Pérez I, Macías-Barragán J, Sifuentes-Franco S.
Multisystem Inflammatory Syndrome in Children (MIS-C) Following SARS-CoV-2 Infection: Role of Oxidative Stress.
Front Immunol. 2021 Oct 19;12:723654. doi: 10.3389/fimmu.2021.723654.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8560690/

Ricke DO.
Hyperactivated Mast Cells Pathogenesis Hypothesis for COVID-19 Cutaneous Manifestations.
JID Innov. 2021 Dec;1(4):100052. doi: 10.1016/j.xjidi.2021.100052.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8381631/

Pérez-Gómez HR, Morfín-Otero R, González-Díaz E, Esparza-Ahumada S, León-Garnica G, Rodríguez-Noriega E.
The Multifaceted Manifestations of Multisystem Inflammatory Syndrome during the SARS-CoV-2 Pandemic.
Pathogens. 2022 May 8;11(5):556. doi: 10.3390/pathogens11050556.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9143280/

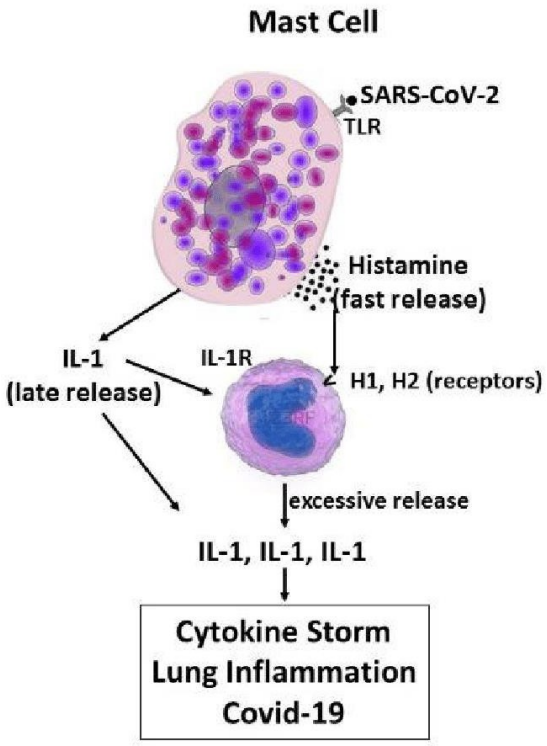
Theoharides TC, Conti P.
COVID-19 and Multisystem Inflammatory Syndrome, or is it Mast Cell Activation Syndrome?
J Biol Regul Homeost Agents. 2020 Sep-Oct,;34(5):1633-1636. doi: 10.23812/20-EDIT3.
https://pubmed.ncbi.nlm.nih.gov/33023287/

Council CR, et al CACTUS Study Team, Landegren N, Palma P, Brodin P.
The Immunology of Multisystem Inflammatory Syndrome in Children with COVID-19.
Cell. 2020 Nov 12;183(4):968-981.e7. doi: 10.1016/j.cell.2020.09.016.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7474869/

[64] Malone RW, et al
COVID-19: Famotidine, Histamine, Mast Cells, and Mechanisms.
Front Pharmacol. 2021 Mar 23;12:633680. doi: 10.3389/fphar.2021.633680.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8021898/

Ricke DO.
Hyperactivated Mast Cells Pathogenesis Hypothesis for COVID-19 Cutaneous Manifestations.
JID Innov. 2021 Dec;1(4):100052. doi: 10.1016/j.xjidi.2021.100052.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8381631/

Conti P, Caraffa A, Tetè G, Gallenga CE, Ross R, Kritas SK, Frydas I, Younes A, Di Emidio P, Ronconi G.
Mast cells activated by SARS-CoV-2 release histamine which increases IL-1 levels causing cytokine storm and inflammatory reaction in COVID-19.



https://www.researchgate.net/publication/344315820_Mast_cells_activated_by_SARS-CoV-2_release_histamine_which_increases_IL-1_levels_causing_cytokine_storm_and_inflammatory_reaction_in_COVID-19

Increased levels of histamine are believed to prevent blood flow through cardiac capillaries by constriction of pericytes, with an increased risk of cardiac pathology due to cell death by anoxia and aneurysms of coronary arteries as a result of increased blood pressure.[65]

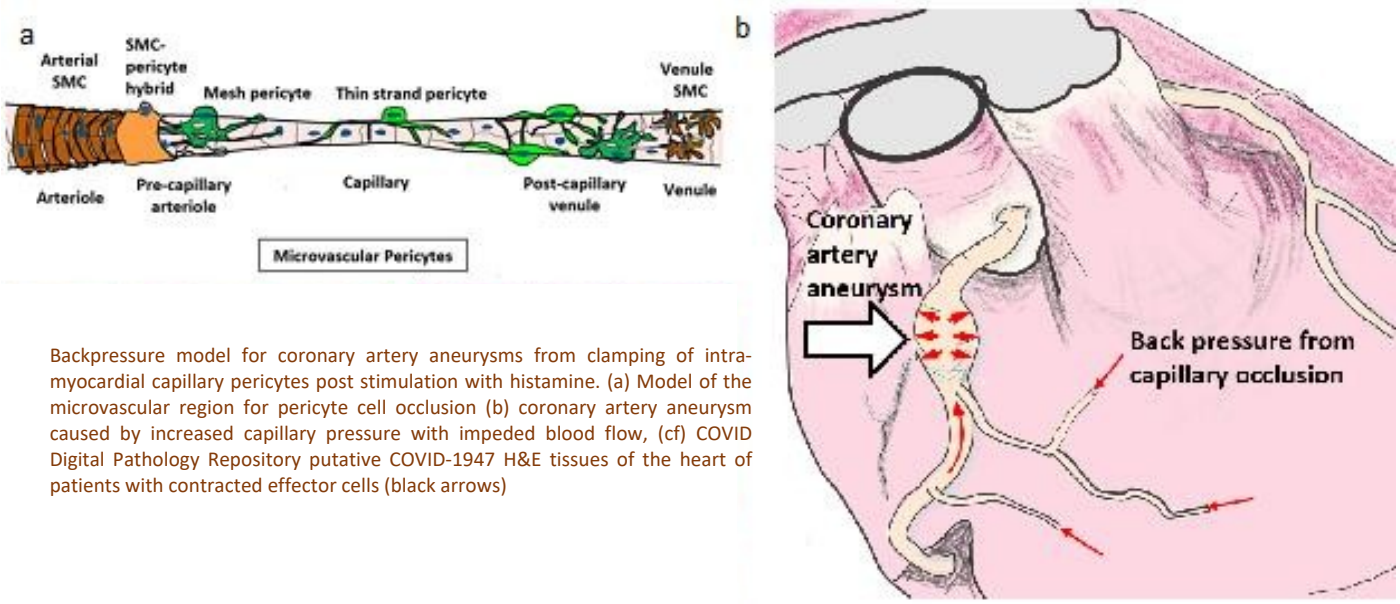
J Biol Regul Homeost Agents. 2020 Sep-Oct,;34(5):1629-1632. doi: 10.23812/20-2EDIT.
https://www.researchgate.net/publication/344315820_Mast_cells_activated_by_SARS-CoV-2_release_histamine_which_increases_IL-1_levels_causing_cytokine_storm_and_inflammatory_reaction_in_COVID-19

[65] Ricke DO, Gherlone N, Fremont-Smith P, Tisdall P, Fremont-Smith M.
Kawasaki disease, multisystem inflammatory syndrome in children: antibody-induced mast cell activation hypothesis.
J Pediatrics Pediatr Med. (2020) 4:1-7. 10.29245/2578-2940/2020/2.1157
https://www.pediatricsresearchjournal.com/articles/kawasaki-disease-multisystem-inflammatory-syndrome-in-children-antibody-induced-mast-cell-activation-hypothesis.pdf

Fremont-Smith M, Gherlone N, Smith N, Tisdall P, Ricke DO.
Models for COVID-19 Early Cardiac Pathology Following SARS-CoV-2 Infection.
Int J Infect Dis. 2021 Dec;113:331-335. doi: 10.1016/j.ijid.2021.09.052.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8473263/

Etiology Models of Antibody Triggered Histamine Intolerance Inducing Kawasaki Disease and Multisystem Inflammatory Syndromes Diseases
https://doi.org/10.32388/52A6VH
https://www.qeios.com/read/52A6VH/pdf

Jiang L, Tang K, Levin M, Irfan O, Morris SK, Wilson K, Klein JD, Bhutta ZA.
COVID-19 and multisystem inflammatory syndrome in children and adolescents.
Lancet Infect Dis. 2020 Nov;20(11):e276-e288. doi: 10.1016/S1473-3099(20)30651-4. Epub 2020 Aug 17. Erratum in: Lancet Infect Dis. 2022 Aug 5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7431129/



Backpressure model for coronary artery aneurysms from clamping of intra-myocardial capillary pericytes post stimulation with histamine. (a) Model of the microvascular region for pericyte cell occlusion (b) coronary artery aneurysm caused by increased capillary pressure with impeded blood flow, (cf) COVID Digital Pathology Repository putative COVID-1947 H&E tissues of the heart of patients with contracted effector cells (black arrows)

https://www.pediatricsresearchjournal.com/articles/kawasaki-disease-multisystem-inflammatory-syndrome-in-children-antibody-induced-mast-cell-activation-hypothesis.html

VACCINE ANTIBODY-DEPENDENT ENHANCEMENT

Understanding the structure of the epitopes of SARS-CoV-2, particularly within the spike, has provided essential information for the development of vaccines that should promote the production of neutralizing antibodies rather than antibodies that might exacerbate the severity of infection with ADE. [66]

In general, it is known that RNA viruses are highly susceptible to random mutations due to the lack of exonuclease proofreading activity of the RNA-dependent RNA polymerases (RdRp) encoded by the virus,[67] with some exceptions such as the *Nidovirales* (to which the genus Coronavirus belongs).

For SARS-CoV, an exonuclease activity with a proofreading function for nsp14 (ExoN) and a homologous nsp14 protein that is also found in SARS-CoV-2 has been described. [68]

The high error rate and subsequent rapid evolution of virus populations, which could lead to the accumulation of amino acid mutations, could affect the transmissibility of the virus, its cellular trophism, and even its pathogenicity. [69] As will be discussed in more detail in the chapter on vaccine variants.

[66] Shrock E, Fujimura E, Kula T, et al.
Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity.
Science. 2020;370(6520):eabd4250. doi:10.1126/science.abd4250
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857405/

[67] Chen J, Wang R, Wang M, Wei GW.
Mutations Strengthened SARS-CoV-2 Infectivity.
J Mol Biol. 2020;432(19):5212-5226. doi:10.1016/j.jmb.2020.07.009
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7375973/

[68] Pachetti M, Marini B, Benedetti F, et al.
Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant.
J Transl Med. 2020;18(1):179. Published 2020 Apr 22. doi:10.1186/s12967-020-02344-6
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7174922/

[69] Giovanetti M, Benedetti F, Campisi G, et al.
Evolution patterns of SARS-CoV-2: Snapshot on its genome variants.
Biochem Biophys Res Commun. 2021;538:88-91. doi:10.1016/j.bbrc.2020.10.102
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836704/

resistant, the rapid mutant selection induced by vaccination may result in an increased risk of ADEs for vaccinees who subsequently contract the infection.
ADE represents a particularly serious and potentially fatal adverse reaction that is still being in-depth study for SARS-Cov-2 infection and its vaccines[70] , while it has been extensively studied in the

[70] Lee, W.S., Wheatley, A.K., Kent, S.J. et al.
Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies.
Nat Microbiol 5, 1185-1191 (2020). https://doi.org/10.1038/s41564-020-00789-5
https://www.nature.com/articles/s41564-020-00789-5

Arvin, A. M., Fink, K., Schmid, M. A. et al.
A perspective on potential antibody-dependent enhancement of SARS-CoV-2.
Nature 584, 353-363 (2020). https://doi.org/10.1038/s41586-020-2538-8
https://www.nature.com/articles/s41586-020-2538-8

Fan Wu, et al
Antibody-dependent enhancement (ADE) of SARS-CoV-2 infection in recovered COVID-19 patients: studies based on cellular and structural biology analysis
medRxiv 2020.10.08.20209114; doi: https://doi.org/10.1101/2020.10.08.20209114
https://www.medrxiv.org/content/10.1101/2020.10.08.20209114v1.full.pdf

Wen J, Cheng Y, Ling R, Dai Y, Huang B, Huang W, Zhang S, Jiang Y.
Antibody-dependent enhancement of coronavirus.
Int J Infect Dis. 2020 Nov;100:483-489. doi: 10.1016/j.ijid.2020.09.015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7483033/

Fierz W, Walz B.
Antibody Dependent Enhancement Due to Original Antigenic Sin and the Development of SARS.
Front Immunol. 2020;11:1120. Published 2020 Jun 5. doi:10.3389/fimmu.2020.01120
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291596/

Wan Y, Shang J, Sun S, et al.
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
J Virol. 2020;94(5):e02015-19. Published 2020 Feb 14. doi:10.1128/JVI.02015-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

de Alwis R, Chen S, Gan ES, Ooi EE.
Impact of immune enhancement on Covid-19 polyclonal hyperimmune globulin therapy and vaccine development.
EBioMedicine. 2020 May;55:102768. doi: 10.1016/j.ebiom.2020.102768
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7161485/

Hotez PJ, Corry DB, Bottazzi ME.
COVID-19 vaccine design: the Janus face of immune enhancement.
Nat Rev Immunol. 2020 Jun;20(6):347-348. doi: 10.1038/s41577-020-0323-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7187801/

Desheva, Yulia & Mamontov, Andrey & Nazarov, Peter
Contribution of antibody-dependent enhancement to the pathogenesis of coronavirus infections.
AIMS Allergy and Immunology.(2020).4. 50-59. 10.3934/Allergy.2020005.
https://www.researchgate.net/publication/344087225_Contribution_of_antibody-dependent_enhancement_to_the_pathogenesis_of_coronavirus_infections.

Wang J and Zand MS.
The potential for antibody-dependent enhancement of SARS-CoV-2 infection: Translational implications for vaccine development.
Journal of Clinical and Translational Science, page 1 of 4. doi: 10.1017/cts.2020.39
https://doi.org/10.1017/cts.2020.39

Karthik K, Senthilkumar TMA, Udhayavel S, Raj GD.
Role of antibody-dependent enhancement (ADE) in the virulence of SARS-CoV-2 and its mitigation strategies for the development of vaccines and immunotherapies to counter COVID-19.
Hum Vaccin Immunother. 2020 Dec 1;16(12):3055-3060. doi: 10.1080/21645515.2020.1796425.
https://www.tandfonline.com/doi/full/10.1080/21645515.2020.1796425

Peron JPS, Nakaya H.
Susceptibility of the Elderly to SARS-CoV-2 Infection: ACE-2 Overexpression, Shedding, and Antibody-dependent Enhancement (ADE).
Clinics (Sao Paulo). 2020;75:e1912. doi:10.6061/clinics/2020/e1912
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7213670/

Jennifer K DeMarco, MSc, William E Severson, PhD, Daniel R DeMarco, PhD, Jon Gabbard, PhD, Kenneth E Palmer, PhD,

Activation of Macrophages Enhances Susceptibility to SARS-CoV-2 Antibody-Dependent Enhancement and Promotes Damage to Downstream Epithelial Cells,
Open Forum Infectious Diseases, Volume 7, Issue Supplement_1, October 2020, Page S319, https://doi.org/10.1093/ofid/ofaa439.701
https://academic.oup.com/ofid/article/7/Supplement_1/S319/6057001

Garber K.
Coronavirus vaccine developers wary of errant antibodies.
Nat Biotechnol. 2020 Jun 5. doi: 10.1038/d41587-020-00016-w.
https://pubmed.ncbi.nlm.nih.gov/32641838/

Cegolon L, Pichierri J, Mastrangelo G, et al.
Hypothesis to explain the severe form of COVID-19 in Northern Italy.
BMJ Glob Health. 2020;5(6):e002564. doi:10.1136/bmjgh-2020-002564
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7295427/

Negro F.
Is antibody-dependent enhancement playing a role in COVID-19 pathogenesis?
Swiss Med Wkly. 2020 Apr 16;150:w20249. doi: 10.4414/smw.2020.20249.
https://smw.ch/article/doi/smw.2020.20249

Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, Shi Z, Zhou Y, Du L, Li F.
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
J Virol. 2020 Feb 14;94(5):e02015-19. doi: 10.1128/JVI.02015-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

Bournazos S, Gupta A, Ravetch JV.
The role of IgG Fc receptors in antibody-dependent enhancement.
Nat Rev Immunol. 2020 Oct;20(10):633-643. doi: 10.1038/s41577-020-00410-0.
https://pubmed.ncbi.nlm.nih.gov/32782358/

Ulrich H, Pillat MM, Tárnok A.
Dengue Fever, COVID-19 (SARS-CoV-2), and Antibody-Dependent Enhancement (ADE): A Perspective.
Cytometry A. 2020 Jul;97(7):662-667. doi: 10.1002/cyto.a.24047.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7300451/

Zhou Y, et al
Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor associated with distinct epitopes on the RBD. Cell Rep. 2021 Feb 2;34(5):108699. doi: 10.1016/j.celrep.2021.108699.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7802522/

Zohar T, Alter G.
Dissecting antibody-mediated protection against SARS-CoV-2.
Nat Rev Immunol. 2020 Jul;20(7):392-394. doi: 10.1038/s41577-020-0359-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7278217/

Mohamed Khosroshahi L, Rokni M, Mokhtari T, Noorbakhsh F.
Immunology, immunopathogenesis and immunotherapeutics of COVID-19; an overview.
Int Immunopharmacol. 2021;93:107364. doi:10.1016/j.intimp.2020.107364
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7784533/

Yang L, Liu S, Liu J, et al.
COVID-19: immunopathogenesis and immunotherapeutics.
Signal Transduct Target Ther. 2020;5(1):128. Published 2020 Jul 25. doi:10.1038/s41392-020-00243-2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7381863/

Ebrahimi N, Aslani S, Babaie F, et al.
Recent findings on the Coronavirus disease 2019 (COVID-19); immunopathogenesis and immunotherapeutics.
Int Immunopharmacol. 2020;89(Pt B):107082. doi:10.1016/j.intimp.2020.107082
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7547582/

Seneff, S., & Nigh, G.
Worse Than the Disease? Reviewing Some Possible Unintended Consequences of the mRNA Vaccines Against COVID-19.
International Journal of Vaccine Theory, Practice, and Research, 2(1), 38-79. https://doi.org/10.56098/ijvtpr.v2i1.23 (Original work published May 10, 2021)
https://dpbh.nv.gov/uploadedFiles/dpbhnvgov/content/Boards/BOH/Meetings/2021/SENEFF~1.PDF

Eroshenko, N., Gill, T., Keaveney, M.K. et al.
Implications of antibody-dependent enhancement of infection for SARS-CoV-2 countermeasures.
Nat Biotechnol 38, 789-791 (2020). https://doi.org/10.1038/s41587-020-0577-1
https://www.nature.com/articles/s41587-020-0577-1

biological mechanism because it was found during preclinical studies with vaccines against SARS- Cov-1[71] , MERS[72] , Dengue[73] , Zika virus[74] , Ebola[75] , HIV[76] , seasonal influenza[77] ,

[71] Yip MS, Leung HL, Li PH, Cheung CY, Dutry I, Li D, Daëron M, Bruzzone R, Peiris JS, Jaume M.
Antibody-dependent enhancement of SARS coronavirus infection and its role in the pathogenesis of SARS.
Hong Kong Med J. 2016 Jun;22(3 Suppl 4):25-31. https://www.hkmj.org/system/files/hkm1603sp4p25.pdf

Jaume M, Yip MS, Kam YW, Cheung CY, Kien F, Roberts A, Li PH, Dutry I, Escriou N, Daeron M, Bruzzone R, Subbarao K, Peiris JS, Nal B, Altmeyer R.
SARS CoV subunit vaccine: antibody-mediated neutralisation and enhancement.
Hong Kong Med J. 2012 Feb;18 Suppl 2:31-6.
https://www.hkmj.org/system/files/hkm1202sp2p31.pdf

Jaume M, Yip MS, Cheung CY, Leung HL, Li PH, Kien F, Dutry I, Callendret B, Escriou N, Altmeyer R, Nal B, Daëron M, Bruzzone R, Peiris JS.
Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway.
J Virol. 2011 Oct;85(20):10582-97. doi: 10.1128/JVI.00671-11.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187504/

Wang SF, Tseng SP, Yen CH, Yang JY, Tsao CH, Shen CW, Chen KH, Liu FT, Liu WT, Chen YM, Huang JC.
Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins.
Biochem Biophys Res Commun. 2014 Aug 22;451(2):208-14. doi: 10.1016/j.bbrc.2014.07.090.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7092860/

[72] Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, Shi Z, Zhou Y, Du L, Li F.
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
J Virol. 2020 Feb 14;94(5):e02015-19. doi: 10.1128/JVI.02015-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

[73] Wan Y, Shang J, Sun S, et al.
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
J Virol. 2020;94(5):e02015-19. Published 2020 Feb 14. doi:10.1128/JVI.02015-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

Halstead SB.
Dengue Antibody-Dependent Enhancement: Knowns and Unknowns.
Microbiol Spectr. 2014 Dec;2(6). doi: 10.1128/microbiolspec.AID-0022-2014.
https://pubmed.ncbi.nlm.nih.gov/26104444/

Halstead SB.
Vaccine-Associated Enhanced Viral Disease: Implications for Viral Vaccine Development.
BioDrugs. 2021 Sep;35(5):505-515. doi: 10.1007/s40259-021-00495-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8427162/

[74] Marques ETA, Drexler JF.
Complex Scenario of Homotypic and Heterotypic Zika Virus Immune Enhancement.
mBio. 2019;10(5):e01849-19. Published 2019 Sep 3. doi:10.1128/mBio.01849-19
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6722417/

[75] Kuzmina NA, Younan P, Gilchuk P, Santos RI, Flyak AI, Ilinykh PA, Huang K, Lubaki NM, Ramanathan P, Crowe JE Jr, Bukreyev A.
Antibody-Dependent Enhancement of Ebola Virus Infection by Human Antibodies Isolated from Survivors.
Cell Rep. 2018 Aug 14;24(7):1802-1815.e5. doi: 10.1016/j.celrep.2018.07.035.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6697154/.

[76] Shmelkov E, Nadas A, Cardozo T.
Could vaccination with AIDSVAX immunogens have resulted in antibody-dependent enhancement of HIV infection in human subjects?
Hum Vaccin Immunother. 2014;10(10):3013-6. doi: 10.4161/21645515.2014.972148.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5443089/

[77] Winarski KL, Tang J, Klenow L, Lee J, Coyle EM, Manischewitz J, Turner HL, Takeda K, Ward AB, Golding H, Khurana S.
Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics.
Proc Natl Acad Sci U S A. 2019 Jul 23;116(30):15194-15199. doi: 10.1073/pnas.1821317116.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6660725/

Mamontov A, Losev I, Korzhevskii D, Guselnikova V, Polevshchikov A, Desheva Y.
Study of Antibody-Dependent Reactions of Mast Cells In Vitro and in a Model of Severe Influenza Infection in Mice.
Front Immunol. 2021 Jul 14;12:689436. doi: 10.3389/fimmu.2021.689436.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8317171/

Ramakrishnan B, Viswanathan K, Tharakaraman K, Dančík V, Raman R, Babcock GJ, Shriver Z, Sasisekharan R.

respiratory syncytial virus[78] , measles[79] , as well as for bacterial infections.[80]

Table 1 Key characteristics of antibody-dependent enhancement (ADE) infected by different viruses.

Virus	Main Host	Types of ADE	Clinical manifestations of ADE	Enhancing epitopes location	Impact of vaccine application
Dengue virus	Humans, Primate,Aedes	1.FcR mediated virus-antibody immune complexes infect monocytes, macrophages, and dendritic cells 2.Through FcR, LILR-B1 regulates the host's antiviral response, inhibits the innate response mediated by the TLR signaling pathway, disrupts the RIG-I/MDA-5 signal cascade, and induces IL-10 production	1. Increased susceptibility to other serotypes 2. Increased viral infection and association with severe dengue fever (DHF/DSS) 3. Infants born to dengue fever-immunized mothers, serious diseases that may be infected when maternal antibodies are reduced	prM protein, E protein DII-FL region	Vaccine raises the risk of ADE for DENV infection.Sero-negative dengue vaccinators are at increased risk of severe dengue, and WHO recommends vaccination only for sero-positive dengue
SRAS-CoV	Rhinolophus sinicus, Paguma larvatas, Humans	FcR-ADE(mainly mediated by FcγRII)	May be related to severe lymphopenia	Spike protein	Infection after immunization may cause severe acute lung injury (ALI)

A Structural and Mathematical Modeling Analysis of the Likelihood of Antibody-Dependent Enhancement in Influenza.
Trends Microbiol. 2016 Dec;24(12):933-943. doi: 10.1016/j.tim.2016.09.003. ì
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5526082/

[78] Jamrozik E, Heriot G, Bull S, Parker M
Oxford-Johns Hopkins Global Infectious Disease Ethics (GLIDE). Vaccine-enhanced disease: case studies and ethical implications for research and public health.
Wellcome Open Res. 2021;6:154. Published 2021 Jun 16. doi:10.12688/wellcomeopenres.16849.1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8250497/

[79] Banoun H.
Measles and Antibody-Dependent Enhancement (ADE): History and Mechanisms.
Explor Res Hypothesis Med. Published online: Apr 29, 2022. doi: 10.14218/ERHM.2022.00018.
https://www.xiahepublishing.com/2472-0712/ERHM-2022-00018

Iankov ID, Pandey M, Harvey M, Griesmann GE, Federspiel MJ, Russell SJ.
Immunoglobulin g antibody-mediated enhancement of measles virus infection can bypass the protective antiviral immune response.
J Virol. 2006;80(17):8530-8540. doi:10.1128/JVI.00593-06
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1563851/

Iankov ID, Penheiter AR, Griesmann GE, Carlson SK, Federspiel MJ, Galanis E.
Neutralization capacity of measles virus H protein specific IgG determines the balance between antibody-enhanced infectivity and protection in microglial cells.
Virus Res. 2013 Mar;172(1-2):15-23. doi: 10.1016/j.virusres.2012.12.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3612881/

[80] Torres VVL, Coggon CF, Wells TJ.
Antibody-Dependent Enhancement of Bacterial Disease: Prevalence, Mechanisms, and Treatment.
Infect Immun. 2021 Mar 17;89(4):e00054-21. doi: 10.1128/IAI.00054-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8090947/

Influenza virus	Humans,Pigs, Birds, Ferrets	FcR-ADE	Increased risk of a (H1N1) pdm09 disease	HA,NA	1.Trivalent inactivated influenza vaccine (TIV) in 2008–09 increased risk of a (H1N1) pdm09 disease 2. The vaccine may be associated with vaccine associated enhanced respiratory disease (VAERD)
Porcine Reproductive and Respiratory Syndrome	Pigs	FcR-ADE (Including FcγRI,FcγRII,FcγRIII, FCεRI)	Promote virus infection and enhance clinical symptoms, Increase the level and duration of viremia	GP5,N Protein	Infected with prrsv after immunization with inactivated vaccine, clinical symptoms increased
Human immunodeficiency virus	Humans	1.FcR-ADE (Including FcγRI,FcγRII,FcγRIII, FCαR) , FcR promotes virus entry by enhancing adhesion to CD4 receptor 2.CR3, C1q complement mediated ADE	1.ADE and plasma viral load is positive correlation. ADE accelerates immunosuppression and disease progression 2.Enhancing antibodies is beneficial to the emergence of ADE susceptible mutants	N-terminal immune dominant domain of gp41, gp120	One of the factors affecting vaccine development,higher rates of infection/risk among vaccinees were observed in RV144 clinical trials, but have not been confirmed to be directly associated with ADE
West nile virus	Horses, Humans,Birds	1.Fcγ receptor dependent ADE 2.CR3 dependent ADE	Increased viral infectivity	Domain I and domain II of the E protein	No vaccine has been marketed. Plasma samples of human WNV infection during rehabilitation can enhance ZIKV infection in vitro and in vivo
Respiratory syncytial virus	Humans	FcR mediate the uptake of viruses into monocytes, macrophages, and dendritic cells, leading to enhanced infection	ADE infection leads to activation of Th2 response and increased expression of TNF-α and IL-6, resulting in aggravated disease ADE infection of lung dendritic cells (DCs) can negatively regulate the function of DC cells, resulting in impaired T cell activation	Glycoproteins G and F	Formalin inactivated RSV vaccine recipients have increased disease and even led to death

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8438590/table/t0010/

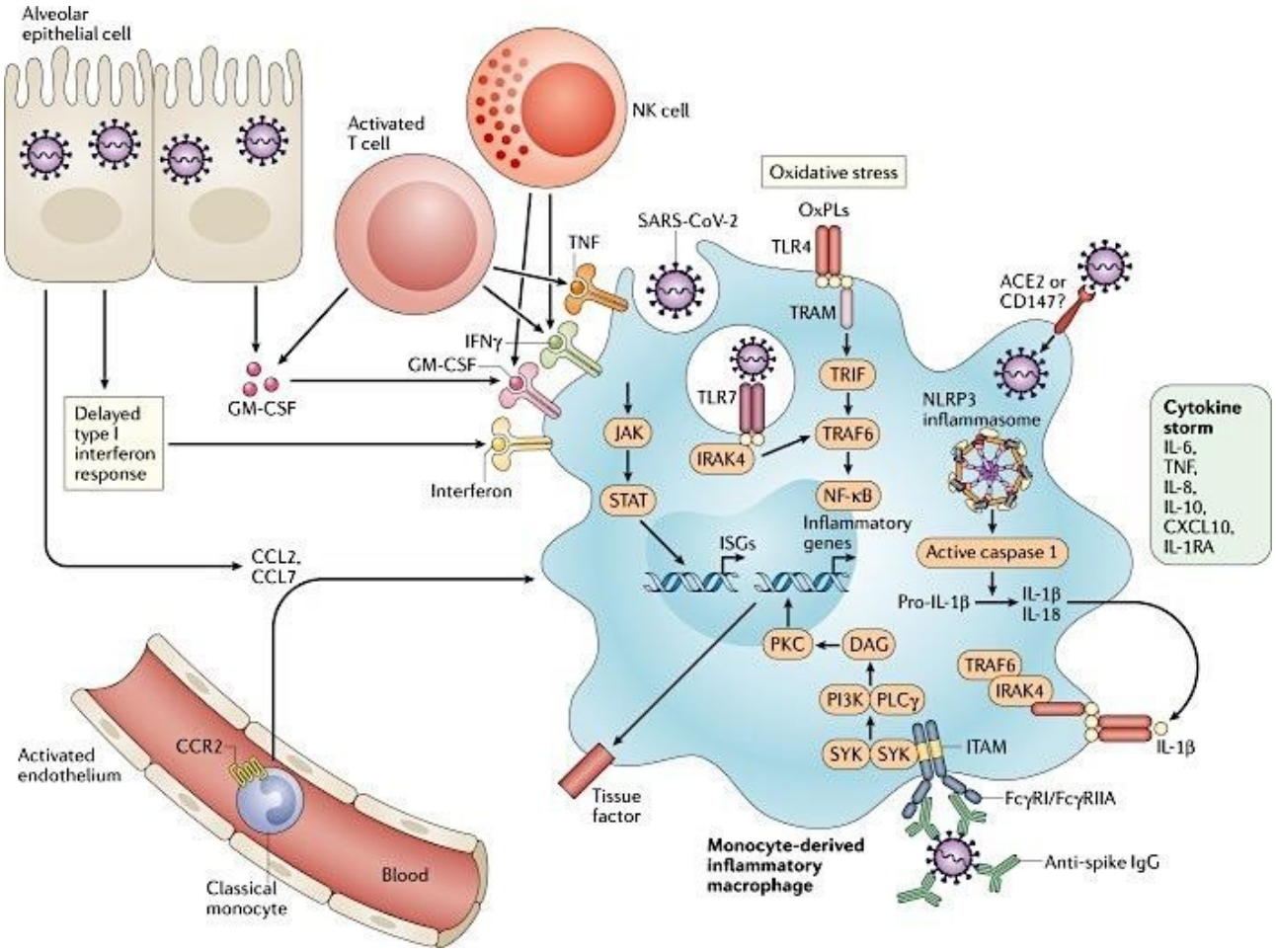
Antibody-dependent enhancement following vaccination may develop through more than one molecular mechanism, which will be explored in more detail later, but it can be summarized now as follows: a proportion of vaccinees are predisposed by vaccination precisely to manifest the serious and fatal complications of the disease from which they want to protect themselves.

When the virus binds to nonneutralizing antibodies, it forms immune complexes that are able to enter cells of the immune system (macrophages, mast cells, ect) through binding to Fc receptors (γ or ε), causing infection of the cells themselves, as already seen for infection ADE.

Specifically, when the vaccinee subsequently becomes infected (or has asymptomatic/chronic infection at the time of vaccination) with a variant of SARS-Cov-2, the virus more readily enters macrophages through the Fc-γ receptor and infects them, and then instead of being processed to be presented to other cells of the immune system, it on the one hand inhibits type I IFN signaling and on the other hand allows pro-inflammatory expression of IL-1, IL-6, and TNF-α, contributing to the cytokine storm syndrome and fatal potentiation of the disease.

In these cases, the development of acute respiratory disease coincides with seroconversion* IgG antiviral.

** The production of specific antibodies, detectable in the blood, in response to infection or immunization. Seroconversion specifically means the change of the serological test result from negative to positive, indicating the presence of antibodies.*



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7201395/

Possible pathways contributing to monocyte-derived macrophage hyperactivation and hyperinflammation in COVID-19.

Several mechanisms probably contribute to the hyperactivation of monocyte-derived macrophages seen in patients with COVID-19.

Delayed production of type I interferon leading to enhanced cytopathic effects and increased detection of microbial threats promotes enhanced release of chemoattractant monocytes by alveolar epithelial cells (and probably also by macrophages and stromal cells), leading to prolonged recruitment of blood monocytes to the lungs. Monocytes differentiate into pro-inflammatory macrophages through activation of the signal transducer Janus kinase (JAK) and activator of transcription pathways (STAT). Activated natural killer (NK) cells and T cells further promote the recruitment and activation of monocyte-derived macrophages through the production of granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF) and interferon-γ (IFNγ). Oxidized phospholipids (OxPLs) accumulate in infected lungs and activate monocyte-derived macrophages through the Toll-like receptor 4 (TLR4)-TRAF6 - NF-κB pathway.

Virus detection may trigger TLR7 activation through recognition of viral single-stranded RNA. It is possible that type I interferons induce the expression of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry receptors, allowing the virus to access the cytoplasm of macrophages and activate the NLRP3 inflammasome, which leads to the secretion of mature IL-1β and/or IL-18. IL-1β can amplify monocyte-derived macrophage activation in an autocrine or paracrine manner, but it can also reduce type I interferon production in infected lungs. The involvement of Fcγ receptors (FcγRs) by anti-spike protein IgG immune complexes may contribute to the increased inflammatory activation of monocyte-derived macrophages. Activated monocyte-derived macrophages contribute to the COVID-19 cytokine storm by releasing huge amounts of pro-inflammatory cytokines. CCL, CC-chemokine ligand; CXCL10, CXC-chemokine ligand 10; ISG, interferon-stimulated gene; ITAM, immunoreceptor tyrosine-based activation motif; TRAM, adaptor molecule related to TRIF.

Many of the viruses associated with ADE exhibit a fusion mechanism between the viral envelope and the cellular,[81] and for influenza A H1N1, vaccine-induced cross-reactive anti-HA2 antibodies in a swine model promote membrane fusion causing vaccine-associated enhanced respiratory illness (VAERD)[82] .
As mentioned above, ADE has been observed in multiple animal models of SARS-CoV-1, and attempts to create vaccines for SARS-CoV-1 have resulted in lung immunopathology (eosinophilic infiltration) in challenge tests in murine and nonhuman primate models[83] .
Increased hepatitis was also observed in a ferret model with a recombinant vaccinia virus Ankara (rMVA) vaccine expressing the Spike protein of SARS-CoV-1; indeed, it is known that SARS-CoV

[81] Smatti MK, Al Thani AA, Yassine HM.
Viral-Induced Enhanced Disease Illness.
Front Microbiol. 2018 Dec 5;9:2991. doi: 10.3389/fmicb.2018.02991.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6290032/

[82] Khurana S, Loving CL, Manischewitz J, King LR, Gauger PC, Henningson J, Vincent AL, Golding H.
Vaccine-induced anti-HA2 antibodies promote virus fusion and enhance influenza virus respiratory disease.
Sci Transl Med. 2013 Aug 28;5(200):200ra114. doi: 10.1126/scitranslmed.3006366.
https://pubmed.ncbi.nlm.nih.gov/23986398/

[83] Tseng CT, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, Atmar RL, Peters CJ, Couch RB.
Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus.
PLoS One. 2012;7(4):e35421. doi: 10.1371/journal.pone.0035421. Epub 2012 Apr 20. Erratum in: PLoS One. 2012;7(8).
doi:10.1371/annotation/2965cfae-b77d-4014-8b7b-236e01a35492.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3335060/

Bolles M, Deming D, Long K, Agnihothram S, Whitmore A, Ferris M, Funkhouser W, Gralinski L, Totura A, Heise M, Baric RS.
A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge.
J Virol. 2011 Dec;85(23):12201-15. doi: 10.1128/JVI.06048-11.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3209347/

Wang Q, Zhang L, Kuwahara K, Li L, Liu Z, Li T, Zhu H, Liu J, Xu Y, Xie J, Morioka H, Sakaguchi N, Qin C, Liu G.
Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-human Primates.
ACS Infect Dis. 2016 May 13;2(5):361-76. doi: 10.1021/acsinfecdis.6b00006. Epub 2016 Apr 11. Erratum in: ACS Infect Dis. 2020 May 8;6(5):1284-1285.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7075522/

Iwata-Yoshikawa N, Uda A, Suzuki T, et al.
Effects of Toll-like receptor stimulation on eosinophilic infiltration in lungs of BALB/c mice immunized with UV-inactivated severe acute respiratory syndrome-related coronavirus vaccine.
J Virol. 2014;88(15):8597-8614. doi:10.1128/JVI.00983-14
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4135953/

Bolles M, Deming D, Long K, et al.
A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge.
J Virol. 2011;85(23):12201-12215. doi:10.1128/JVI.06048-11
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3209347/

Jaume M, Yip MS, Kam YW, Cheung CY, Kien F, Roberts A, Li PH, Dutry I, Escriou N, Daeron M, Bruzzone R, Subbarao K, Peiris JS, Nal B, Altmeyer R.
SARS CoV subunit vaccine: antibody-mediated neutralisation and enhancement.
Hong Kong Med J. 2012 Feb;18 Suppl 2:31-6.
https://www.hkmj.org/system/files/hkm1202sp2p31.pdf

Menachery VD, Yount BL Jr, Sims AC, et al.
SARS-like WIV1-CoV poised for human emergence.
Proc Natl Acad Sci U S A. 2016;113(11):3048-3053. doi:10.1073/pnas.1517719113
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4801244/

Jaume M, Yip MS, Cheung CY, et al.
Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway.
J Virol. 2011;85(20):10582-10597. doi:10.1128/JVI.00671-11
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187504/

can infect hepatocytes and cause hepatitis in humans, and this study found increased damage liver in animals reinfected in the post-vaccine challenge test. [84]

Jaume et al. [85] point out the possible dangers associated with vaccinations against the Spike protein of SARS- CoV-1 due to Fc receptor-mediated infection of immune cells, and this leads to the prediction that vaccines against SARS-CoV-1[86] , MERS-CoV[87] or SARS-CoV-2 vaccines have higher risks of inducing ADE in humans upon post-vaccinal reinfection,[88] regardless of the vaccine technology[89] or the type of precision drug (e.g. monoclonal antibody) selected[90] .
COVID-19 vaccines can induce vaccine-associated disease enhancement (VADE), with the production of suboptimal and nonprotective titers of neutralizing antibodies or the induction of a pro-inflammatory type 2 T-helper response.
Second, enhanced respiratory disease (ERD), in which lung symptoms are more severe due to monocytic and eosinophilic peribronchial infiltration, could occur.[91]

---

[84] Weingartl H, et al
Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets.
J Virol. 2004 Nov;78(22):12672-6. doi: 10.1128/JVI.78.22.12672-12676.2004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC525089/

[85] Jaume M,
Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway.
J Virol. 2011 Oct;85(20):10582-97. doi: 10.1128/JVI.00671-11.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187504/

[86] Graham RL, Donaldson EF, Baric RS.
A decade after SARS: strategies for controlling emerging coronaviruses.
Nat Rev Microbiol. 2013;11(12):836-848. doi:10.1038/nrmicro3143
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5147543/

[87] Agrawal AS, et al
Immunization with inactivated Middle East Respiratory Syndrome coronavirus vaccine leads to lung immunopathology on challenge with live virus.
Hum Vaccin Immunother. 2016 Sep;12(9):2351-6. doi: 10.1080/21645515.2016.1177688.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5027702/

[88] Ricke DO.
Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies.
Front Immunol. 2021;12:640093. Published 2021 Feb 24. doi:10.3389/fimmu.2021.640093
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7943455/

[89] Rauch S, Jasny E, Schmidt KE, Petsch B.
New Vaccine Technologies to Combat Outbreak Situations.
Front Immunol. 2018;9:1963. Published 2018 Sep 19. doi:10.3389/fimmu.2018.01963
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6156540/

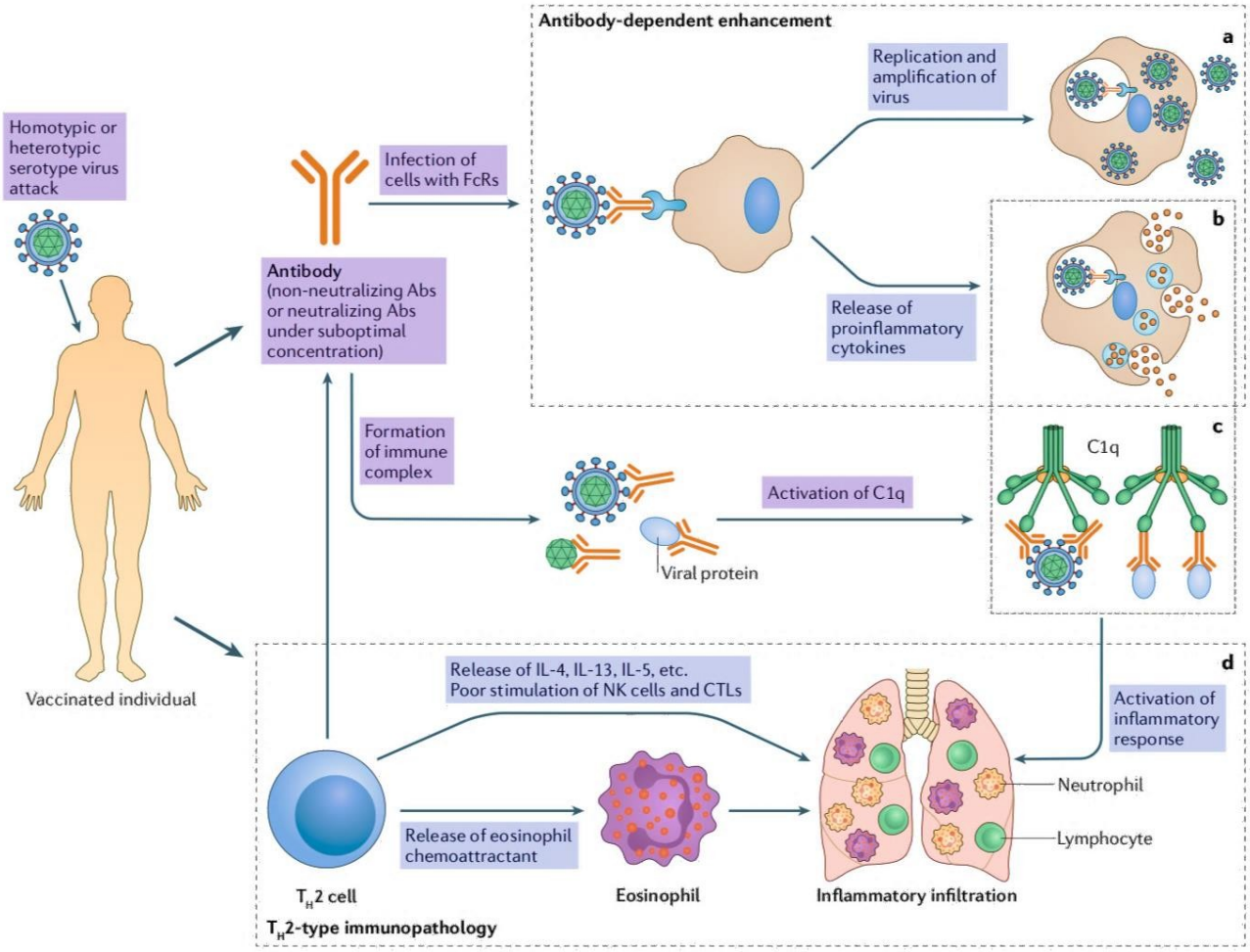
Badgujar KC, Badgujar VC, Badgujar SB.
Vaccine development against coronavirus (2003 to present): An overview, recent advances, current scenario, opportunities and challenges.
Diabetes Metab Syndr. 2020;14(5):1361-1376. doi:10.1016/j.dsx.2020.07.022
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7371592/

[90] Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, He L, Chen Y, Wu J, Shi Z, Zhou Y, Du L, Li F.
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
J Virol. 2020 Feb 14;94(5):e02015-19. doi: 10.1128/JVI.02015-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

Menachery VD, et al
A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence.
Nat Med. 2015 Dec;21(12):1508-13. doi: 10.1038/nm.3985. Epub 2015 Nov 9. Erratum in: Nat Med. 2016 Apr;22(4):446. Erratum in: Nat Med. 2020 Jul;26(7):1146.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4797993/

[91] Halstead SB, Katzelnick L.
COVID-19 Vaccines: Should We Fear ADE?
J Infect Dis. 2020;222(12):1946-1950. doi:10.1093/infdis/jiaa518
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7454712/

In fact, studies with inactivated SARS-CoV vaccines and respiratory syncytial virus vaccines have reported VADE via a Th2 cellular response and eosinophilic lung infiltration, which may be worsened in older vaccinees.[92]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566580/
Mechanisms of vaccine-associated disease enhancement.
Vaccination induces humoral and cellular immune response in immunized individuals. Under normal conditions, when homologous virus enters an immunized body, it will be neutralized or eliminated by vaccine-induced neutralizing antibodies (Abs) or specific T cells, respectively. In the context of vaccine-associated disease enhancement, vaccines primarily induce nonneutralizing Abs or low titers of neutralizing Abs (suboptimal concentration) or type 2 helper T cell (TH2 cell)-oriented responses. When these vaccinated individuals are infected with serotypic homotypic or heterotypic viruses, antibodies will immediately recognize the viruses and mediate antibody-dependent disease exacerbation in two ways. First, virus-antibody complexes could enter Fc receptor (FcR) carrier cells, such as dendritic cells and monocytes, through FcR-mediated internalization, which is called antibody-dependent enhancement (ADE). For viruses with innate trophism for FcR-bearing cells, such as dengue virus, ADE will produce higher viral loads than under conditions without antibodies.
a) After entry, the virus, regardless of whether it replicates or not, can activate a harmful immune response, resulting in the release of proinflammatory cytokines.
b) Apart from ADE, antibody-antigen complexes can stimulate the complement pathway through activation of the C1q pathway, thus further enhancing inflammatory responses
c) Vaccine-associated disease enhancement may also involve a Th2-driven immune response. Activated Th2 cells contribute to the activation of antibody production. However, they release interleukin-4 (IL-4), IL-13 and IL-5, as well as eosinophil chemotactic agents, resulting in eosinophil infiltration and proinflammatory cytokine production in the lung.
d) Natural killer (NK) cells and CD8 cytotoxic T lymphocytes+ (CTL) are poorly stimulated in Th2 cell-directed immune responses. Exaggerated release of cytokines (part b), activation of the complement pathway (part c), and excessive mobilization of eosinophils contribute to infiltration of the lung by eosinophils, neutrophils, and lymphocytes and production of inflammatory cytokines (part d), leading to acute lung injury or acute respiratory distress syndrome.

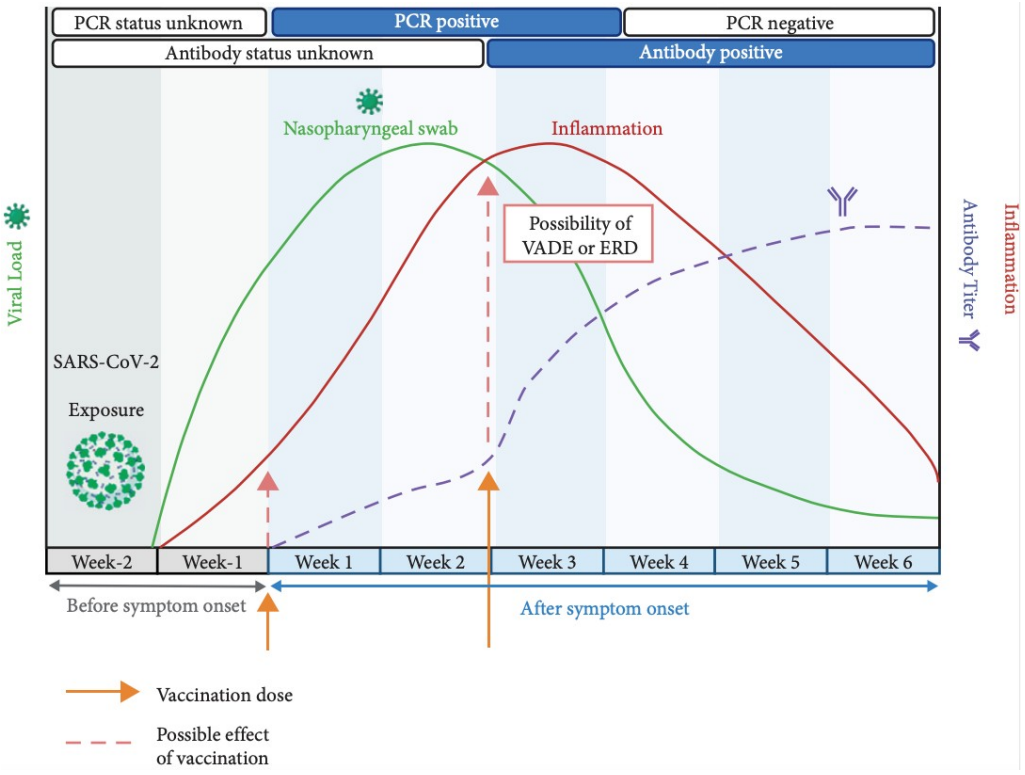
[92] Su S, Du L, Jiang S.
Learning from the past: development of safe and effective COVID-19 vaccines.
Nat Rev Microbiol. 2021;19(3):211-219. doi:10.1038/s41579-020-00462-y
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566580/

Tunjungputri et al[93] reported two cases of patients hospitalized with confirmed COVID-19 pneumonia with a history of recent CoronaVac vaccination. The first patient with a relatively milder course of the disease had received two doses of CoronaVac, while the second patient with a more progressive course of the disease had received only one dose before developing symptoms and being hospitalized, leading to the hypothesis that the vaccination may have potentiated the inflammatory process and slatentized the previously asymptomatic COVID-19 disease.

Therefore, the authors suggest assessing whether vaccine recipients are infected with SARS-CoV-2 even in an asymptomatic form, as vaccine administration may exacerbate inflammation and disease progression, and advise that if SARS-Cov-2 infection is confirmed, antibody titer should be checked before vaccination as neutralizing antibodies mediate ADE when in suboptimal concentrations. [94]

However, there is currently a lack of knowledge about the levels of baseline antibody titers and the minimum level of neutralizing antibodies that would confer protection against COVID-19.

Studies report that neutralizing antibodies are detectable from day 10 and peak at week 3 (IgG) and week 4 (IgM),[95] and in the patients in the case-control study cited above, vaccinations had been performed less than 3 weeks after antibody titers were measured, further reinforcing the idea that neutralizing antibodies had not yet been optimally formed, posing the risk of VADE or ERD.



[93] Tunjungputri RN, Tetrasiwi EN, Veronica M, Pandelaki J, Ibrahim F, Nelwan EJ.
Vaccine-Associated Disease Enhancement (VADE): Considerations in Postvaccination COVID-19.
Case Rep Med. 2021;2021:9673453. Published 2021 Oct 29. doi:10.1155/2021/9673453
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8570879/

[94] Fan Wu, et al
Antibody-dependent enhancement (ADE) of SARS-CoV-2 infection in recovered COVID-19 patients: studies based on cellular and structural biology analysis
medRxiv 2020.10.08.20209114; doi: https://doi.org/10.1101/2020.10.08.20209114
https://www.medrxiv.org/content/10.1101/2020.10.08.20209114v1.full.pdf

[95] Ni L et al
Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals.
Immunity. 2020 Jun 16;52(6):971-977.e3. doi: 10.1016/j.immuni.2020.04.023.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196424/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8570879/pdf/CRIM2021-9673453.pdf
A schematic time sequence of events showing exposure to SARS-CoV-2, PCR status, vaccine administration, symptom development, inflammation, and possibility of improvement in vaccine-associated disease (VADE) and enhanced respiratory disease (ERD). After exposure to SARS-CoV-2, inflammation (red line) may occur until the onset of symptoms. During this period, the inflammatory response may be amplified by the presence of VADE or ERD (pink arrow) when the patient had already been exposed to vaccination (orange arrow).

Vaccines with a high risk of inducing VADE or ERD include inactivated viral vaccines, such as CoronaVac, which may involve nonneutralizing antigenic targets and/or protein S in nonneutralizing conformations for antibodies that can induce increased inflammation.[96]

Specifically, regarding the impact of the vaccine antigen production process in the risk of ADEs, it has been shown that formaldehyde treatment of the vaccine spike leads to cross-linking in this protein such that about half of the trimers are in an "RBD up" conformation, also known as the prefusion conformation.
By blocking the "up" conformation with formaldehyde, the trimers are no longer free to take on both conformations, and thus the neutralizing epitopes of RBD are likely to have lower immunogenicity because of their reduced exposure in half of the trimers.
Consequently, antibody titers developed against SARS-CoV-2 from a formaldehyde-treated vaccine may be sub-neutralizing toward circulating viruses.
In addition, the epitope of the spike protein responsible for AED (see figures below) observed in a preclinical study of formaldehyde-inactivated SARS-CoV-1 vaccine[97] , was also found to be present in SARS-CoV-2.
The amino acid sequence of the ADE epitope is LYQDVNC and is located at $_{S597-603}$ in the spike protein of SARS- CoV-1 and $_{S611-617}$ in the spike protein of SARS-CoV-2.
Inactivation with beta-propiolactone at high concentrations of SARS-CoV-2 also causes viral aggregation and chemical modification of viral amino acids with loss of antigenic potential[98] .
Finally, protein vaccines that use nonhuman cell lines to produce the protein of interest could generate viral proteins with glycosylation patterns different from those produced during natural infection.
Grant et al. found that glycans cover about 40 percent of the surface area of the SARS- CoV-2 spike protein, as will be discussed in more detail in the section on the Glycobiology of Viral Infections, with implications for binding to the human leukocyte antigen (HLA) complex and subsequent antigen-specific immune responses. [99]

[96] Lee WS, Wheatley AK, Kent SJ, DeKosky BJ.
Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies.
Nat Microbiol. 2020 Oct;5(10):1185-1191. doi: 10.1038/s41564-020-00789-5.
https://www.nature.com/articles/s41564-020-00789-5

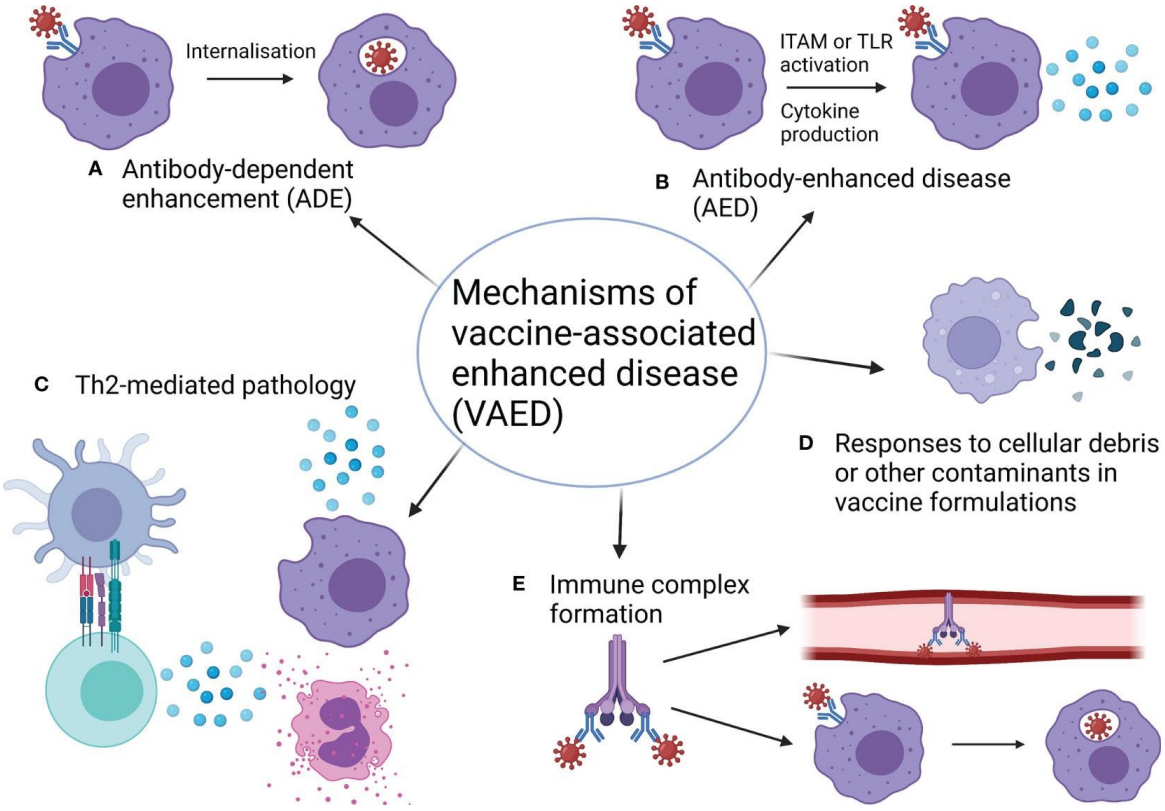
[97] Wang Q, Zhang L, Kuwahara K, Li L, Liu Z, Li T, Zhu H, Liu J, Xu Y, Xie J, Morioka H, Sakaguchi N, Qin C, Liu G.
Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-human Primates.
ACS Infect Dis. 2016 May 13;2(5):361-76. doi: 10.1021/acsinfecdis.6b00006. Epub 2016 Apr 11. Erratum in: ACS Infect Dis. 2020 May 8;6(5):1284-1285.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7075522/

[98] Gupta D, Parthasarathy H, Sah V, et al.
Inactivation of SARS-CoV-2 by β-propiolactone causes aggregation of viral particles and loss of antigenic potential.
Virus Res. 2021;305:198555. doi:10.1016/j.virusres.2021.198555
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8416322/

[99] Grant OC, Montgomery D, Ito K, Woods RJ.
Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition.
Sci Rep. 2020 Sep 14;10(1):14991. doi: 10.1038/s41598-020-71748-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7490396/

Many influenza vaccine antigens are produced in fertilized chicken eggs, and a glycosylation site in influenza H3N2 has been found to alter antibody binding such that weak neutralizing responses are induced in both ferrets and humans.[100]
Because differential glycosylation patterns between vaccine antigens and wild-type antigens can induce weakly neutralizing antibodies in response to particular epitopes, this is another theoretical concern for VAED if the pathogen in question is found to facilitate ADE or AED.[101]

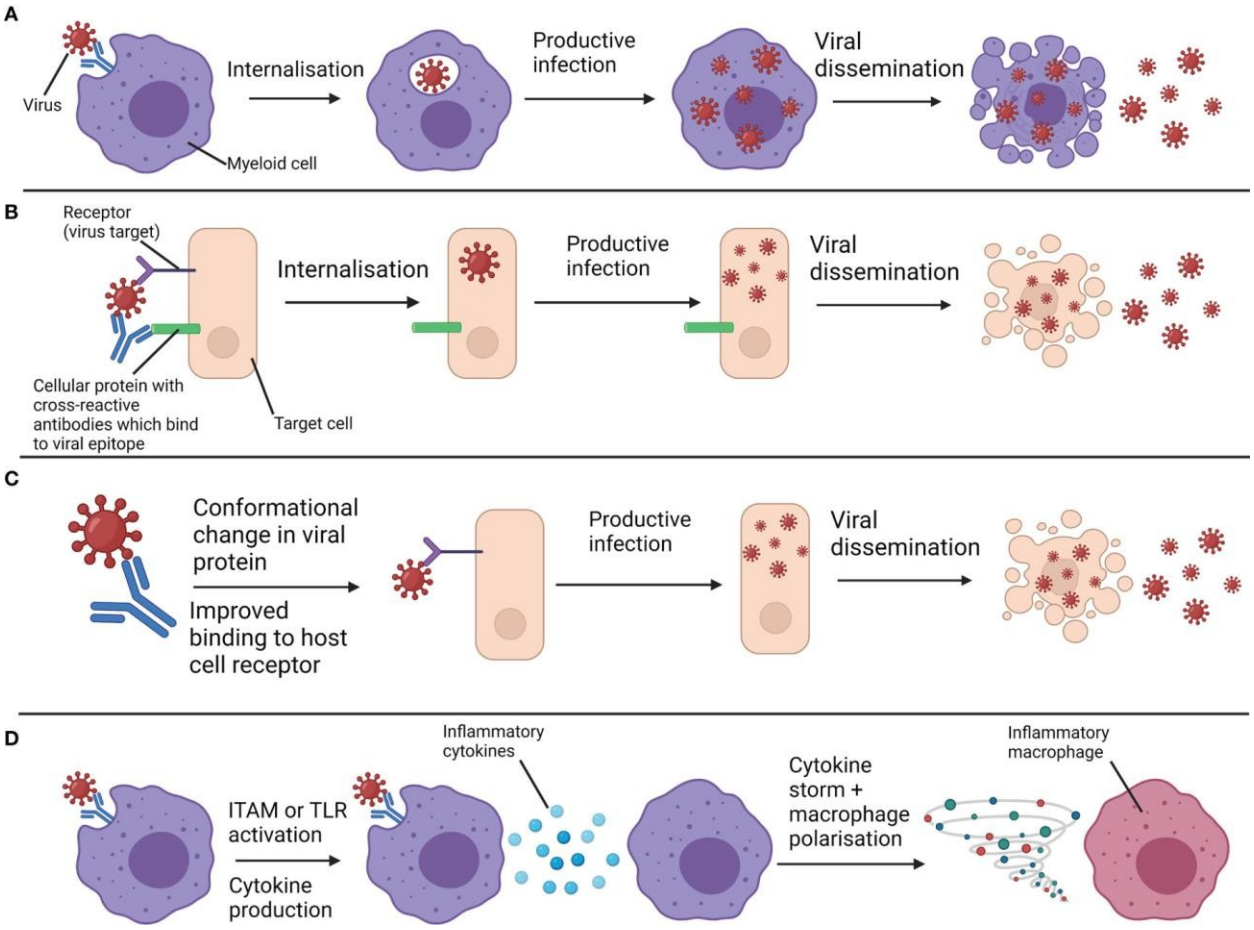
*In some studies, a distinction is made between ADE and AED to better elucidate the biological mechanism. In these cases, antibody-dependent enhancement **(ADE)** occurs when antibodies increase the ability of a virus to infect cells.*
*Antibody-enhanced disease **(AED)** occurs when antibodies exacerbate inflammation, resulting in pathology.*



https://www.frontiersin.org/articles/10.3389/fimmu.2022.882972/full
Mechanisms of vaccine-associated enhanced disease. **(A)** Antibody-dependent enhancement (ADE) occurs when antibodies increase the ability of a virus to infect cells (see also Figure 2 below). **(B)** Antibody-enhanced disease (AED) occurs when antibodies exacerbate inflammation, resulting in pathology (see also Figure 2 below). **(C)** Distorted Th2 responses can be pathogenic for some infections, and therefore vaccines that induce Th2 responses in this case can cause pathology. Usually Th2 pathology is associated with eosinophil infiltration. **(D)** Components of vaccine formulations such as bovine serum albumin (BSA) and cellular debris may mediate pathogenic cellular responses to these components when encountered again as contaminants in the test material. Although these components are normally removed during vaccine preparation, some preclinical studies have not included appropriate washing and centrifugation steps to facilitate this. **(E)** Immune complexes between viral proteins, antibodies, and/or complement can lead to accumulation of deposits in blood vessels and organs or facilitate increased virus uptake through myeloid cells, causing ADE.

[100] Zost SJ, Parkhouse K, Gumina ME, et al.
Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains.
Proc Natl Acad Sci U S A. 2017;114(47):12578-12583. doi:10.1073/pnas.1712377114
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5703309/

[101] Gartlan C, Tipton T, Salguero FJ, Sattentau Q, Gorringe A, Carroll MW.
Vaccine-Associated Enhanced Disease and Pathogenic Human Coronaviruses.
Front Immunol. 2022;13:882972. doi:10.3389/fimmu.2022.882972
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9014240/



https://www.frontiersin.org/articles/10.3389/fimmu.2022.882972/full
Overview of antibody-dependent enhancement (ADE) and antibody-enhanced disease (AED). (A) Non-neutralizing antibodies or sub-neutralizing antibody concentrations bind to viruses and interact with Fc receptors on myeloid cells. This facilitates internalization of viruses. Viruses that can productively infect myeloid cells can proliferate and spread after their uptake, enhancing infection. This is a form of ADE. (B) Cross-reactive antibodies bind to both virus and host cell components, bringing viruses into close contact with their receptor. Receptor-mediated uptake and increased infection follow. This is another form of ADE. (C) Antibodies against a particular epitope result in a conformational change in a viral protein that enhances infection through improved binding to the host cell receptor. This is another form of ADE. (D) Virus-bound antibodies interact with Fc receptors on myeloid cells and activate immunoreceptor tyrosine activation motifs (ITAMs) associated with these receptors, or facilitate viral uptake and subsequent activation of endosomal toll-like receptors (TLRs). Through one of these mechanisms, inflammatory cytokines and chemokines are produced, exacerbating inflammation to a pathogenic extent and polarizing myeloid cells toward more inflammatory phenotypes. These are forms of AED. Productive infection of myeloid cells is not required for this mechanism.

A preliminary report by Dr. Farshi, reported on the outcomes of SARS CoV-2 infection in 33 vaccinated African green monkeys and 200 guinea pigs with mRNA vaccines against SARS CoV-2: two of these monkeys and 9 mice manifested a cytokine storm (increased IL-6) with lung immunopathology (ARDS). [102] Dr. Farshi notes that vaccine companies usually try to achieve maximum immunity in the lungs of vaccinated people because the lung is the most critical organ for the severe form of CoVID-19.
However, this approach does not consider that severe-fatal COVID-19 is induced by the cytokine storm, leading to an error in studies of vaccine effects known as "survivor aircraft bias" for

[102] Cytokine Storm Response to COVID-19 Vaccinations
Esmaeil F, J Cytokine Biol 2020, 6:
https://www.omicsonline.org/open-access-pdfs/cytokine-storm-response-to-covid19-vaccinations.pdf

Khan WH, Hashmi Z, Goel A, Ahmad R, Gupta K, Khan N, Alam I, Ahmed F, Ansari MA.
COVID-19 Pandemic and Vaccines Update on Challenges and Resolutions.
Front Cell Infect Microbiol. 2021 Sep 10;11:690621. doi: 10.3389/fcimb.2021.690621.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8461057/

an analogy with military aircraft, where the study of damage focuses on the survivors of the severe form (in this case lung damage) and not on the cause of deaths (cytokine storm).
If, on the other hand, cytokine storm syndrome is assessed following challenge testing, the results indicate a significant incidence (about 6 percent for monkeys and 4.5 percent for guinea pigs), which extrapolated to the world population, more than 65 percent of whom have been vaccinated with at least one dose,[103] lead to an estimated number of the severe-fatal forms by disease potentiation that is extremely alarming).
Confirming this concern, it was reported in Science (August 2021) that 60% of Israeli patients severely ill with COVID-19 were vaccinated with the BNT162b2 vaccine, and this could be considered a potential form of ADE. Similarly, 70% of new Israeli cases of COVID-19 were reported, at least once, in vaccinated people, confirming that recovery from previous SARS-Cov-2 infection is more protective against variant infection than vaccination. [104]
A case control that presented an abnormal immunologic response to BNT162b2 vaccine was also described as a potential predisposing cause for accelerated mortality induced by SARS CoV-2 infection.[105]
More recently published studies investigating the biological mechanism of ADE support
The increased risk of ADE in vaccinees following SARS-Cov-2 infection.[106]

Insight Dr. Mauro

Mantovani [107]

Immune System and T Lymphocytes ***Video***
T lymphocytes and heterologous and memory immunity toward SARS -Cov-2 ***Video***
A.D.E. and the Immune System ***Video***
Covid-19, ADE phenomenon and SPIKE protein ***Video***
The immunological memory ***ppt***
Covid-19 Vaccines and Immunity ***ppt***

[103] Hannah Ritchie, et al (2020) - "Coronavirus Pandemic (COVID-19)." Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/coronavirus' [Online Resource].
https://ourworldindata.org/covid-vaccinations

[104] A grim warning from Israel: vaccination blunts, but does not defeat delta
16 AUG 2021 Science doi: 10.1126/science.abl9630
https://www.science.org/content/article/grim-warning-israel-vaccination-blunts-does-not-defeat-delta

Mizrahi B, Lotan R, Kalkstein N, Peretz A, Perez G, Ben-Tov A, Chodick G, Gazit S, Patalon T.
Correlation of SARS-CoV-2-breakthrough infections to time-from-vaccine.
Nat Commun. 2021 Nov 4;12(1):6379. doi: 10.1038/s41467-021-26672-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8569006/

[105] Hansen T, Titze U, Kulamadayil-Heidenreich NSA, Glombitza S, Tebbe JJ, Röcken C, Schulz B, Weise M, Wilkens L.
First case of postmortem study in a patient vaccinated against SARS-CoV-2.
Int J Infect Dis. 2021 Jun;107:172-175. doi: 10.1016/j.ijid.2021.04.053.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8051011/

[106] Danchin A, Pagani-Azizi O, Turinici G, Yahiaoui G.
COVID-19 Adaptive Humoral Immunity Models: Weakly Neutralizing Versus Antibody-Disease Enhancement Scenarios.
Acta Biotheor. 2022 Aug 13;70(4):23. doi: 10.1007/s10441-022-09447-1.
https://pubmed.ncbi.nlm.nih.gov/35962852/

Hirschbühl K, et al.
High viral loads: what drives fatal cases of COVID-19 in vaccinees? - an autopsy study.
Mod Pathol. 2022 Aug;35(8):1013-1021. doi: 10.1038/s41379-022-01069-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8974809/

[107] Institute of Biological Medicine https://www.imbio.it/

ADE risk assessment for anti-SARS-Cov-2 vaccines.

The use of the concept of ADE to denote increased severity of disease must be strictly differentiated from the ADE of infection, i.e., virus binding, uptake and replication, cytokine release or other antibody activity detected in vitro. [108]

The first principle for assigning ADE potential is that an antibody-dependent effect *in vitro* does not represent or predict ADE as a disease without evidence of a role of the antibody in the pathogenesis of a more severe clinical outcome.

A second principle is that animal models for evaluating human polyclonal antibodies or monoclonal antibodies (mAbs) should be evaluated with caution, because the FcRs recruited by IgG are species specific, as is complement activation.

Antibodies can have very different properties in animals, which are not predictive of those in the human host, because the effector functions of antibodies are modified by the different species-specific interactions between the antibody and immune cells. Animals may also develop antibodies against a therapeutic antibody that limit its efficacy or cause immunopathology.

In addition, the pathogenesis of a virus strain in an animal model does not fully reflect human infection because most viruses are highly species specific. These differences may incorrectly support the protective or immunopathological effects of vaccines and antibodies.

A third principle is that the nature of the antibody response depends on the form of the viral protein recognized by the immune system, thus determining which epitopes are presented. Protective and nonprotective antibodies can be induced by different forms of the same protein.

A fourth principle is that the mechanisms of pathogenesis in the human host differ substantially among viruses, or even among strains of a particular virus. Therefore, results on the effects of passive antibodies or vaccine-induced immunity cannot be confidently extrapolated from one viral pathogen to another.

The following table summarizes the Information provided and the limitations of approaches for evaluating antibody-mediated protection against SARS-CoV-2 and the risk of antibody-dependent disease potentiation.

[108] Arvin AM, et al
A perspective on potential antibody-dependent enhancement of SARS-CoV-2.
Nature. 2020 Aug;584(7821):353-363. doi: 10.1038/s41586-020-2538-8.
https://www.nature.com/articles/s41586-020-2538-8.pdf

Test modality	Information provided	Limitations
In vitro: cell culture Infect relevant human cells with or without antibodies	Virus neutralization Virus uptake, productive infection or cytokines	Cell lines lack primary cell receptor characteristics Primary human cells are difficult to culture and have donor variability • Receptor expression must be maintained
In vivo: animal models Infection of animals with or without antibody or vaccine intervention	Protection against or increase of viral replication or disease	Lack of disease models of human illness Lack of models predictive of enhanced disease in humans Viral replication as a proxy of disease requires clinical validation Need to assess T cells for contribution to pathology or reducing ADE With human mAbs: • Differential engagement of animal FcγRs • Different expression patterns of FcγRs in humans and animals • Potential generation of anti-human antibodies
Human: clinical and epidemiological studies	Correlations of outcomes with • Previous HCoV infection • Treatment with plasma from convalescent patients • Kinetics of adaptive immune responses	No markers to differentiate severe disease from enhanced disease Limited knowledge of antibody or T cell epitope specificities during natural SARS-CoV-2 or other HCoV infection, and of outcomes of infection with new coronaviruses

https://www.nature.com/articles/s41586-020-2538-8.pdf

In addition to the limitations presented above of the study in animal models for risk assessment of ADEs, it should be noted that in preclinical studies of SARS-Cov-2 vaccines, nonhuman primates, which do not manifest the severe/fatal complications of Covid-19[109] , and the challenge test was carried out with the infectious virus that has the same (or very similar) reference spike sequence as the currently marketed vaccine constructs, and thus does not allow for a proper assessment of either the efficacy or the risk of ADE in case of infection with vaccine-resistant variants. [110]

It should be mentioned that although from the point of view of symptoms, vaccine ADE is not distinguishable from COVID-19 ADE, it is known from evidence with other vaccines that induce disease enhancement that vaccine ADE presents with characteristic pulmonary immunopathology with eosinophil infiltration, and therefore it is possible to make a differential diagnosis of the two disease processes. [111]

[109] Kanduc D.
Lack of Molecular Mimicry between Nonhuman Primates and Infectious Pathogens: The Possible Genetic Bases.
Glob Med Genet. 2021;8(1):32-37. doi:10.1055/s-0041-1724106
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7964256/

Kanduc D.
Lack of Molecular Mimicry between Nonhuman Primates and Infectious Pathogens: The Possible Genetic Bases.
Glob Med Genet. 2021 Mar;8(1):32-37. doi: 10.1055/s-0041-1724106. Epub 2021 Feb 19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7499017/

[110] COVID-19 the vaccine update p.93

[111] Respiratory complications-immunopathology p. 47

To date, only a few very partial in *vitro* and *in vivo* studies are available[112] suggesting its risk in humans. Although the issue was brought to the attention of the EMA as early as March 2020 by the Coalition for Epidemic Preparedness Innovations (CEPI) and the Brighton Collaboration (BC) Safety Platform for Emergency vACcines (SPEAC) 113 no useful results are available from regulatory agencies to date. While for vaccine ADE the manufacturers have not yet published reliable studies, for COVID-19 the studies available to date suggest that severe/fatal complications associated with SARS-Cov-2 infection are a consequence of ADE.

ADE explains why the elderly are at greater risk than children (as long as they do not have maternal antibodies to SARS-Cov-2, because then they may be susceptible to ADE if they become infected[114]) and healthy adults, as they have a greater amount of nonneutralizing antibodies from coronavirus infections or older vaccinations (e.g., flu shots), and have an underperforming immune system in fighting infection.

It follows that Sars-Cov-2, because of its ability to form quasispecies, may concretely be responsible for the phenomenon of disease enhancement in vaccinees, which necessarily needed to be investigated and ruled out before proceeding with human trials. [115]

Cardozo et al. in the article *"Informed consent disclosure to vaccine trial subjects of risk of COVID-19 vaccines worsening clinical disease"*[116] point out that current data on COVID-19 vaccines are limited but do not reveal evidence of ADE of disease: nonhuman primate studies of Moderna's mRNA-1273 vaccine showed excellent protection without detectable immunopathology *[117] .

* see Critical issues in preclinical studies for a discussion of methodological errors on p. 79

[112] Dapeng Li, et al
The functions of SARS-CoV-2 neutralizing and infection-enhancing antibodies in vitro and in mice and nonhuman primates
bioRxiv 2020.12.31.424729; doi: https://doi.org/10.1101/2020.12.31.424729
https://www.biorxiv.org/content/10.1101/2020.12.31.424729v1.full

Zhou Y, Liu Z, Li S, et al.
Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor associates with distinct epitopes on the RBD.
Cell Rep. 2021;34(5):108699. doi:10.1016/j.celrep.2021.108699
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7802522/

Fan Wu, et al
Antibody-dependent enhancement (ADE) of SARS-CoV-2 infection in recovered COVID-19 patients: studies based on cellular and structural biology analysis
medRxiv 2020.10.08.20209114; doi:https://doi.org/10.1101/2020.10.08.20209114
https://www.medrxiv.org/content/10.1101/2020.10.08.20209114v1

[113] Lambert PH, Ambrosino DM, Andersen SR, et al.
Consensus summary report for CEPI/BC March 12-13, 2020 meeting: Assessment of risk of disease enhancement with COVID-19 vaccines.
Vaccine. 2020;38(31):4783-4791. doi:10.1016/j.vaccine.2020.05.064
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7247514/

[114] Ricke DO.
Two Different Antibody-Dependent Enhancement (ADE) Risks for SARS-CoV-2 Antibodies.
Front Immunol. 2021 Feb 24;12:640093. doi: 10.3389/fimmu.2021.640093.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7943455/

[115] Su S, Du L, Jiang S.
Learning from the past: development of safe and effective COVID-19 vaccines.
Nat Rev Microbiol. 2021;19(3):211-219. doi:10.1038/s41579-020-00462-y
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566580/

[116] Cardozo T, Veazey R.
Informed consent disclosure to vaccine trial subjects of risk of COVID-19 vaccines worsening clinical disease
[published online ahead of print, 2020 Oct 28]. Int J Clin Pract. 2020;e13795. doi:10.1111/ijcp.13795
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7645850/pdf/IJCP-9999-e13795.pdf

[117] Corbett KS, Flynn B, Foulds KE, et al.
Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates.
N Engl J Med. 2020;383(16):1544-1555. doi:10.1056/NEJMoa2024671
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7449230/

Phase 1 studies of several vaccines reported no immunopathology in subjects given candidate vaccines, although it is unlikely that these subjects had become infected with circulating viruses. [118]
The authors point out, however, that all preclinical studies to date have been carried out with Wuhan-Hu- 1 virus or closely related strains, while the D614G mutant was the most widely circulating form, and several observations suggest that this alternative form may be antigenically distinct from the original Wuhan strain, not so much in composition but in viral spike conformation and exposure of neutralization epitopes.
Similarly, phase 1 and 2 clinical trials of candidate vaccines were designed only to test immunogenicity (i.e., vaccine antibody formation) as an efficacy endpoint and not to evaluate the effect of subject exposure to circulating virus after vaccination, which is when ADE/immunopathology is expected to occur.
Therefore, the absence of evidence of ADEs in the COVID-19 vaccine trial data to date does not excuse researchers from informing participants in clinical trials of the vaccine of the risk of disease enhancement, as it represents a realistic and not theoretical risk to subjects.
Informed consent forms for COVID-19 vaccine trials are not publicly available for privacy reasons. In addition, they vary from one clinical site to another, and the sample consent forms on which they are based do not have to be disclosed until the trial is completed, or even afterwards.
However, these consent forms are usually identical in content to the "Risks to Participants" section of the trial protocols, which have been publicly released by Pfizer, Moderna, and Johnson & Johnson for their COVID-19 vaccine studies ([119] and Supplement).
Because these three vaccines are representative of the diversity of vaccines being tested, it is highly likely that the consent form inferred from these protocols will be similar or identical to those of all vaccine studies currently underway.
All three protocols mention the risk of disease enhancement by the vaccine, but all three list this risk last or second to last in the list of risks, after Ad26-Cov2 vector risks, adenovirus vectors in general, vaccination risks in general, pregnancy and birth control risks (said to be "unknown"), risks from blood draws and risks from collecting nasal swab samples (for the J&J), after allergy, fainting, local site injection reaction, general systemic adverse reactions and laboratory abnormalities for the Moderna vaccine, and after local site injection reactions and general systemic adverse events for the Pfizer vaccine.
In addition, both Moderna and J&J call the risk of disease enhancement caused by the vaccine "theoretical."
Finally, citing risk, Pfizer and Moderna cite previous evidence of disease enhancement caused by RSV and Dengue vaccine, as well as feline coronavirus (Pfizer) and measles (Moderna), however, SARS and MERS are not mentioned. J&J discusses SARS and MERS, but argues that vaccine-induced disease enhancement is due to non-neutralizing antibodies and unbalanced Th2 cell responses and that Ad26 vaccination does not show this profile.
Thus, the authors point out that overall, the Pfizer, Moderna and J&J trial protocols provided to participants of the COVID-19 vaccine clinical trials, when compared with the documentation on antibody-dependent disease enhancement presented in their discussion and widely available to any experienced professional in the field, do not allow the participant to understand that administration of the vaccine can lead to mild illness to severe and lasting disease or even death.

---

[118] Mulligan, M.J., Lyke, K.E., Kitchin, N. et al.
Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults.
Nature 586, 589-593 (2020). https://doi.org/10.1038/s41586-020-2639-4
https://www.nature.com/articles/s41586-020-2639-4

[119] McNamara D.
Three Major COVID Vaccine Developers Release Detailed Trial Protocols.
https://www.medscape.com/viewarticle/937845

Disclosure of the specific risk of disease enhancement from vaccination requires a specific, separate informed consent form and a demonstration of patient understanding in order to meet medical ethics standards.
The informed consent process for ongoing COVID-19 vaccine trials does not appear to meet this standard. Although the global COVID-19 health emergency warrants accelerated candidate vaccine trials with known responsibilities, this acceleration does not justify the lack of additional attention needed to strengthen informed consent procedures specific to COVID-19 vaccine risks.
Similar considerations are reported regarding the informed consent of the "Astrazeneca" vaccine in the *Covid-19* document *the vaccine* from p. 87

Insight

ANTIBODIES AND VIRAL INFECTION

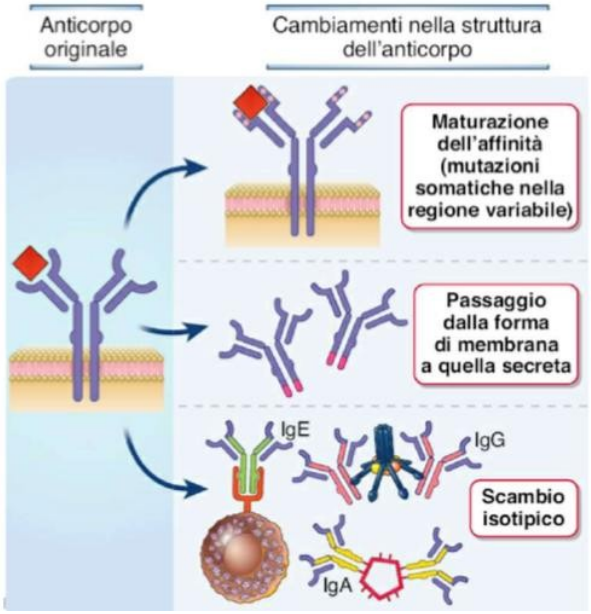
Antibodies are very stable molecules due to their structure, which is based on the repetition of a compact globular unit, folded back on itself, about 110 amino acids long, called the "immunoglobulin domain."
This domain is present in many molecules of the immune system, such as TCR (cell receptor, T lymphocyte antigen receptor) and MHC (major histocompatibility complex) molecules, in adhesion molecules, such as ICAM-1 (Intercellular adhesion molecule-1) and VCAM-1 (Vascular adhesion molecule-1), and in many other proteins, often involved in cell-cell interaction.
We therefore speak of the "immunoglobulin superfamily" to refer to the set of proteins that contain at least one immunoglobulin domain.

Functions of membrane antibodies and soluble antibodies

Antibodies expressed on the membrane of B lymphocytes function as cellular receptors for antigen. The carboxy-terminal end of the H chains terminates with a transmembrane domain and a small intracellular tail, to which molecules involved in the transmission within the cell of the signal generated by antigen binding associate with noncovalent binding.
When two or more membrane antibodies interact with antigen, an activation signal is generated in B lymphocytes, which, together with accessory signals provided by CD4 helper T lymphocytes, induces B lymphocyte proliferation and differentiation.
This mechanism of selective activation of B lymphocytes that have encountered the antigen is essential for the induction of the specific antibody response, also called the "humoral response."
Terminal differentiation of antigen-activated B lymphocytes gives rise to plasma cells, which secrete soluble antibodies containing the same variable regions as the antibody expressed in the membrane by the precursor B lymphocyte.
Immunoglobulins secreted into serum and biological fluids by plasma cells, have the function of binding a multiplicity of antigens due to the diversity of variable regions, and activating a limited number of effector functions by means of a few types of constant regions. Antibodies can bind soluble antigens, for example, toxins, and neutralize them to prevent them from performing their host-damaging function.[120]

[120] Antibodies and genetic mechanisms of antibody diversity

https://www.biologyonline.com/dictionary/immunoglobulin



L'anticorpo originale espresso in membrana è solo il prototipo per una serie di processi volti a fornire risposte umorali sempre più efficaci.

- **Maturazione dell'affinità**: mutazioni nella regione variabile (indicate dai punti gialli) affinano la specificità del sito di legame per l'antigene, senza modificare le funzioni effettrici che dipendono dalla regione costante.
- **Anticorpi secreti**: gli anticorpi espressi sulla superficie cellulare possiedono un dominio transmembrana che li àncora nel doppio strato lipidico. Da questi, si passa alla produzione di anticorpi in forma secreta, che tuttavia hanno lo stesso sito di legame per l'antigene. Gli anticorpi secreti possono o non possono mostrare mutazioni nella regione variabile (cioè la secrezione degli anticorpi può avvenire sia prima che dopo la maturazione dell'affinità).
- **Scambio isotipico**: questo processo modifica la regione costante (come indicato dal cambiamento del colore al verde al giallo o al rosa) senza cambiare l'affinità di legame per l'antigene. Lo scambio isotipico si osserva sia negli anticorpi di membrana sia in quelli secreti.

https://pls.scienze.unipd.it/biologia-biotecnologie/wp-content/uploads/sites/5/2020/10/Immunologia-PL2020_compressed.pdf

The classes of antibodies

Multiple classes of antibodies (i.e., IgM, IgA, IgG, and IgE) are involved in immune responses to viral infections (Figure below). [121] These classes are characterized by their intrinsic biophysical properties, functions, tissue distributions, and half-lives.

Together with IgD[122] **, IgM** immunoglobulins are normally the first to be expressed during naïve B-cell development, comprising the majority of antibodies produced between B-cell activation and switching (switching class).

IgM accounts for about 10 percent of all antibodies in serum and demonstrates relatively low affinity compared with IgG, due to limited affinity maturation through somatic mutations.

---

[121] Galipeau Y, Greig M, Liu G, Driedger M, Langlois MA.
Humoral Responses and Serological Assays in SARS-CoV-2 Infections.
Front Immunol. 2020 Dec 18;11:610688. doi: 10.3389/fimmu.2020.610688. PMID: 33391281;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7775512/

Sun Y, Huang T, Hammarström L, Zhao Y.
The Immunoglobulins: New Insights, Implications, and Applications.
Annu Rev Anim Biosci. 2020 Feb 15;8:145-169. doi: 10.1146/annurev-animal-021419-083720.
https://pubmed.ncbi.nlm.nih.gov/31846352/

Antibodies lecture
https://didattica-2000.archived.uniroma2.it/immunotlb/deposito/Anticorpi.pdf
https://pls.scienze.unipd.it/biologia-biotecnologie/wp-content/uploads/sites/5/2020/10/Immunologia-PL2020_compressed.pdf
http://amsacta.unibo.it/3067/40/13_anticorpi_I_ed_ebook.pdf
https://www.biopills.net/anticorpi/
https://it.wikipedia.org/wiki/Anticorpo

[122] Gutzeit C, Chen K, Cerutti A.
The enigmatic function of IgD: some answers at last.
Eur J Immunol. 2018;48(7):1101-1113. doi:10.1002/eji.201646547
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6033660/

Sheir, Donia
Role of IgD in prevention and treatment of SARS CoV-2 infection "The Hidden Soldier."
Microbial Biosystems. (2020). 5. 108-114. 10.21608/mb.2020.33453.1019.
https://www.researchgate.net/publication/342857337_Role_of_IgD_in_prevention_and_treatment_of_SARS_CoV-2_infection_The_Hidden_Soldier

However, IgM antibodies demonstrate high avidity for the target antigen because they form pentamers that use multimeric interactions with the target antigen to facilitate neutralization, and they are mainly found in the circulation where they can facilitate antigen opsonization. [123]
Recent studies have also revealed several roles of secretory IgM in the mucosa of the gastrointestinal and respiratory tracts, which will be discussed in more detail later.[124]
Human **IgA** immunoglobulins, which can be further subdivided into the subclasses IgA1 and IgA2[125] , generally exceed serum IgM levels and are significantly more present in mucosal surfaces and secretions (e.g., saliva, breast milk, etc.), where they are central to mucosal immunity.
IgA immunoglobulins form dimers upon secretion, which contributes to their increased avidity. Although IgA antibodies do not fix complement as effectively as IgM, IgA antibodies secreted by plasma cells in the respiratory tract play a key role in mucosal immunity through neutralization of the pathogen, a process that facilitates aggregation and prevents initial infection of host cells, thus conferring sterilizing immunity to a pathogen.[126]
IgG antibodies begin to appear later in the immune response because they undergo affinity maturation through somatic mutations that result in high affinity for the target antigen and a greater ability to neutralize pathogens[127] .
In addition to their role in antigen neutralization, IgG antibodies also have other critically important roles, particularly Fc-mediated effector functions such as cellular activations and antibody-dependent cellular cytotoxicity (ADCC).[128]
IgG immunoglobulins are monomeric and account for about 75% of all antibodies in serum. They are associated with lasting immunity, given their long half-life in the blood and association with differentiated memory B cells[129] .

---

[123] Boes M. Role of natural and immune IgM antibodies in immune responses.
Mol Immunol. 2000 Dec;37(18):1141-9. doi: 10.1016/s0161-5890(01)00025-6.
https://pubmed.ncbi.nlm.nih.gov/11451419/

[124] Planchais C, Mouquet H.
Easy pan-detection of human IgA immunoglobulins.
J Immunol Methods. 2020 Sep-Oct;484-485:112833. doi: 10.1016/j.jim.2020.112833
https://pubmed.ncbi.nlm.nih.gov/32771390/

[125] Breedveld A, van Egmond M.
IgA and FcαRI: Pathological Roles and Therapeutic Opportunities.
Front Immunol. 2019;10:553. doi:10.3389/fimmu.2019.00553
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6448004/

van Gool MMJ, van Egmond M.
IgA and FcαRI: Versatile Players in Homeostasis, Infection, and Autoimmunity.
Immunotargets Ther. 2021;9:351-372. Published 2021 Jan 5. doi:10.2147/ITT.S266242
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7801909/

[126] Freihorst J, Ogra PL.
Mucosal immunity and viral infections.
Ann Med. 2001 Apr;33(3):172-7. doi: 10.3109/07853890109002074.
https://pubmed.ncbi.nlm.nih.gov/11370770/
[127] Zan H, Casali P.
Immunoglobulin Somatic Hypermutation and Class-Switch DNA Recombination. In: Mackay IR, Rose NR, Diamond B, Davidson A, editors. Encyclopedia of Medical Immunology: Autoimmune Diseases. New York, NY: Springer New York; (2014). 10.1007/978-0-387-84828-0_556

[128] Vidarsson G, Dekkers G, Rispens T.
IgG subclasses and allotypes: from structure to effector functions.
Front Immunol. 2014;5:520. Published 2014 Oct 20. doi:10.3389/fimmu.2014.00520
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4202688/

Lu LL, Suscovich TJ, Fortune SM, Alter G.
Beyond binding: antibody effector functions in infectious diseases.
Nat Rev Immunol. 2018;18(1):46-61. doi:10.1038/nri.2017.106
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6369690/
[129] Schroeder HW Jr, Cavacini L.
Structure and function of immunoglobulins.
J Allergy Clin Immunol. 2010;125(2 Suppl 2):S41-S52. doi:10.1016/j.jaci.2009.09.046
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3670108/

IgG can also bind C1q, activating the classic complement pathway of the innate immune system [130].

IgG antibodies can be divided into multiple subtypes (i.e., IgG1, IgG2, IgG3 and IgG4), each with slightly different roles in humoral immunity. For example, IgG1, IgG3 and occasionally IgG4 (following repeated exposure) are secreted in response to protein antigens, while IgG2 responds almost exclusively to polysaccharide antigens.[131]

Since different pathogens cause different ratios of IgG subtypes, these can be used as characteristic profiles to monitor vaccine efficacy (in the sense of vaccine antibody production) with regard to correlates of protection[132] .

Finally, **IgE** antibodies predominantly mediate allergic reactions and immune responses against parasitic infections, and constitute less than 0.01% of all total antibodies.

IgE antibodies are monomeric and show a strong affinity for FcεRI receptors expressed on numerous innate immune cells (e.g., mast cells, basophils, eosinophils), allowing the generation of a generalized inflammatory response through innate activation of the immune system.[133]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6369690/
Effector functions of antibodies.
Antibodies are capable of deploying a plethora of effector functions during the course of an infection. These include but are not limited to the following: **a |** The direct neutralization of toxins or microorganisms. **b |** The neutralization of microbial virulence factors, such as those involved in quorum sensing and biofilm formation. **c |** The entrapment of pathogens in mucins. **d |** The activation of complement to drive clearance or destruction of phagocytes, generate chemoattractants or anaphylatoxins such as C3a and C5a or complement fragment opsonins such as C3b or induce lysis through the membrane attachment complex. **e |** The activation of neutrophil opsonophagocytosis, oxidative bursts, production of lytic and chemoattractant enzymes, or the formation of neutrophil extracellular traps (NETs) of chromatin and antimicrobial proteins. **f |** The induction of macrophage opsonophagocytosis, oxidative bursts, or release of antimicrobial peptides. **g |** The activation of natural killer (NK) cell degranulation to kill infected cells**. h |** The enhancement of antigen uptake, processing, and presentation by dendritic cells (DCs) to T cells. **i |** The presentation of antigens by follicular dendritic cells (FDCs) to B lymphocytes. **j |** The degranulation of mast cells, basophils, and eosinophils to release vasoactive substances, chemoattractants, and T helper type 2 ($_{TH2}$) cytokines in the context of allergens or parasitic infections. Fc, crystallizable fragment; MBL, mannose-binding lectin; pMHC, peptide-MHC complex.

---

[130] Holers VM.
Complement and its receptors: new insights into human disease.
Annu Rev Immunol. 2014;32:433-59. doi: 10.1146/annurev-immunol-032713-120154.
https://pubmed.ncbi.nlm.nih.gov/24499275/

[131] Vidarsson G, Dekkers G, Rispens T.
IgG subclasses and allotypes: from structure to effector functions.
Front Immunol. 2014;5:520. Published 2014 Oct 20. doi:10.3389/fimmu.2014.00520
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4202688/

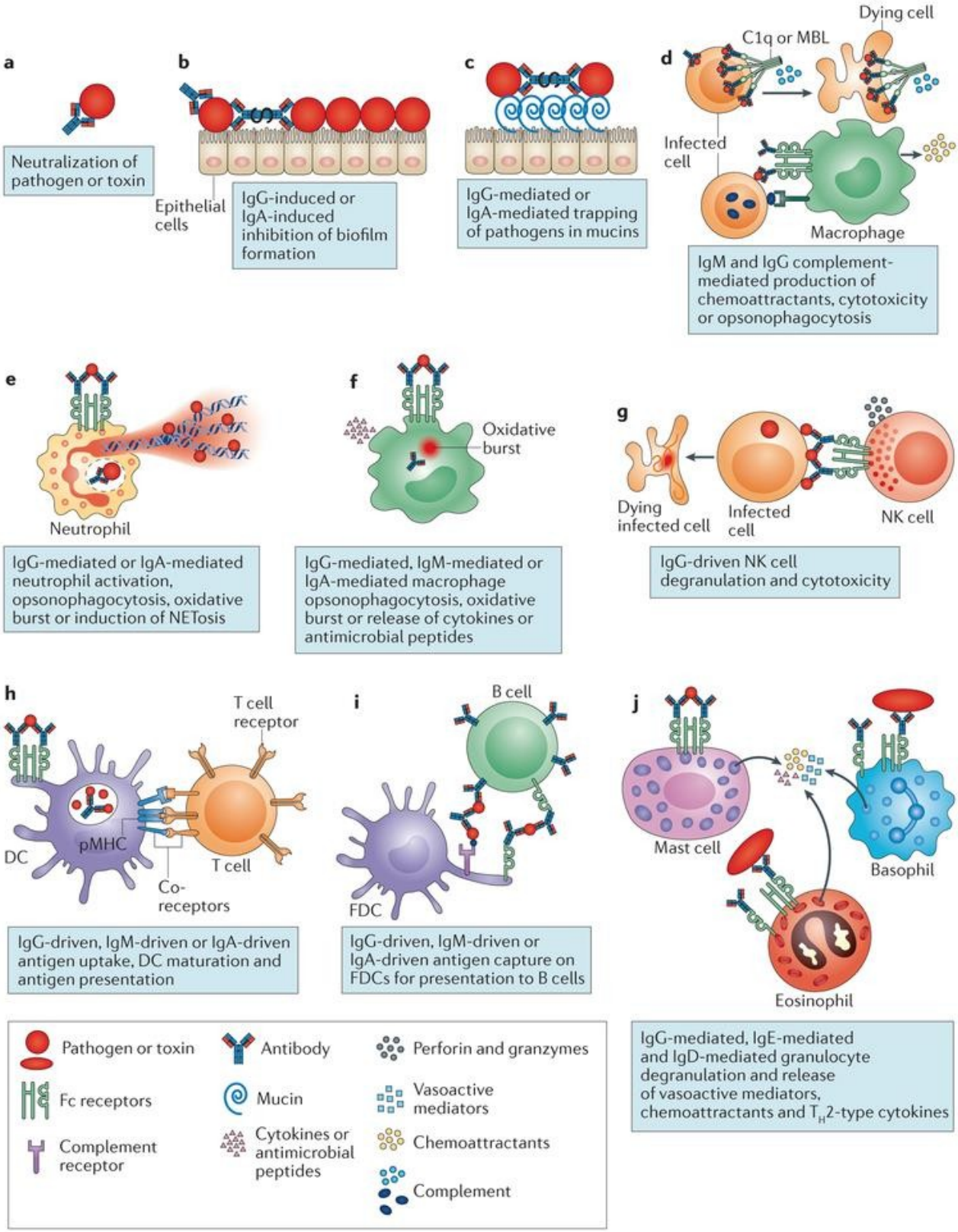
[132] Immunological correlates of vaccination-induced protection

Soininen A, Seppälä I, Nieminen T, Eskola J, Käyhty H.
IgG subclass distribution of antibodies after vaccination of adults with pneumococcal conjugate vaccines.
Vaccine. 1999 Apr 9;17(15-16):1889-97. doi: 10.1016/s0264-410x(98)00475-7.
https://pubmed.ncbi.nlm.nih.gov/10217586/

Isa MB, Martínez L, Giordano M, Passeggi C, de Wolff MC, Nates S.
Comparison of immunoglobulin G subclass profiles induced by measles virus in vaccinated and naturally infected individuals.
Clin Diagn Lab Immunol. 2002;9(3):693-697. doi:10.1128/cdli.9.3.693-697.2002
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC119984/

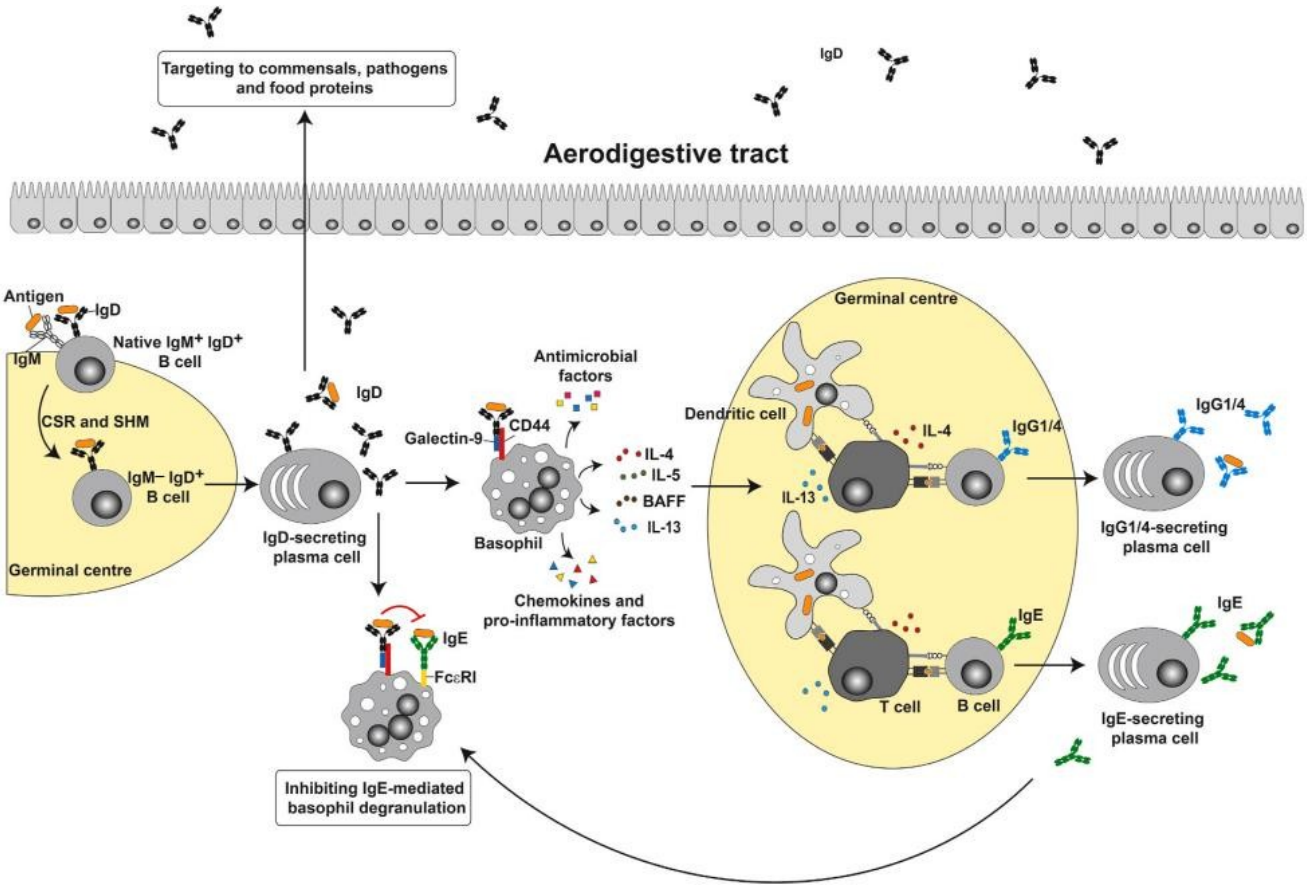
[133] Amarasekera M.
Immunoglobulin E in health and disease.
Asia Pac Allergy. 2011;1(1):12-15. doi:10.5415/apallergy.2011.1.1.12
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3206235/

Lu LL, Suscovich TJ, Fortune SM, Alter G.
Beyond binding: antibody effector functions in infectious diseases.
Nat Rev Immunol. 2018 Jan;18(1):46-61. doi: 10.1038/nri.2017.106.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6369690/


a
Neutralization of pathogen or toxin
b
Epithelial cells
IgG-induced or IgA-induced inhibition of biofilm formation
c
IgG-mediated or IgA-mediated trapping of pathogens in mucins
d
Dying cell
C1q or MBL
Infected cell
Macrophage
IgM and IgG complement-mediated production of chemoattractants, cytotoxicity or opsonophagocytosis
e
Neutrophil
IgG-mediated or IgA-mediated neutrophil activation, opsonophagocytosis, oxidative burst or induction of NETosis
f
Oxidative burst
IgG-mediated, IgM-mediated or IgA-mediated macrophage opsonophagocytosis, oxidative burst or release of cytokines or antimicrobial peptides
g
Dying infected cell
Infected cell
NK cell
IgG-driven NK cell degranulation and cytotoxicity
h
T cell receptor
DC
pMHC
T cell
Co-receptors
IgG-driven, IgM-driven or IgA-driven antigen uptake, DC maturation and antigen presentation
i
B cell
FDC
IgG-driven, IgM-driven or IgA-driven antigen capture on FDCs for presentation to B cells
j
Mast cell
Basophil
Eosinophil
IgG-mediated, IgE-mediated and IgD-mediated granulocyte degranulation and release of vasoactive mediators, chemoattractants and T_H2-type cytokines
Pathogen or toxin
Fc receptors
Complement receptor
Antibody
Mucin
Cytokines or antimicrobial peptides
Perforin and granzymes
Vasoactive mediators
Chemoattractants
Complement

Immunoglobulin D

Immunoglobulin D (IgD) has remained a mysterious class of antibodies for nearly half a century.
IgD was initially thought to be a recently evolved Ig isotype expressed only by some mammalian species, but recent findings in fish and amphibians show that IgD was present in the ancestor of all jawed vertebrates and has important immunological functions.
The structure of IgD has been very dynamic throughout evolution. Mammals can express IgD through alternative splicing and class change recombination (CSR).
Cell-dependent and T-lymphocyte-independent IgM to active IgD class switching occurs in a unique subset of human upper aerodigestive mucosal B cells, which provides a layer of mucosal protection toward many pathogens and their virulence factors.
Circulating IgD can bind to myeloid cells, such as basophils, and induce antimicrobial, inflammatory, and B-cell-stimulating factors, contributing to immune surveillance but also to inflammation and tissue damage when this pathway is hyperactivated under pathological conditions. Recent research shows that IgD is an important immunomodulator that orchestrates an ancestral surveillance system at the interface between immunity and inflammation. [134]



https://pubmed.ncbi.nlm.nih.gov/34237381/
The function of sIgD in the aerodigestive mucosa. In the lymphoepithelial organs of the aerodigestive mucosa, FO B cells (IgD+ IgM+) enter a GC program by undergoing CSR from IgM to IgD and extended SHM. The resulting B lymphocytes (IgD+ IgM-) further differentiate into IgD-secreting plasma cells, which release IgD locally or colonize distal mucosal districts (e.g., lacrimal, salivary, and mammary glands, as well as

[134] Chen K, Cerutti A.
New insights into the enigma of immunoglobulin D.
Immunol Rev. 2010;237(1):160-179. doi:10.1111/j.1600-065X.2010.00929.x
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3048779/

Wan Z, Zhao Y, Sun Y.
Immunoglobulin D and its encoding genes: An updated review.
Dev Comp Immunol. 2021 Nov;124:104198. doi: 10.1016/j.dci.2021.104198. Epub 2021 Jul 5.
https://pubmed.ncbi.nlm.nih.gov/34237381/

the middle ear) through the general circulation (not shown in this figure). IgD secreted by these cells binds to local mucosal antigens, including microbiota, pathogens and their products, and food antigens. sIgD also binds to basophils (and mast cells) via a receptor composed of galectin-9 and CD44. When engaged by antigen, basophil-bound IgD induces basophil release of chemokines, antimicrobial and pro-inflammatory factors, as well as IL-4, IL-5 and IL-13, which facilitates the generation of Th2 follicular cells that secrete IL-4 and IL-13. These GC T cells then enhance B cell production of the initial antigen-specific IgG1 (in mice) or IgG4 (in humans) and IgE. In addition, cross-linking of IgD by antigens also constrains IgEmediated basophil degranulation.

ADE STUDY

Serological tests to detect SARS-Cov-2

Serological tests are designed to detect the presence of IgG antibodies to a specific pathogen, in this case SARS-CoV-2.

A positive serologic test result is indicative of past exposure to one or more antigenic epitopes of the pathogen and therefore is not an indicator of active infection. In addition, if the pathogen of interest shares antigenic epitope sequences with proteins of other microbes or even those of vaccine antigens, a test may be a false positive.

In the course of a natural SARS-CoV-2 infection, viral RNA levels decline rapidly during the second week until they become undetectable[135] and the antibody assay then becomes the primary and most accurate way to detect a recently resolved or previous infection.

Serologic testing is also critical for the detection of asymptomatic and previously undiagnosed infections in the population.

The most commonly used serological tests include lateral flow immunoassays (LFIA), enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays (CLIA) (Table 1).[136]

Depending on the test used, they can detect IgM, IgA, IgG or total antibodies[137] . In addition, tests vary in the specific antibodies they detect; these include antibodies to RBD, nucleocapsid (N) protein, spike (S) protein, or nucleocapsid and spike (NS) proteins.[138]

---

[135] Wölfel R, et al
Virological assessment of hospitalized patients with COVID-2019.
Nature. 2020 May;581(7809):465-469. doi: 10.1038/s41586-020-2196-x. Epub 2020 Apr 1. Erratum in: Nature. 2020 Dec;588(7839):E35.
https://www.nature.com/articles/s41586-020-2196-x

Wang Y, Zhang L, Sang L, et al.
Kinetics of viral load and antibody response in relation to COVID-19 severity.
J Clin Invest. 2020;130(10):5235-5244. doi:10.1172/JCI138759
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7524490/

Borremans B, Gamble A, Prager KC, et al.
Quantifying antibody kinetics and RNA detection during early-phase SARS-CoV-2 infection by time since symptom onset.
Elife. 2020;9:e60122. Published 2020 Sep 7. doi:10.7554/eLife.60122
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7508557/

[136] http://omero.farm.unipi.it/matdidFarm/9/Capitolo%204-Immunodosaggi.pdf

[137] Mekonnen D, Mengist HM, Derbie A, Nibret E, Munshea A, He H, Li B, Jin T.
Diagnostic accuracy of serological tests and kinetics of severe acute respiratory syndrome coronavirus 2 antibody: A systematic review and meta-analysis.
Rev Med Virol. 2021 May;31(3):e2181. doi: 10.1002/rmv.2181.
https://pubmed.ncbi.nlm.nih.gov/33152146/

Woof JM, Mestecky J.
Mucosal immunoglobulins.
Immunol Rev. 2005 Aug;206:64-82. doi: 10.1111/j.0105-2896.2005.00290.x.
https://pubmed.ncbi.nlm.nih.gov/16048542/

[138] Ong DSY, Fragkou PC, Schweitzer VA, et al.
How to interpret and use COVID-19 serology and immunology tests.
Clin Microbiol Infect. 2021;27(7):981-986. doi:10.1016/j.cmi.2021.05.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8106522/

Kevadiya BD, Machhi J, Herskovitz J, et al.
Diagnostics for SARS-CoV-2 infections.
Nat Mater. 2021;20(5):593-605. doi:10.1038/s41563-020-00906-z

The quality and usefulness of a serological test is assessed primarily by its degree of sensitivity and specificity.[139] *Sensitivity* describes the ability of a serologic test to provide a positive result from samples that contain antibodies to SARS-CoV-2 ("true positives"). Therefore, a highly sensitive test has a very low frequency of false negatives.

Specificity, on the other hand, describes the ability of a test to provide a negative result when a sample contains no antibodies to SARS-CoV-2. In this case, a high-specificity SARS-CoV-2 serological test should result in few false positives, including those resulting from cross-reactivity to any of the other six human coronaviruses.[140]

Serological tests that analyze IgM, which naturally have a lower affinity for viral antigen than IgG, will have a higher risk of producing false positives and therefore should require a higher specificity threshold.

Test thresholds for specificity and sensitivity are arbitrary values established experimentally, and they differ among serologic test types and testing methods.

Thresholds for SARS-CoV-2 are determined primarily on the basis of test results from negative control samples collected before the pandemic, as well as positive control samples that have been confirmed by a certified (i.e., validated against sequencing) RT-PCR clinical diagnostic test.[141] Currently, there are no international reference standards for defining the sensitivity and specificity of serological testing, which makes it very difficult to compare different serological tests and assays without conducting a direct experimental comparison.

Recent studies have attempted to compare multiple test kits[142] but, while this represents progress, what ultimately emerged was the need to prepare a well-characterized set of standardized sera that

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8264308/

[139] Brouwer PJM, Caniels TG, van der Straten K, et al.
Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability.
Science. 2020;369(6504):643-650. doi:10.1126/science.abc5902
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7299281/

[140] FDA. EUA Authorized Serology Test Performance. FDA (2020). Available at: https://www.fda.gov/medical-devices/emergency-situations-medical- devices/eua-authorized-serology-test-performance.

Cheng MP, Yansouni CP, Basta NE, et al.
Serodiagnostics for Severe Acute Respiratory Syndrome-Related Coronavirus 2 : A Narrative Review.
Ann Intern Med. 2020;173(6):450-460. doi:10.7326/M20-2854
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7281623/

[141] GISAID. Genomic epidemiology of novel coronavirus - Available at: https://nextstrain.org/ncov/gisaid/global/6m

[142] Haselmann V, Kittel M, Gerhards C, et al.
Comparison of test performance of commercial anti-SARS-CoV-2 immunoassays in serum and plasma samples.
Clin Chim Acta. 2020;510:73-78. doi:10.1016/j.cca.2020.07.007
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7343640/

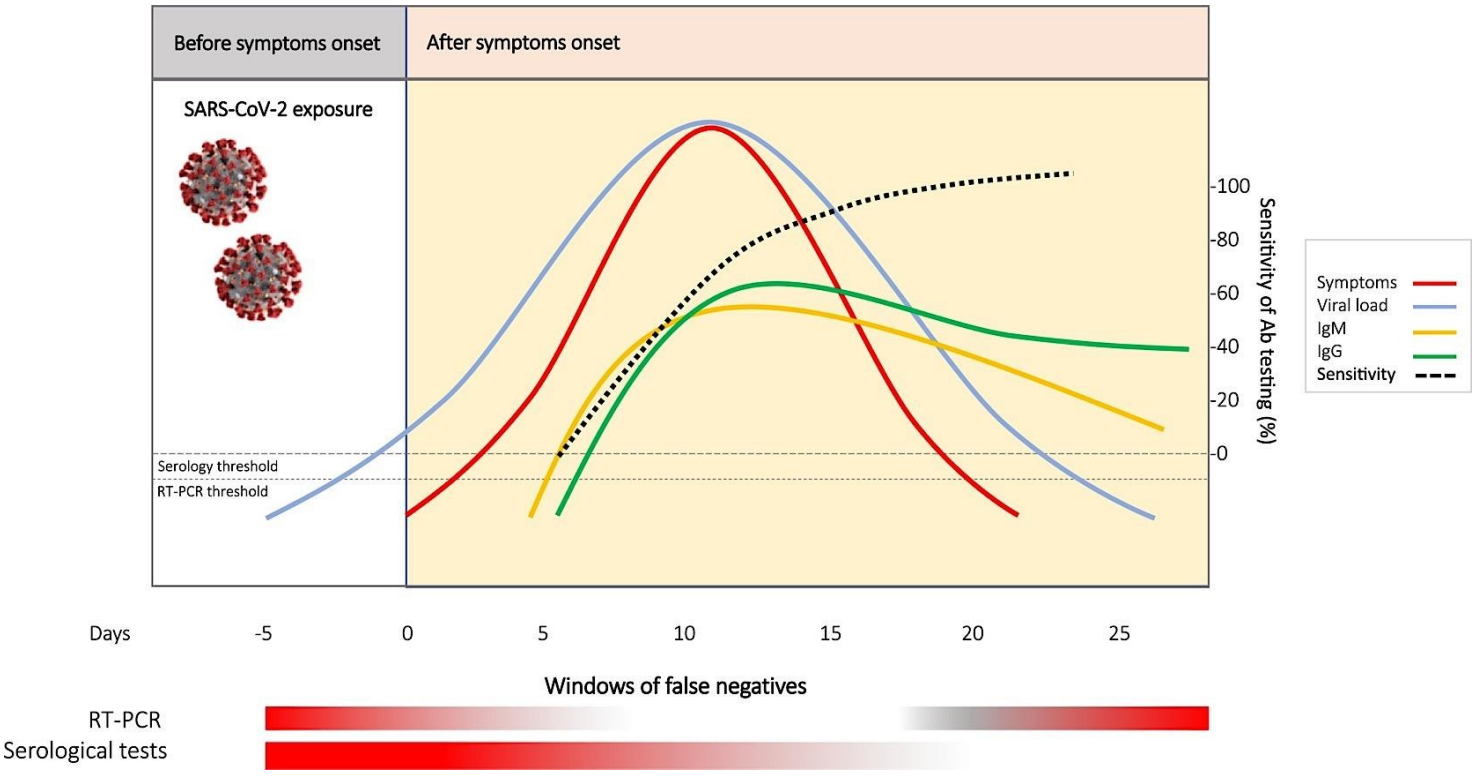
Theel ES.
Performance Characteristics of High-Throughput Serologic Assays for Severe Acute Respiratory Syndrome Coronavirus 2 with Food and Drug Administration Emergency Use Authorization: A Review.
Clin Lab Med. 2022;42(1):15-29. doi:10.1016/j.cll.2021.10.006
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8563341/

Wilkins D, Aksyuk AA, Ruzin A, et al.
Validation and performance of a multiplex serology assay to quantify antibody responses following SARS-CoV-2 infection or vaccination.
Clin Transl Immunology. 2022;11(4):e1385. Published 2022 Apr 26. doi:10.1002/cti2.1385
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9040421/

Choi HW, Jeon CH, Won EJ, Kang SJ, Lee SY, Kee SJ.
Performance of Severe Acute Respiratory Syndrome Coronavirus 2 Serological Diagnostic Tests and Antibody Kinetics in Coronavirus Disease 2019 Patients.
Front Microbiol. 2022;13:881038. Published 2022 Apr 14. doi:10.3389/fmicb.2022.881038
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9048255/

could be tested against any approved serological test kit, so that the sensitivity and specificity of these kits could be compared.[143]

Other variables that require standardization for serologic testing and comparison of kits include the duration of PSO (post-symptom onset) for sample collection from patients, as the sensitivity and specificity of commercial tests can vary depending on when the sample is collected[144] and the method by which samples are sometimes inactivated for laboratory safety.[145]



[143] Adams ER, Ainsworth M, Anand R, et al.
Antibody testing for COVID-19: A report from the National COVID Scientific Advisory Panel.
Wellcome Open Res. 2020;5:139. Published 2020 Jun 11. doi:10.12688/wellcomeopenres.15927.1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7941096/

[144] Ortiz AT, Torrente FF, Twigg A, et al.
The Influence of Time on the Sensitivity of SARS-CoV-2 Serological Testing.
Preprint. Res Sq. 2022;rs.3.rs-1286644. Published 2022 Feb 17. doi:10.21203/rs.3.rs-1286644/v1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8863153/

Muecksch F, Wise H, Batchelor B, et al.
Longitudinal analysis of clinical serology assay performance and neutralising antibody levels in COVID19 convalescents.
Preprint. medRxiv. 2020;2020.08.05.20169128. Published 2020 Aug 6. doi:10.1101/2020.08.05.20169128.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7418752/

La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM.
Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays.
Reprod Biomed Online. 2020;41(3):483-499. doi:10.1016/j.rbmo.2020.06.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7293848/

[145] Cheng MP, Yansouni CP, Basta NE, et al.
Serodiagnostics for Severe Acute Respiratory Syndrome-Related Coronavirus 2 : A Narrative Review.
Ann Intern Med. 2020;173(6):450-460. doi:10.7326/M20-2854
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7281623/

Hu X, An T, Situ B, et al.
Heat inactivation of serum interferes with the immunoanalysis of antibodies to SARS-CoV-2.
J Clin Lab Anal. 2020;34(9):e23411. doi:10.1002/jcla.23411
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7361150/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7293848/
The temporal relationship between viral load, symptoms, and diagnostic test positivity. The onset of symptoms (day 0) is usually 5 days after infection (day -5). At this early stage corresponding to the window or asymptomatic period, the viral load may be below the RT- PCR threshold and the test may give false-negative results. The same is true at the end of the disease when the patient is recovering. Seroconversion may generally be detectable between 5-7 days and 14 days after the onset of symptoms; therefore, in the early stage of the disease, serologic tests are more likely to give false-negative results. The dashed black line in the graph illustrates the sensitivity of the chemiluminescent test as derived from the data sheet of a commercial test (Abbott Diagnostics, USA). Ig, immunoglobulin; RT-PCR, reverse transcription-PCR; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Sensitivity and specificity thresholds are also important for epidemiological considerations unique to certain situations and environments.
These thresholds can be modified to allow greater sensitivity of the test at the expense of specificity, or the reverse, whereby specificity is favored at the expense of sensitivity.
For example, in a region with a high seroprevalence of SARS-CoV-2, sensitivity may be prioritized over specificity to ensure that the majority of positive cases are identified.
Conversely, if the prevalence *[146] in a given region is very low, then higher specificity and relatively lower sensitivity would be favored so that fewer patients may give false-positive results while still detecting most true positives.
It is essential to have the correct balance between sensitivity and specificity, as the epidemiological implications of disproportionate false negatives or false positives can be profound. A test with too many false positives will keep people isolated longer than necessary, creating otherwise avoidable social and economic tensions.
A test with too many false negatives will result in an underestimation of the prevalence of the disease, which can lead to premature relaxation of disease surveillance and waves of resurgent infections as patients misidentified as false negatives unknowingly continue to transmit the disease. [147]

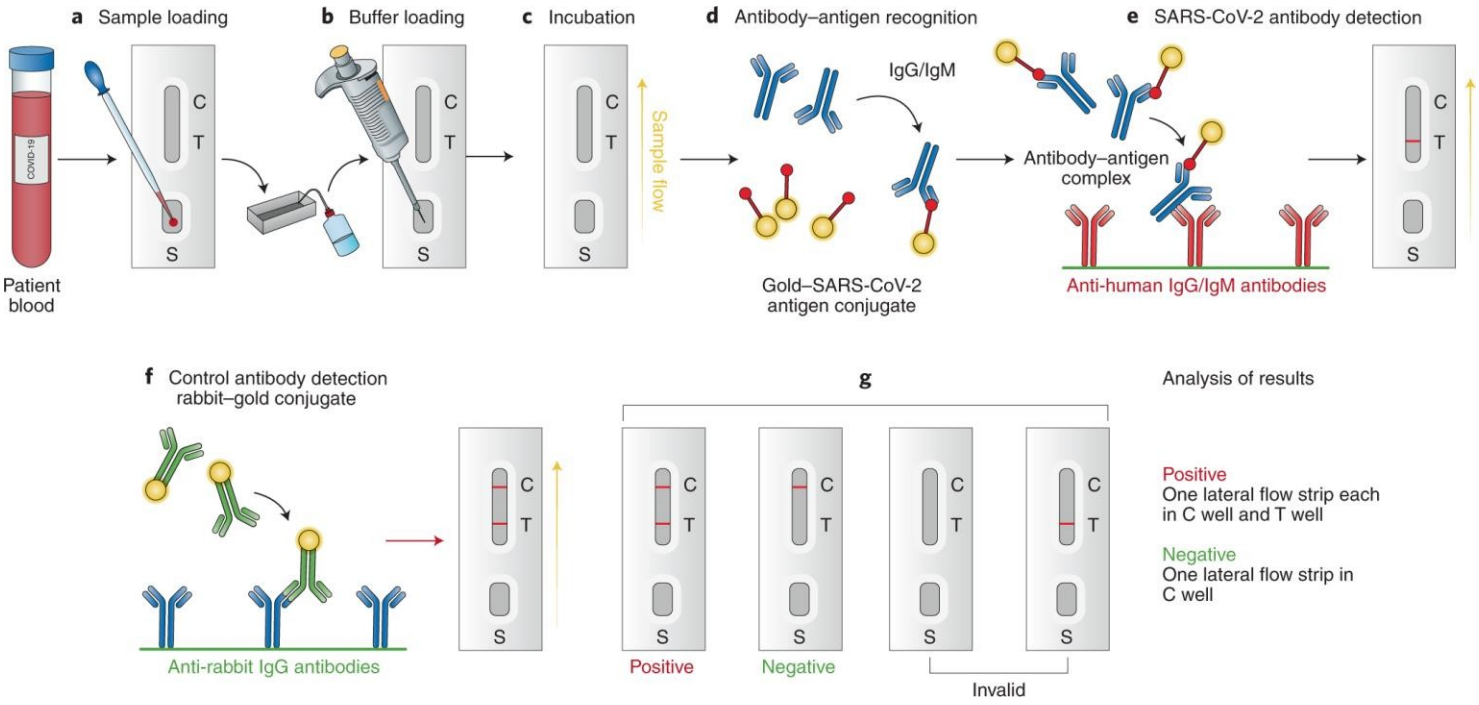
* *Prevalence takes into account existing cases, while incidence refers to new cases.*

https://www.nature.com/articles/s41563-020-00906-z/
Commonly used immunoassays contain recombinant antigens specific for SARS-CoV-2 immobilized on nitrocellulose membranes. Anti-human mouse IgM and IgG antibodies conjugated with colored latex beads are immobilized on conjugated swabs. The test sample contacts the membrane inside the assay. The colored antibodies form conjugated latex complexes with human antiviral antibodies. This complex immobilized on the membrane is captured by the recombinant antigen specific for SARS-CoV-2. If SARS-CoV-2-specific IgG/IgM is present in the sample, this leads to a colored band, indicating a positive test result. The complex is captured on the membrane by the goat anti-mouse antibody, forming a red control line. An embedded control line is displayed in the test window. The absence of a colored band demonstrates a negative result. a-e, The workflow begins with patient serum added to the sample flow well (S) (a), saline buffer is added drop by drop (b), and the sample incubated (c) until antibody-antigen recognition (d) and SARS- CoV-2 antibody detection (e). f, Rabbit-gold antibody shows in the control well (C). g, A positive test band (T) indicates the presence of COVID-19 antibody, and results without a positive C band are invalid. Notably, this test describes a post-immune response and can show negative results for individuals who have been recently infected. It can also detect the virus in previously infected but asymptomatic individuals.

[146] https://www.quadernodiepidemiologia.it/epi/freq/inc_pre.htm

https://toolbox.eupati.eu/resources/concetti-epidemiologici-incidenza-e-prevalenza/?lang=it

[147] Bryant JE, et al
Serology for SARS-CoV-2: Apprehensions, opportunities, and the path forward.
Sci Immunol. 2020 May 19;5(47):eabc6347. doi: 10.1126/sciimmunol.abc6347.
https://www.science.org/doi/10.1126/sciimmunol.abc6347



Serological testing and origin of false positives

In addition to false positives due to cross-reactivity with human coronavirus and pre-existing antibodies[148] , serologic testing can also lead to false positives due to the presence of cross-reactive autoimmune antibodies.[149] Diagnostic concerns arise from the fact that, due to polyclonal hypergamma-globulinemia, patients with immune-mediated diseases and particularly those with autoimmune conditions may produce false-positive results for SARS-CoV2-IgG and IgM.

The virus-specific antibody detection test for SARS-CoV-IgG and IgM has already shown false-positive results in patients with the following autoimmune diseases: systemic lupus erythematosus, Sjogren's syndrome, systemic sclerosis, mixed connective tissue disease, and rheumatoid arthritis.[150]

Specifically, samples from rheumatoid arthritis patients with high levels of rheumatoid factor (RF) isotype IgM and IgG were found to produce a false-positive signal in several assays.

Because RF binds to the constant parts of IgG, this could precipitate other antibodies present in the immunoassay in a nonspecific manner. These nonspecific positive signals could not only provide false indications of protective immunity to SARS-CoV-2 for an individual with RF, but could also provide an incorrect picture of the proportion of the population exposed to infection during broader screenings, especially if the diagnoses or RF status of the population from which the samples were collected is unknown.

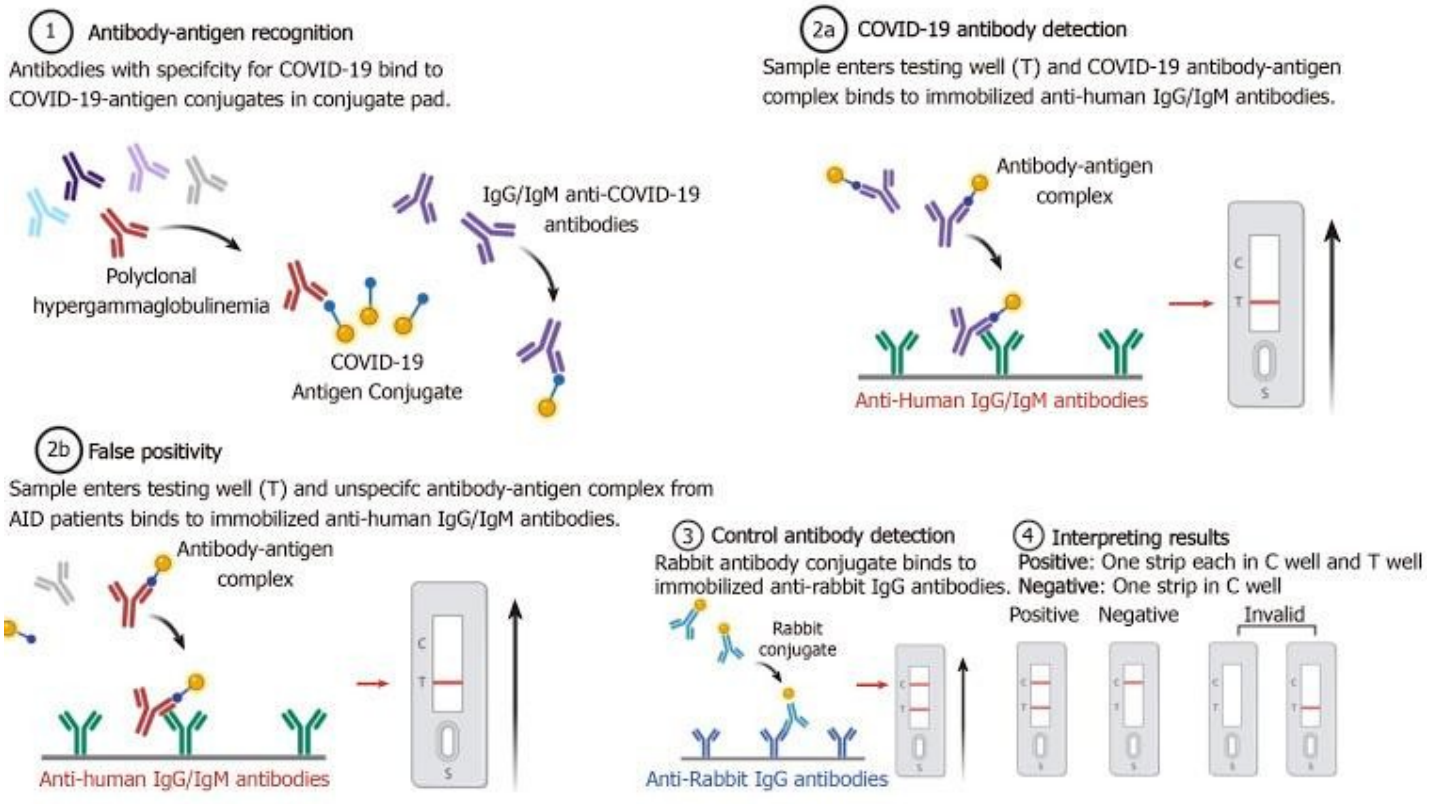
[148] Latiano A, Tavano F, Panza A, et al.
False-positive results of SARS-CoV-2 IgM/IgG antibody tests in sera stored before the 2020 pandemic in Italy.
Int J Infect Dis. 2021;104:159-163. doi:10.1016/j.ijid.2020.12.067
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7834192/

[149] Georgiev T, Angelov AK.
Complexities of diagnosis and management of COVID-19 in autoimmune diseases: Potential benefits and detriments of immunosuppression.
World J Clin Cases. 2020;8(17):3669-3678. doi:10.12998/wjcc.v8.i17.3669
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7479565/

[150] Wang Y, Sun S, Shen H, Jiang L, Zhang M, Xiao D, Liu Y, Ma X, Zhang Y, Guo N, Jia T. Cross-reaction of SARS-CoV antigen with autoantibodies in autoimmune diseases.
Cell Mol Immunol. 2004 Aug;1(4):304-7. PMID: 16225774.
http://www.cmi.ustc.edu.cn/1/4/304.pdf

Kharlamova et al[151] found that most false positive signals were detected in IgM assays, as noted by others[152] , consistent with the low affinity of IgM antibodies, compared with IgG class switching and the higher affinity of mature antibodies.

The serum of patients with SLE (systemic lupus erythematosus) has a high abundance of autoantibodies, including RF, ANA, and antibodies against dsDNA[153] , and although SLE is a less prevalent disease than RA (rheumatoid arthritis), serum samples from these patients contributed significantly to the false-positive signals in the study.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7479565/
Viral antibody detection test and hypothetical case of false-positive cross-reactivity in patients with autoimmune diseases and polyclonal related hypergammaglobulinemia

[151] Kharlamova N, et al
False Positive Results in SARS-CoV-2 Serological Tests for Samples From Patients With Chronic Inflammatory Diseases.
Front Immunol. 2021 May 3;12:666114. doi: 10.3389/fimmu.2021.666114.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8126683/

[152] Whitman JD, Hiatt J, Mowery CT, et al.
Evaluation of SARS-CoV-2 serology assays reveals a range of assay performance.
Nat Biotechnol. 2020;38(10):1174-1183. doi:10.1038/s41587-020-0659-0
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7740072/

Latiano A, Tavano F, Panza A, et al.
False-positive results of SARS-CoV-2 IgM/IgG antibody tests in sera stored before the 2020 pandemic in Italy.
Int J Infect Dis. 2021;104:159-163. doi:10.1016/j.ijid.2020.12.067
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7834192/

[153] Dema B, Charles N.
Autoantibodies in SLE: Specificities, Isotypes and Receptors.
Antibodies (Basel). 2016;5(1):2. Published 2016 Jan 4. doi:10.3390/antib5010002
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6698872/

For seroprevalence studies, the specificity of the test is critical,[154] and this is particularly concerning in sub-Saharan Africa, where the prevalence of COVID-19 is low compared with other continents[155] and the endemicity of *P. falciparum* malaria is high.[156]

In such a context, from a review published by Vanroye et al[157] , most of the Rapid Diagnostic Tests (RDTs) products evaluated would result in a high number of false-positive results, probably higher than the number of true positives, due to cross-reactivity with *P. falciparium*.

Of note, the more geographically widespread *P. vivax* was also associated with cross-reactivity, whereas the WHO guidelines mentioned testing for cross-reactivity only for *P. falciparum* and *P. ovale*
[158].

A similar impact can be expected in Schistosomiasis and Dengue endemic areas. [159]

Although not an objective of the study (and not systematically evaluated), the authors[119] observed deficiencies in product instructions for use (without mention of antigen detection for 11 out of 13 products), manufacturing (bacterial contamination of the swab vial, assembly errors and package labeling) and performance (weak control and test lines).

These shortcomings have also been noted before and may be caused by high customer demand and insufficient regulatory oversight, which, however, can seriously affect the usability of products in the market. [160]

---

[154] Mulchandani R, Jones HE, Taylor-Phillips S, et al.
Accuracy of UK Rapid Test Consortium (UK-RTC) "AbC-19 Rapid Test" for detection of previous SARS-CoV-2 infection in key workers: test accuracy study.
BMJ. 2020;371:m4262. Published 2020 Nov 11. doi:10.1136/bmj.m4262
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7656121/.

European Centre for Disease Prevention and Control. Considerations for the use of antibody tests for SARS-CoV-2 - first update. 10 February 2022. Stockholm: ECDC; 2022.
https://www.ecdc.europa.eu/sites/default/files/documents/Considerations-for-the-use-of-antibody-tests-for-SARS-CoV2-first-update.pdf

[155] World Health Organization WHO Coronavirus (COVID-19) Dashboard.

[156] Gatton ML, Ciketic S, Barnwell JW, et al.
An assessment of false positive rates for malaria rapid diagnostic tests caused by non-Plasmodium infectious agents and immunological factors.
PLoS One. 2018;13(5):e0197395. Published 2018 May 14. doi:10.1371/journal.pone.0197395
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5951549/

World malaria report 2019

[157] Vanroye F, Bossche DVD, Brosius I, Tack B, Esbroeck MV, Jacobs J.
COVID-19 Antibody Detecting Rapid Diagnostic Tests Show High Cross-Reactivity When Challenged with Pre-Pandemic Malaria, Schistosomiasis and Dengue Samples.
Diagnostics (Basel). 2021 Jun 25;11(7):1163. doi: 10.3390/diagnostics11071163.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8305106/

[158] Coronavirus disease (COVID-19) Pandemic - Emergency Use Listing Procedure (EUL) open for IVDs

COVID-19 Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0

[159] Yek C, Nam VS, Leang R, et al.
The Pandemic Experience in Southeast Asia: Interface Between SARS-CoV-2, Malaria, and Dengue.
Front Trop Dis. 2021;2:788590. doi:10.3389/fitd.2021.788590
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8975143/

[160] West R, Kobokovich A, Connell N, Gronvall GK.
COVID-19 Antibody Tests: A Valuable Public Health Tool with Limited Relevance to Individuals.
Trends Microbiol. 2021;29(3):214-223. doi:10.1016/j.tim.2020.11.002
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836413/

Morshed M, Sekirov I, McLennan M, et al.
Comparative Analysis of Capillary vs Venous Blood for Serologic Detection of SARS-CoV-2 Antibodies by RPOC Lateral Flow Tests.
Open Forum Infect Dis. 2021;8(3):ofab043. Published 2021 Jan 28. doi:10.1093/ofid/ofab043
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7928643/

Pallett SJC, Jones R, Pallett MA, et al.
Characterising differential antibody response is integral to future SARS-CoV-2 serostudies.
J Infect. 2020;81(6):e28-e30. doi:10.1016/j.jinf.2020.07.029

In addition, interference with autoantibodies, heterotropic antibodies, and alloantibodies (antibodies from allogeneic tissue) can lead to false positives in pregnant women, patients with autoimmune diseases, transplants, and blood transfusions. [161]

To further understand the endogenous interference factors of ELISA on the detection of serum SARS-CoV- 2 IgM and IgG, the test was used in sera from 200 patients without COVID-19 infection, including the rheumatoid factor (RF) positive group, antinuclear antibody (ANA) positive group, the pregnant women's group and normal senior, with 50 samples in each group and 100 normal controls.[162]

The level of SARS-CoV-2 IgG in pregnant women was significantly higher than that in the normal control group ($p = 0.000$), but there was no significant difference between the other groups.

SARS-CoV-2 IgM levels in the group of pregnant women, normal elderly, ANA positive and RF positive were significantly higher than those in the normal control group ($p < 0.05$), with significantly higher false-positive rates in these groups ($p = 0.036$, $p = 0.004$, $p = 0.000$, compared with the normal control group). Serum RF caused a false positive for SARS-CoV-2 IgM in a concentration-dependent manner, especially when its concentration was above 110.25 IU/L.

Antibody responses in COVID-19 patients

IgG subclasses modulate immune responses through the engagement of different FcRs,[163] and ectopic expression of FcγRIIa and FcγRIIb, but not FcγRI or FcγRIIIa, has been shown to be able to induce the ADE of SARS-CoV infection[164] .

Allelic polymorphisms in FcγRIIa are associated with SARS pathology, and individuals with an isoform of FcγRIIa that binds to both IgG1 and IgG2 develop more severe disease than individuals with FcγRIIa that binds only to IgG2.[165]

In addition, recent studies on antibody responses in patients with COVID-19 have associated higher titers of anti-N IgM and IgG at all time points after symptom onset with a worse disease outcome [166].

---

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7392850/

[161] Rifkin SB, Owens LE, Greenwald JL.
Factors associated with false-positive results from fingerstick OraQuick ADVANCE rapid HIV 1/2 antibody test.
J Int Assoc Physicians AIDS Care (Chic). 2012 Nov-Dec;11(6):356-60. doi: 10.1177/1545109712454194
https://journals.sagepub.com/doi/10.1177/1545109712454194

Reported Causes of False-positive EIA and Immunoblot Tests for HIV Antibody

[162] Liu W, Long X, Wan K, et al.
The endogenous factors affecting the detection of serum SARS-CoV-2 IgG/IgM antibodies by ELISA.
J Med Virol. 2022;94(5):1976-1982. doi:10.1002/jmv.27557
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9015225/

[163] Galipeau Y, Greig M, Liu G, Driedger M, Langlois MA.
Humoral Responses and Serological Assays in SARS-CoV-2 Infections.
Front Immunol. 2020;11:610688. Published 2020 Dec 18. doi:10.3389/fimmu.2020.610688
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7775512/

[164] Jaume M, Yip MS, Cheung CY, et al.
Anti-severe acute respiratory syndrome coronavirus spike antibodies trigger infection of human immune cells via a pH- and cysteine protease-independent FcγR pathway.
J Virol. 2011;85(20):10582-10597. doi:10.1128/JVI.00671-11
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187504/

[165] Yuan FF, Tanner J, Chan PK, et al.
Influence of FcgammaRIIA and MBL polymorphisms on severe acute respiratory syndrome.
Tissue Antigens. 2005;66(4):291-296. doi:10.1111/j.1399-0039.2005.00476.x
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190181/

[166] Wenting Tan, et al.
Viral Kinetics and Antibody Responses in Patients with COVID-19
medRxiv 2020.03.24.20042382; doi: https://doi.org/10.1101/2020.03.24.20042382
https://www.medrxiv.org/content/10.1101/2020.03.24.20042382v1.full.pdf

In particular, higher titers of anti-S and anti-N IgG and IgM correlate with more severe clinical pictures and advanced age[167] , suggesting potentially harmful effects of antibodies in some patients.
However, 70% of patients who recovered from mild COVID-19 had measurable neutralizing antibodies that persisted even after the follow-up visit[168] . Therefore, the knowledge that can be gained from studying the characteristics of antibodies related to recovery rather than worsening of the disease is indispensable for identifying the type of antibodies to be evaluated for ADE risk during the disease and following vaccination.[169]
From studies conducted with SARS-Cov-1 vaccines, it was seen that vaccines expressing the N protein did not provide protection, and on the contrary increased infection-induced pneumonia with increased lung infiltration of eosinophils and altered $_{TH}$ 2 cell responses,[170] with induction of ERD.

https://www.frontiersin.org/articles/10.3389/fimmu.2020.610688/full
Overview of antibody isotype characteristics and an approximate time sequence from SARS-CoV-2 infection to possible immunity. Each antibody isotype is represented with its typical form and associated heavy chain. A brief description of their major function is also included, as well as a representation of the upregulated and downregulated cytokines required for each class change. The approximate time sequence of the appearance and subsequent decrease of each isotype in relation to viral RNA is shown. The curves and values are based on recent serologic studies discussed in this review. Because limited literature is available on the involvement of IgE in pathogenesis and antibody-mediated immunity to SARS-CoV-2, as such the representation of IgE time sequence is purely hypothetical.

[167] Jiang, Hw., Li, Y., Zhang, Hn. et al.
SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses.
Nat Commun 11, 3581 (2020). https://doi.org/10.1038/s41467-020-17488-8
https://www.nature.com/articles/s41467-020-17488-8

Fan Wu, et al
Antibody-dependent enhancement (ADE) of SARS-CoV-2 infection in recovered COVID-19 patients: studies based on cellular and structural biology analysis
medRxiv 2020.10.08.20209114; doi: https://doi.org/10.1101/2020.10.08.20209114
https://www.medrxiv.org/content/10.1101/2020.10.08.20209114v1.full.pdf

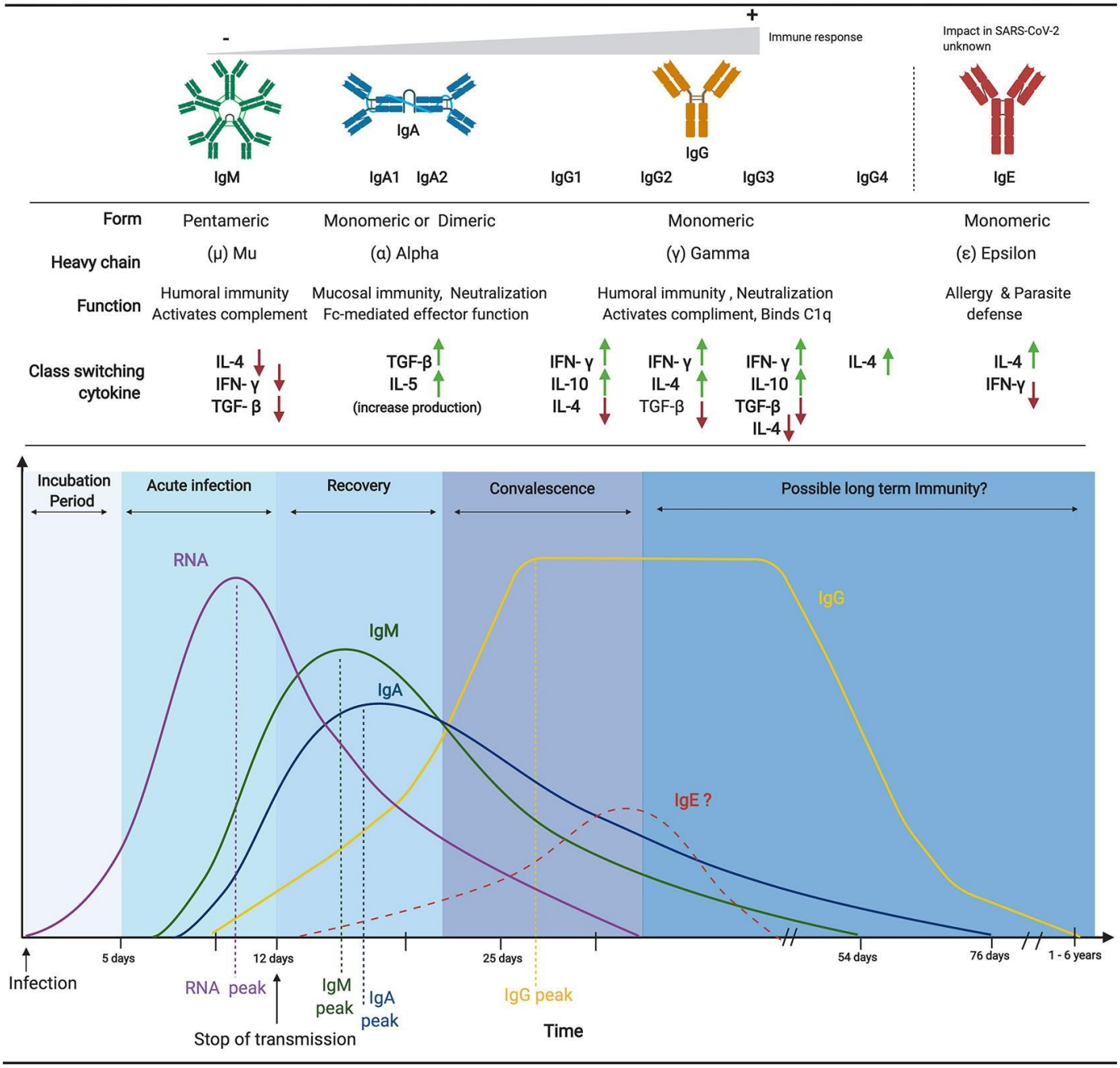
[168] Fan Wu, et al
Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications medRxiv 2020.03.30.20047365; doi:https://doi.org/10.1101/2020.03.30.20047365
https://www.medrxiv.org/content/10.1101/2020.03.30.20047365v2.full.pdf

[169] Zheng J, Deng Y, Zhao Z, et al.
Characterization of SARS-CoV-2-specific humoral immunity and its potential applications and therapeutic prospects.
Cell Mol Immunol. 2022;19(2):150-157. doi:10.1038/s41423-021-00774-w
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8513558/

[170] Deming D, et al
Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants.
PLoS Med. 2006 Dec;3(12):e525. doi: 10.1371/journal.pmed.0030525. Erratum in: PLoS Med. 2007 Feb;4(2):e80.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1716185/.

Yasui F, et al
Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV.
J Immunol. 2008 Nov 1;181(9):6337-48. doi: 10.4049/jimmunol.181.9.6337.
https://www.jimmunol.org/content/181/9/6337.long

Antibody overview and timeline in Sars-CoV-2 infection



Cross-reactivity and heterologous immunity

The cross-reactivity of endemic coronavirus antibodies against SARS-CoV-2 is of potential clinical relevance. The original antigenic sin hypothesis suggests that preexisting immunity results in the reactivation of a response to a previous strain, as opposed to the formation of a direct response against the current strain, and this may reduce the formation of neutralizing antibodies, thus dampening the effective elimination of the new virus.

Pioneering studies on influenza responses, based on epidemiology, modeling, and antibody repertoire profiling, suggest that antibodies generated by childhood exposure to influenza are "programmed" and exert an important effect on the nature of the antibody response elicited by the

subsequent exposures in humans[171] . It has therefore been suggested that this phenomenon may also exist in SARS-CoV-2 infection. [172]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8546681/
The impact of OAS on the effectiveness of the immune response. OAS is influenced not only by the temporality of an antigenic exposure but also by the "collection" of antigens associated with the exposure. **(A)** Exposure to hCoV (related viruses such as HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-HKU1) prepares the immune system for blue, purple and green antigens. This will result in long-lived memory B cells with specificity for these antigens, respectively. Reexposure to hCoV will result in a robust antibody response to each of these antigens. SARS-CoV-2 expresses the same

---

[171] Stamper CT, Wilson PC.
What Are the Primary Limitations in B-Cell Affinity Maturation, and How Much Affinity Maturation Can We Drive with Vaccination? Is Affinity Maturation a Self-Defeating Process for Eliciting Broad Protection?.
Cold Spring Harb Perspect Biol. 2018;10(5):a028803. Published 2018 May 1. doi:10.1101/cshperspect.a028803
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5899041/

Anderson EM, Goodwin EC, Verma A, et al.
Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection.
Cell. 2021;184(7):1858-1864.e10. doi:10.1016/j.cell.2021.02.010
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7871851/

Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M.
Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period.
Science. 2020;368(6493):860-868. doi:10.1126/science.abb5793
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164482/

Gostic KM, Ambrose M, Worobey M, Lloyd-Smith JO.
Potent protection against H5N1 and H7N9 influenza via childhood hemagglutinin imprinting.
Science. 2016;354(6313):722-726. doi:10.1126/science.aag1322
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5134739/

Yewdell JW, Santos JJS.
Original Antigenic Sin: How Original? How Sinful?
Cold Spring Harb Perspect Med. 2021 May 3;11(5):a038786. doi: 10.1101/cshperspect.a038786.
http://perspectivesinmedicine.cshlp.org/content/11/5/a038786.full

[172] Brown EL, Essigmann HT.
Original Antigenic Sin: the Downside of Immunological Memory and Implications for COVID-19.
mSphere. 2021;6(2):e00056-21. Published 2021 Mar 10. doi:10.1128/mSphere.00056-21
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8546681/

Roncati L, Palmieri B.
What about the original antigenic sin of the humans versus SARS-CoV-2?
Med Hypotheses. 2020;142:109824. doi:10.1016/j.mehy.2020.109824
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7204740/

Reina J.
Possible effect of the "original antigenic sin" in vaccination against new variants of SARS-CoV-2.
Rev Clin Esp (Barc). 2022;222(2):91-92. doi:10.1016/j.rceng.2021.05.005
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8437764/

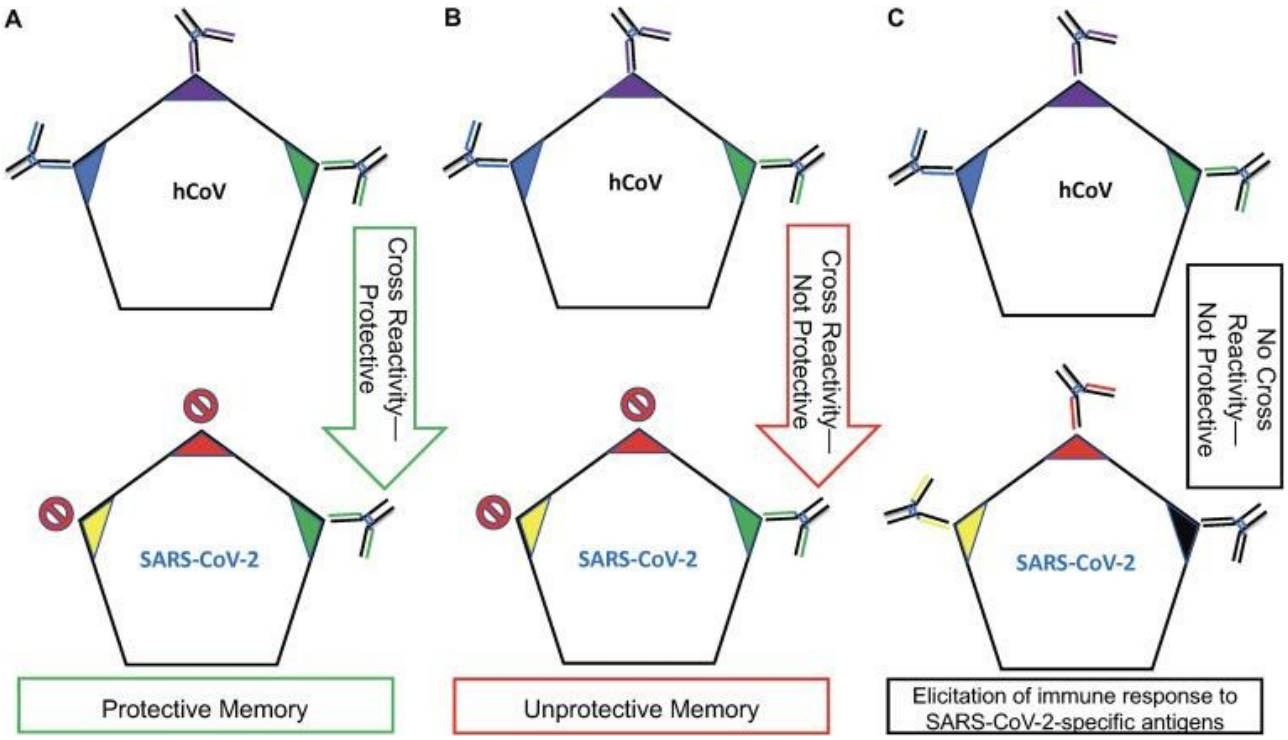
Noori M, Nejadghaderi SA, Rezaei N.
"Original antigenic sin: A potential threat beyond the development of booster vaccination against novel SARS-CoV-2 variants [published online ahead of print, 2021 May 3].
Infect Control Hosp Epidemiol. 2021;1-2. doi:10.1017/ice.2021.199
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8144803/

Fierz W, Walz B.
Antibody Dependent Enhancement Due to Original Antigenic Sin and the Development of SARS.
Front Immunol. 2020;11:1120. Published 2020 Jun 5. doi:10.3389/fimmu.2020.01120
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291596/

Kohler H, Nara P.
A Novel Hypothesis for Original Antigenic Sin in the Severe Disease of SARS-CoV-2 Infection.
Monoclon Antib Immunodiagn Immunother. 2020 Aug;39(4):107-111. doi: 10.1089/mab.2020.0029.
https://pubmed.ncbi.nlm.nih.gov/32762609/

Petráš M, Králová Lesná I.
SARS-CoV-2 vaccination in the context of original antigenic sin.
Hum Vaccin Immunother. 2022;18(1):1949953. doi:10.1080/21645515.2021.1949953
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8290366/

hCoV Green antigen and two new antigens, yellow and red. The anti-green B-cell memory response will prevent or significantly limit the ability of naive B lymphocytes with specificity to yellow and red antigens to develop. In this scenario, the anti-green antibody is protective; therefore, while OAS prevented/diminished the elicitation of antibodies with specificity to yellow and red antigens, the anti-green antigen memory response confers some level of protection against a SARS-CoV-2 infection. **(B)** This scenario is identical to that described for panel A with the exception that the anti-green response elicited against hCoV is not neutralizing for SARS-CoV-2. In this example, the memory response to green is not protective while at the same time inhibiting/interfering with the ability to create a new response to yellow and red antigens that could potentially provide protection. **(C)** This scenario describes two separate exposures. Since no antigen is shared between hCoV and SARS-CoV- 2, the anti-SARS-CoV-2 response will be a primary exposure, not positively or negatively affected by previous exposure to hCoV.



Although cross-reactive antibodies are not neutralizing, however, neutralization is not the only mechanism by which antibodies confer protection.

In fact, cross-reactive antibodies could interact with receptors for the Fc region of antibodies present on the surface of innate immune cells and promote activities of protective effector function, including antibody-dependent cell phagocytosis and antibody-dependent cellular cytotoxicity[173] .

To this end, a subset of monoclonal antibodies isolated from SARS-CoV patients, cross-reactive with SARS-CoV-2 but not neutralizing, was studied and found to confer protection in a mouse model[174] .

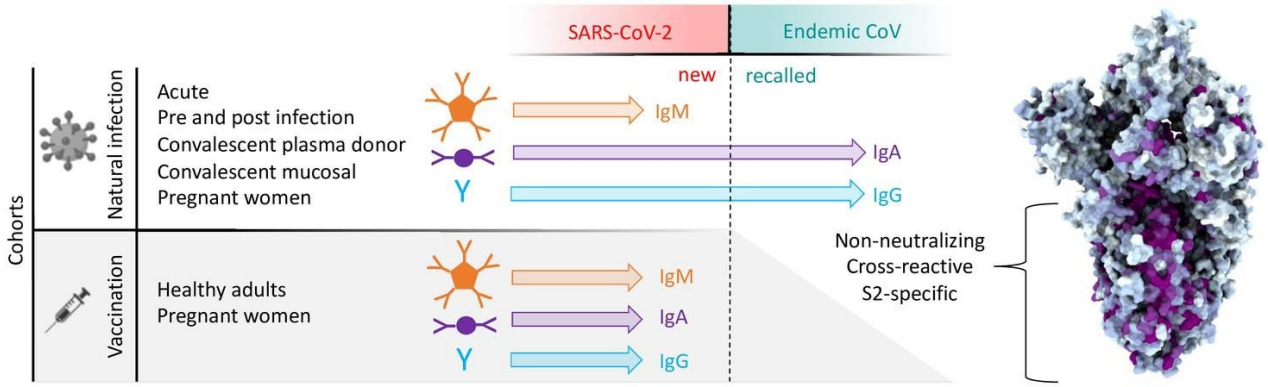
In addition, antibodies attacking human coronavirus OC43 were robustly reactivated in response to SARS-CoV-2 infection but not to stabilized spike vaccination, so it is believed that the

[173] Fox JM, Roy V, Gunn BM, et al.
Optimal therapeutic activity of monoclonal antibodies against chikungunya virus requires Fc-FcγR interaction on monocytes.
Sci Immunol. 2019;4(32):eaav5062. doi:10.1126/sciimmunol.aav5062
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6698136/

Earnest JT, Basore K, Roy V, et al.
Neutralizing antibodies against Mayaro virus require Fc effector functions for protective activity.
J Exp Med. 2019;216(10):2282-2301. doi:10.1084/jem.20190736
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6781005/

[174] Shiakolas AR, Kramer KJ, Wrapp D, et al.
Cross-reactive coronavirus antibodies with different epitope specificities and Fc effector functions.
Cell Rep Med. 2021;2(6):100313. doi:10.1016/j.xcrm.2021.100313
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8139315/

S2 subdomain of the spike protein (i.e., non-overlapping RBD epitopes) is probably responsible for the IgG response-dominated activation. [175]
Further discussion on the difference between the natural and vaccine immune response will be available in subsequent papers.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8562549/
Antibody responses to SARS-CoV-2 and endemic CoV spike proteins were measured in different cohorts. While antibodies to SARS-CoV-2 were induced in all isotypes, only IgA and IgG responses to endemic CoV were robustly enhanced and only among naturally infected but unvaccinated individuals. These recalled and cross-reactive responses to endemic CoV primarily recognized the better conserved S2 domain and were not neutralizing. Although no other antiviral activities of broadly cross-reactive S2-specific antibodies are known, the differing antigenicity of natural infection and vaccination with stabilized pre-fusion spike has potential implications for the extent and level of protection afforded by each.

Neutralization test

Multiple serological tests (hemagglutination inhibition test, complement fixation test, fluorescent antibody test, etc.) are used to assess virus-antibody interactions. [176] However, only a few tests, such as the PRNT Plaque Reduction Neutralization Test (PRNT), measure virus neutralization during the viral attachment process and entry into host cells.[177]

---

[175] Crowley AR, Natarajan H, Hederman AP, et al.
Boosting of Cross-Reactive Antibodies to Endemic Coronaviruses by SARS-CoV-2 Infection but not Vaccination with Stabilized Spike.
Preprint. medRxiv. 2021;2021.10.27.21265574. Published 2021 Oct 28. doi:10.1101/2021.10.27.21265574.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8562549/

Li D, et al
In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies.
Cell. 2021 Aug 5;184(16):4203-4219.e32. doi: 10.1016/j.cell.2021.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232969/

Zhou Y, et al
Enhancement versus neutralization by SARS-CoV-2 antibodies from a convalescent donor, associates with distinct epitopes on the RBD.
Cell Rep. 2021 Feb 2;34(5):108699. doi: 10.1016/j.celrep.2021.108699.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7802522/

[176] Morales-Núñez JJ, Muñoz-Valle JF, Torres-Hernández PC, Hernández-Bello J.
Overview of Neutralizing Antibodies and Their Potential in COVID-19.
Vaccines (Basel). 2021 Nov 23;9(12):1376. doi: 10.3390/vaccines9121376.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8706198/

[177] Mendoza EJ, Manguiat K, Wood H, Drebot M.
Two Detailed Plaque Assay Protocols for the Quantification of Infectious SARS-CoV-2.
Curr Protoc Microbiol. 2020 Jun;57(1):ecpmc105. doi: 10.1002/cpmc.105.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7300432/

Roehrig JT, Hombach J, Barrett AD.
Guidelines for Plaque-Reduction Neutralization Testing of Human Antibodies to Dengue Viruses.
Viral Immunol. 2008 Jun;21(2):123-32. doi: 10.1089/vim.2008.0007.
https://pubmed.ncbi.nlm.nih.gov/18476771/

PRNT allows measurement of the effects of antibodies on viral infectivity by plate inoculating the virus with susceptible cells, as shown in Figure A; therefore, it is considered the gold standard for evaluating the neutralizing ability of antibodies against SARS-CoV-2.
Cells are cultured in semisolid media that limit the spread of virus; each virus that initiates infection produces a localized area of infection, known as a plaque, that can be visualized and counted. After plaque counting, the percentage reduction in total virus infectivity can be determined.[178]
This test has a significant disadvantage in that it is usually performed with live (infectious) viruses, and it is necessary to work in a Biosafety Level 3 (BSL-3) laboratory with very qualified and experienced people.[179]

Laboratories: biosafety levels, description, pathogens processed

Classification of pathogens into risk groups Classification of infectious agents in relation to biosafety Biohazard

Classification Art. 268 - Legislative Decree No. 81/2008

a) Group 1 biological agent: an agent that is unlikely to cause disease in human subjects;

b) Group 2 biological agent: an agent that can cause disease in human subjects and pose a risk to workers; it is unlikely to spread in the community; effective prophylactic or therapeutic measures are usually available;

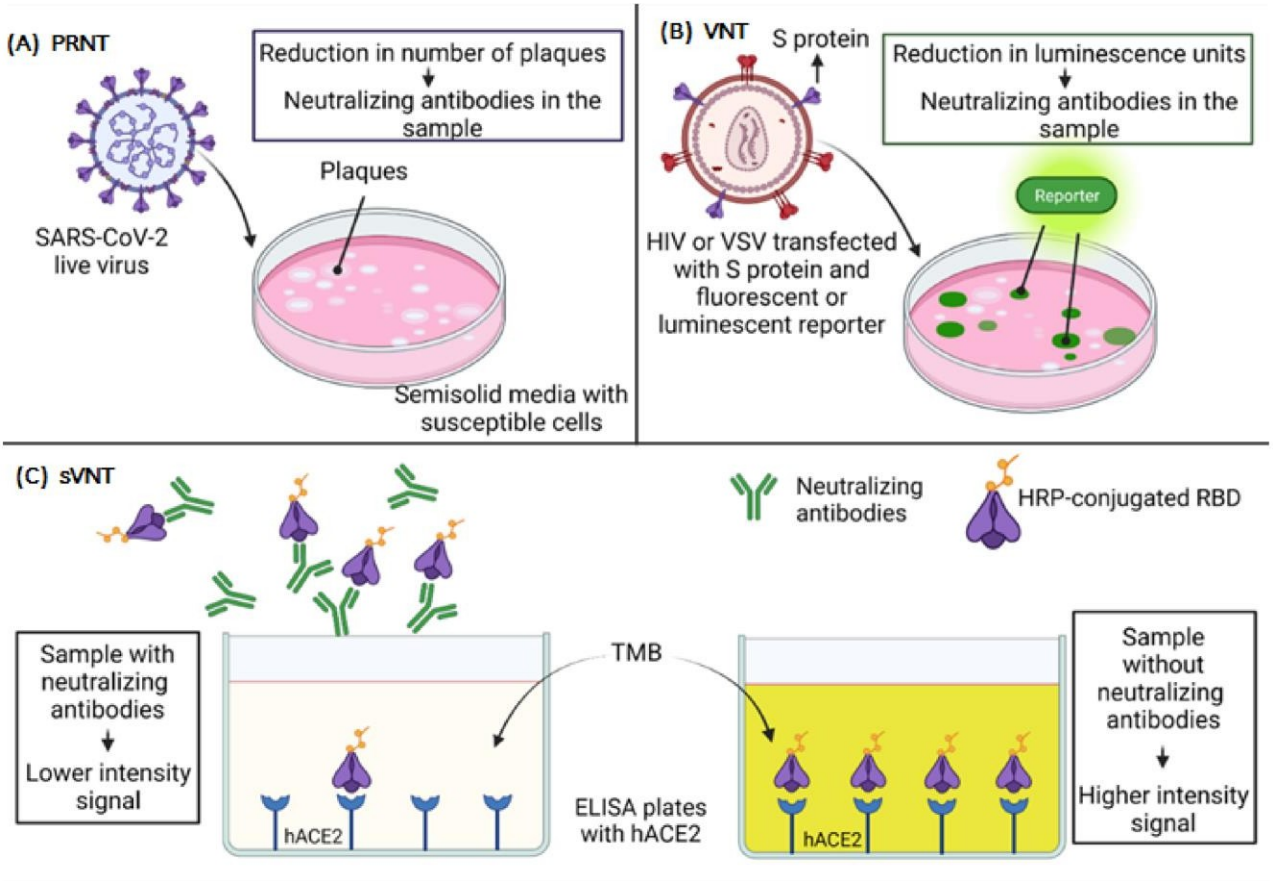
c) Group 3 biological agent: an agent that can cause serious illness in human subjects and poses a serious risk to workers; the biological agent can spread in the community, but effective prophylactic or therapeutic measures are usually available;

d) Group 4 biological agent: a biological agent that can cause serious disease in human subjects and poses a serious risk to workers and may present a high risk of propagation in the community; no effective prophylactic or therapeutic measures are usually available.

[178] Abe KT, et al
A simple protein-based surrogate neutralization assay for SARS-CoV-2.
JCI Insight. 2020 Oct 2;5(19):e142362. doi: 10.1172/jci.insight.142362.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7566699/

[179] Perera RAet at the
Serological assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), March 2020.
Euro Surveill. 2020 Apr;25(16):2000421. doi: 10.2807/1560-7917.ES.2020.25.16.2000421.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7189648/

Okba NMA, et al
Severe Acute Respiratory Syndrome Coronavirus 2-Specific Antibody Responses in Coronavirus Disease Patients.
Emerg Infect Dis. 2020 Jul;26(7):1478-1488. doi: 10.3201/eid2607.200841.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323511/



https://www.mdpi.com/2076-393X/9/12/1376/htm
Neutralization assays. **(A)** In a PRNT assay, cells susceptible to infection are cultured in semisolid media and infected with live SARS-CoV-2 virus. The semisolid media allow the infection to be localized and visualized as plaques. The plaques are counted and a reduction in the number of plaques means that neutralizing antibodies are present in the sample. **(B)** VNT is similar to a PRNT, but instead of a live virus, an HIV-like virus or VSV is transfected with the S protein of SARS-CoV-2 and a luminescent reporter. The luminescent units are sites of infection, and a reduction in luminescent units means that neutralizing antibodies are present. **(C)** The sVNT uses an immobilized hACE2 in ELISA plates and an HRP-conjugated RBD; if neutralizing antibodies are present in the sample, the HRP-conjugated RBD will not bind to hACE2 and there will be no signal or a low intensity signal. PRNT, plaque-reducing neutralization test; VNT, virus neutralization test; sVNT, surrogate virus neutralization test; HIV, human immunodeficiency virus; VSV, vesicular stomatitis virus; hACE2, enzyme of
human angiotensin 2 conversion; HRP, horseradish peroxidase; RBD, receptor binding domain; TMB, tetramethylbenzidine.

Virus neutralization tests (VNT - Fig. B) can also be performed with viral vectors pseudotyped with the S protein of SARS-CoV-2,[180] as we will elaborate later.
This technique does not require a BSL-3 laboratory; however, it does require specialized laboratories and is a very complicated and time-consuming procedure,[181]

[180] Crawford KHD, et al
Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays.
Viruses. 2020 May 6;12(5):513. doi: 10.3390/v12050513.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291041/

Ferrara F, Temperton N.
Pseudotype Neutralization Assays: From Laboratory Bench to Data Analysis.
Methods Protoc. 2018 Jan 22;1(1):8. doi: 10.3390/mps1010008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6526431/

Septisetyani EP, Prasetyaningrum PW, Anam K, Santoso A.
SARS-CoV-2 Antibody Neutralization Assay Platforms Based on Epitopes Sources: Live Virus, Pseudovirus, and Recombinant S Glycoprotein RBD.
Immune Netw. 2021 Nov 23;21(6):e39. doi: 10.4110/in.2021.21.e39.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8733193/

[181] Crawford KHD, et al
Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays.
Viruses. 2020 May 6;12(5):513. doi: 10.3390/v12050513.

Because the SARS-CoV-2 S protein is required for the virus to enter a cell, it is possible to transfect specific cells capable of producing and expressing pseudotypic lentiviral particles for use in infecting susceptible cells expressing the ACE2 receptor.[182]
Such pseudotyping can be achieved with lentiviral particles derived from human immunodeficiency virus (HIV)[183] and vesicular stomatitis virus (VSV)[184] , which allow the measurement of spike-mediated entry into cells with fluorescent reporters or luciferase, used to assess the neutralizing ability of human antibodies.
Compared with live virus assays (live virus assay), pseudovirus-based neutralization assays (PBNAs) are less labor-intensive because data are obtained through luminescent reading.
In contrast, live virus assays require manual reading of results under a microscope[185] and neutralization

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291041/

Ferrara F, Temperton N.
Pseudotype Neutralization Assays: From Laboratory Bench to Data Analysis.
Methods Protoc. 2018 Jan 22;1(1):8. doi: 10.3390/mps1010008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6526431/

[182] Wang S, et al
Antibody-dependent enhancement (ADE) of SARS-CoV-2 pseudoviral infection requires FcγRIIB and virus-antibody complex with bivalent interaction.
Commun Biol. 2022 Mar 24;5(1):262. doi: 10.1038/s42003-022-03207-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8948278/

Chmielewska AM, Czarnota A, Bieńkowska-Szewczyk K, Grzyb K.
Immune response against SARS-CoV-2 variants: the role of neutralization assays.
NPJ Vaccines. 2021 Nov 29;6(1):142. doi: 10.1038/s41541-021-00404-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8630184/

Fan Wu, et al
Antibody-dependent enhancement (ADE) of SARS-CoV-2 infection in recovered COVID-19 patients: studies based on cellular and structural biology analysis
medRxiv 2020.10.08.20209114; doi:https://doi.org/10.1101/2020.10.08.20209114
https://www.medrxiv.org/content/10.1101/2020.10.08.20209114v1.full

Renn A, Fu Y, Hu X, Hall MD, Simeonov A.
Fruitful Neutralizing Antibody Pipeline Brings Hope To Defeat SARS-Cov-2.
Trends Pharmacol Sci. 2020 Nov;41(11):815-829. doi: 10.1016/j.tips.2020.07.004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7572790/

[183] Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z.
Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV.
Nat Commun. 2020 Mar 27;11(1):1620. doi: 10.1038/s41467-020-15562-9. Erratum in: Nat Commun. 2021 Apr 1;12(1):2144.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7100515/

Chen X, et al
Human monoclonal antibodies block the binding of SARS-CoV-2 spike protein to angiotensin converting enzyme 2 receptor.
Cell Mol Immunol. 2020 Jun;17(6):647-649. doi: 10.1038/s41423-020-0426-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7167496/

[184] Xiong HL, et al
Robust neutralization assay based on SARS-CoV-2 S-protein-bearing vesicular stomatitis virus (VSV) pseudovirus and ACE2-overexpressing BHK21 cells.
Emerg Microbes Infect. 2020 Dec;9(1):2105-2113. doi: 10.1080/22221751.2020.1815589.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7534347/

Hoffmann M, Ket al
SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.
Cell. 2020 Apr 16;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102627/

[185] Nie J, Li Q, Wu J, Zhao C, Hao H, Liu H, Zhang L, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Fan C, Huang W, Xu M, Wang Y.
Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2.
Emerg Microbes Infect. 2020 Dec;9(1):680-686. doi: 10.1080/22221751.2020.1743767.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7144318/

is measured as luminescence reduction in relative light units (RLU).[186]

Another approach is to evaluate neutralization by replication of recombinant VSVs (rVSVs).[187] Compared with pseudotypes, recombinant VSVs encoding for the S protein are easier to produce. The rVSVs have been previously used with other lethal viruses and with SARS-CoV and MERS-CoV.
In this case, the native glycoprotein gene in the VSV genome is replaced with the gene encoding for protein S. The VSV genome is also modified to express a green fluorescent protein (GFP) that acts as a reporter, enabling assessment of infection. [188]

An additional test for neutralization is the Immuno-Cov™ developed by Vandergaast et al. [189], in which a VSV is modified to express the S protein, but a "dual split protein" luciferase (DSP) system is used to quantify virus neutralization.
The DSP system uses a chimeric split green fluorescent protein (GFP) and a split Renilla luciferase (RL)[190] . Fusion between two cell lines expressing complementary parts of the reporter system allows measurement of virus-induced cell fusion on 96-well plate.

Septisetyani EP, Prasetyaningrum PW, Anam K, Santoso A.
SARS-CoV-2 Antibody Neutralization Assay Platforms Based on Epitopes Sources: Live Virus, Pseudovirus, and Recombinant S Glycoprotein RBD.
Immune Netw. 2021 Nov 23;21(6):e39. doi: 10.4110/in.2021.21.e39.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8733193/

[186] Chen Q, Nie J, Huang W, Jiao Y, Li L, Zhang T, Zhao J, Wu H, Wang Y.
Development and optimization of a sensitive pseudovirus-based assay for HIV-1 neutralizing antibodies detection using A3R5 cells.
Hum Vaccin Immunother. 2018 Jan 2;14(1):199-208. doi: 10.1080/21645515.2017.1373922.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5791569/

[187] Case JB, et al
Neutralizing Antibody and Soluble ACE2 Inhibition of a Replication-Competent VSV-SARS-CoV-2 and a Clinical Isolate of SARS-CoV-2.
Cell Host Microbe. 2020 Sep 9;28(3):475-485.e5. doi: 10.1016/j.chom.2020.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332453/

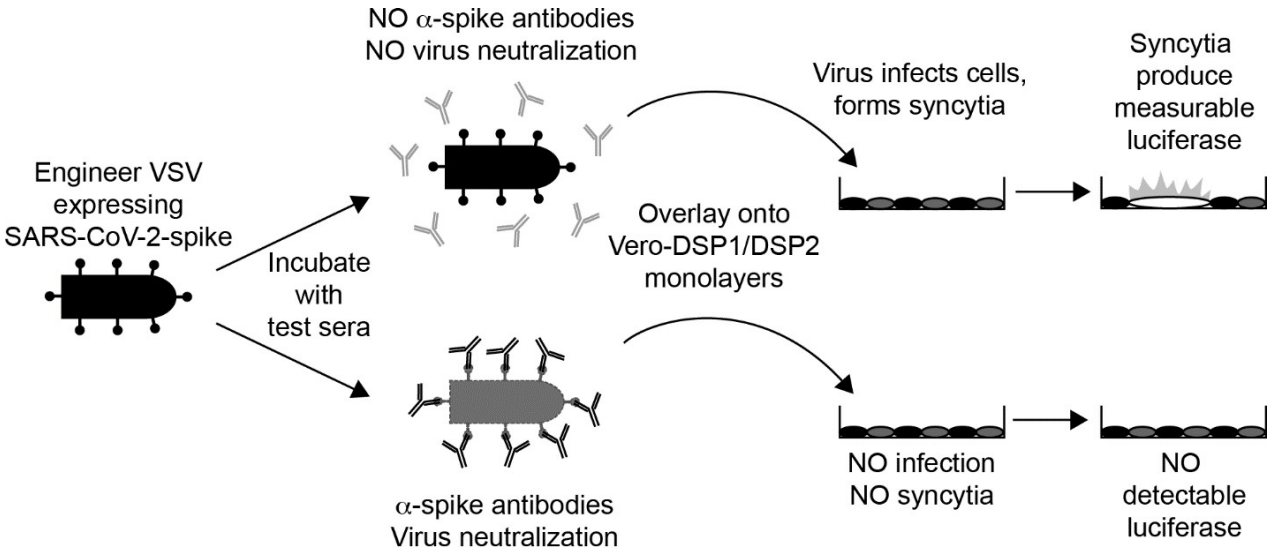
Dieterle ME, et al
A Replication-Competent Vesicular Stomatitis Virus for Studies of SARS-CoV-2 Spike-Mediated Cell Entry and Its Inhibition.
Cell Host Microbe. 2020 Sep 9;28(3):486-496.e6. doi: 10.1016/j.chom.2020.06.020.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332447/

Li H, Zhao C, Zhang Y, Yuan F, Zhang Q, Shi X, Zhang L, Qin C, Zheng A.
Establishment of replication-competent vesicular stomatitis virus-based recombinant viruses suitable for SARS-CoV-2 entry and neutralization assays.
Emerg Microbes Infect. 2020 Dec;9(1):2269-2277. doi: 10.1080/22221751.2020.1830715.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7594855/

[188] Case JB, et al
Neutralizing Antibody and Soluble ACE2 Inhibition of a Replication-Competent VSV-SARS-CoV-2 and a Clinical Isolate of SARS-CoV-2.
Cell Host Microbe. 2020 Sep 9;28(3):475-485.e5. doi: 10.1016/j.chom.2020.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332453/
[189] Vandergaast R, et al
Development and validation of IMMUNO-COV™: a high-throughput clinical assay for detecting antibodies that neutralize SARS-CoV-2.
bioRxiv [Preprint]. 2020 May 27:2020.05.26.117549. doi: 10.1101/2020.05.26.117549.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7302210/

Vandergaast R, et al
IMMUNO-COV v2.0: Development and Validation of a High-Throughput Clinical Assay for Measuring SARS-CoV-2-Neutralizing Antibody Titers.
mSphere. 2021 Jun 30;6(3):e0017021. doi: 10.1128/mSphere.00170-21
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8265629/

[190] Nakane S, Matsuda Z.
Dual Split Protein (DSP) Assay to Monitor Cell-Cell Membrane Fusion.
Methods Mol Biol. 2015;1313:229-36. doi: 10.1007/978-1-4939-2703-6_17
https://pubmed.ncbi.nlm.nih.gov/25947669/



https://www.biorxiv.org/content/10.1101/2020.05.26.117549v1.full.pdf
Overview of the IMMUNO-COVTM test. A SARS-CoV-2 spike expressing VSV (VSV-SARSCoV-2-S- 19CT) is incubated with test sera samples. In the absence of neutralizing SARS-CoV-2 antibodies **(top)**, the virus maintains infectivity and infects Vero-DSP1/DSP2 monolayers. If the test sample contains SARS-CoV-2 neutralizing antibodies **(bottom)**, the antibodies bind to the spike protein causing neutralization of the virus by blocking cell entry. VSV-SARS-CoV-2-S- Δ 1 9 C T induces syncytium formation in VeroDSP1/DSP2 monolayers, which reconstitutes a fully functional luciferase reporter that is used to quantify virus-induced syncytium formation. An elevated luciferase signal means that the test sample has not neutralized the virus, while reduced luciferase indicates the presence of SARS- CoV-2 neutralizing antibodies in the test sample.

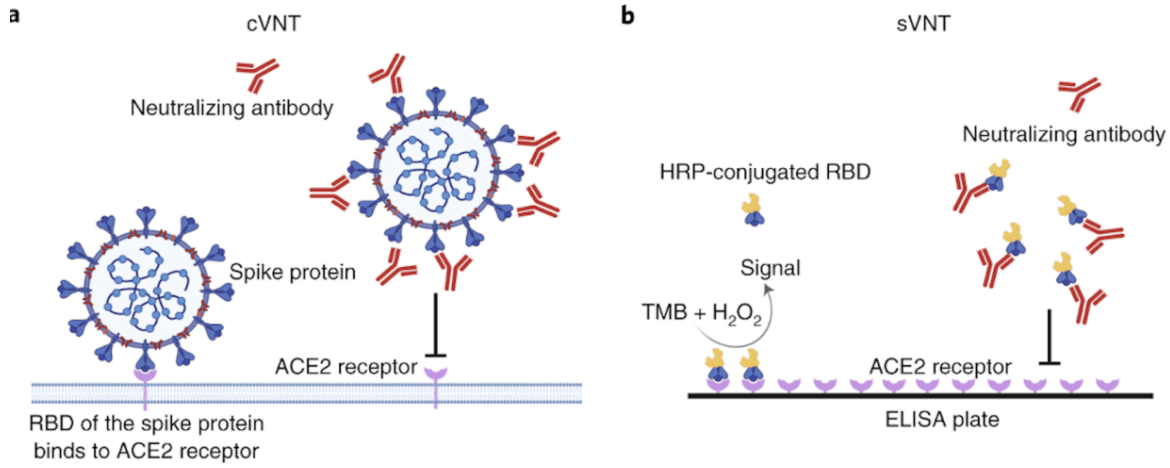
Finally, a novel surrogate virus neutralization test (sVNT) was developed based on the principle of enzyme-linked immunosorbent assay (ELISA) blocking that mimics virus-cell interaction to detect the presence of NAbs (neutralizing antibodies) in a sample.
In this case, human ACE2 protein (hACE2) is immobilized in the plate, and horseradish peroxidase (HRP)-conjugated RBD is used for detection.
In case there is a high presence of NAbs, there will be a lower signal intensity. In contrast, if there are no NAbs, HRP-conjugated RBD binds to hACE2 and the signal will be higher[191] .
This test is the first of its kind to be approved by the FDA for diagnostic use and could be a strategy within the reach of most clinical laboratories.

[191] Meyer B, Reimerink J, Torriani G, Brouwer F, Godeke GJ, Yerly S, Hoogerwerf M, Vuilleumier N, Kaiser L, Eckerle I, Reusken C.
Validation and clinical evaluation of a SARS-CoV-2 surrogate virus neutralisation test (sVNT).
Emerg Microbes Infect. 2020 Dec;9(1):2394-2403. doi: 10.1080/22221751.2020.1835448
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7605318/

Tan CW, Chia WN, Qin X, Liu P, Chen MI, Tiu C, Hu Z, Chen VC, Young BE, Sia WR, Tan YJ, Foo R, Yi Y, Lye DC, Anderson DE, Wang LF.
A SARS-CoV-2 surrogate virus neutralization assay based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol. 2020 Sep;38(9):1073-1078. doi: 10.1038/s41587-020-0631-z.
https://pubmed.ncbi.nlm.nih.gov/32704169/ https://clpmag.com/uncategorized/study-new-covid-19-serological-test-fast-effective/

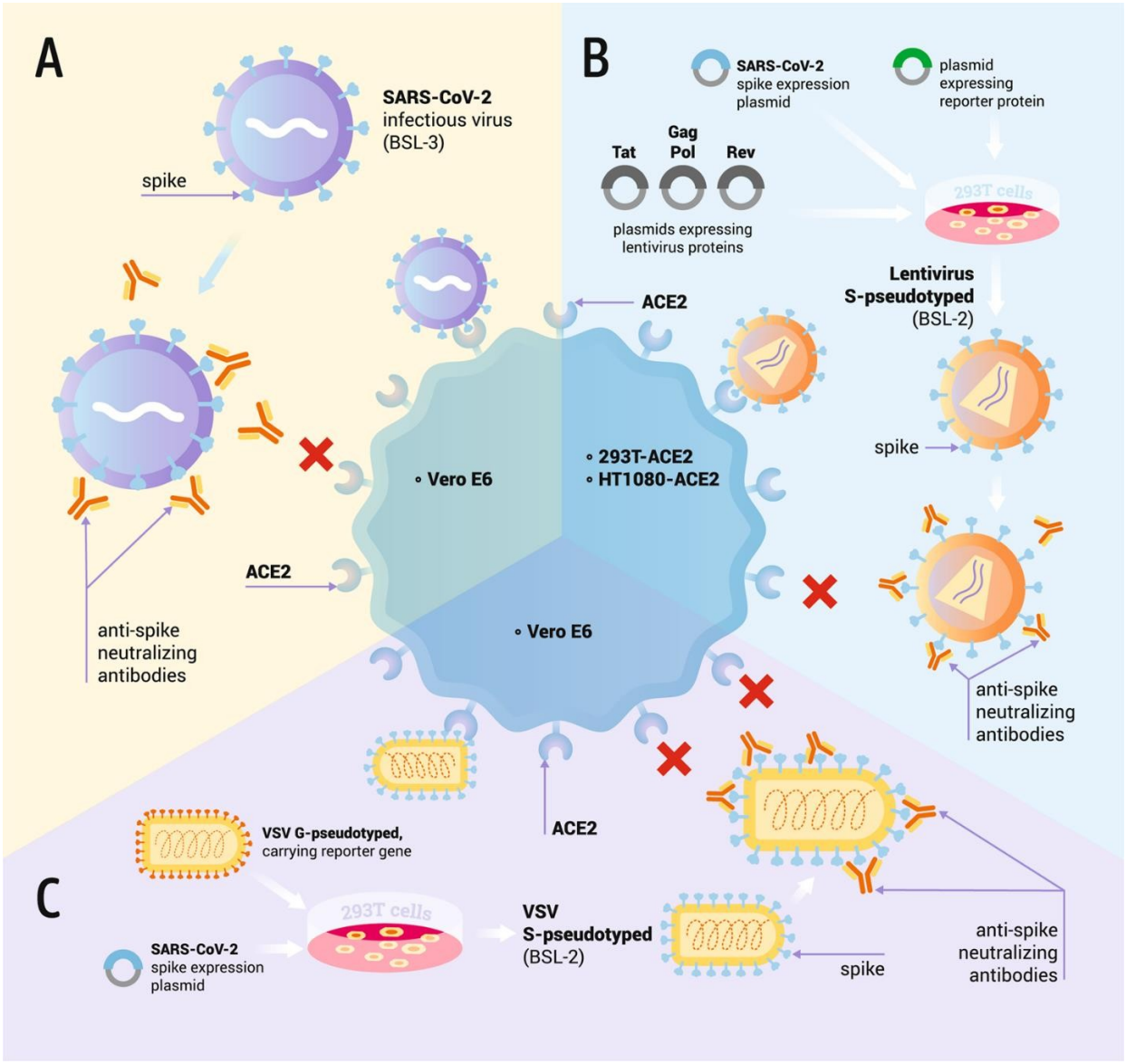
Sholukh AM, et al
Evaluation of Cell-Based and Surrogate SARS-CoV-2 Neutralization Assays.
J Clin Microbiol. 2021 Sep 20;59(10):e0052721. doi: 10.1128/JCM.00527-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8451402/

Oguntuyo KY, et al
Quantifying Absolute Neutralization Titers against SARS-CoV-2 by a Standardized Virus Neutralization Assay Allows for Cross-Cohort Comparisons of COVID-19 Sera.
mBio. 2021 Feb 16;12(1):e02492-20. doi: 10.1128/mBio.02492-20.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8545089/



https://pubmed.ncbi.nlm.nih.gov/32704169/

Principle and initial validation of SARS-CoV-2 sVNT. **a,** The mechanism of cVNT. Anti-SARS-CoV-2 neutralizing antibodies prevent the SARS-CoV-2 spike protein from binding to hACE2 receptor proteins on the host cell surface. **b,** In the sVNT assay, anti-SARS-CoV-2 neutralizing antibodies block HRP-conjugated RBD protein from binding to hACE2 protein pre-coated on an ELISA plate



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8630184/

Examples of the approaches used to test antibody-mediated SARS-CoV-2 neutralization.
A) SARS-CoV-2 amplified from cell culture infects cells expressing the ACE2 receptor, e.g., Vero E6. When incubated with sera, neutralizing antibodies bind to the virus surface and block the interaction between the virus spike protein (S) and the ACE2 receptor, inhibiting virus entry. **B)** 293T cells are transfected with plasmids encoding for structural and nonstructural lentiviral proteins and the SARS-CoV-2 spike and reporter protein. The pseudotyped spike lentivirus vector produced enters cells overexpressing ACE2 (e.g., 293-ACE2 or HT1080-ACE2). Spike-ACE2 interaction can be blocked by neutralizing antibodies that bind the SARS-CoV-2 spike. Expression of the reporter protein allows measurement of the rate of infection. **(C)** 293T cells are transfected with a plasmid encoding for the SARS-CoV-2 spike, followed by infection with pseudotyped VSV G carrying a reporter gene at the G protein sequence locus. The cells produce pseudotyped VSV particles that can enter ACE2-expressing cells, e.g., Vero E6. The pseudovirus with the G gene deleted is only able to perform a single cycle of infection. Cell entry can be inhibited by neutralizing SARS-CoV-2 antibodies that bind the spike. Expression of the reporter protein allows measurement of the rate of infection.

For a better understanding of the topic, the following background is provided.

NEUTRALIZATION TESTING AND CONSTRUCTION OF PSEUDOTYPED VIRUSES

A *Pseudovirus Neutralization Assay* (also known as pseudotyped virus neutralization assay or pseudotype-based neutralization assay) is a laboratory method used to study the effect of antibodies or drugs to neutralize the ability of viruses to enter cells and thus prevent infection.
[192]

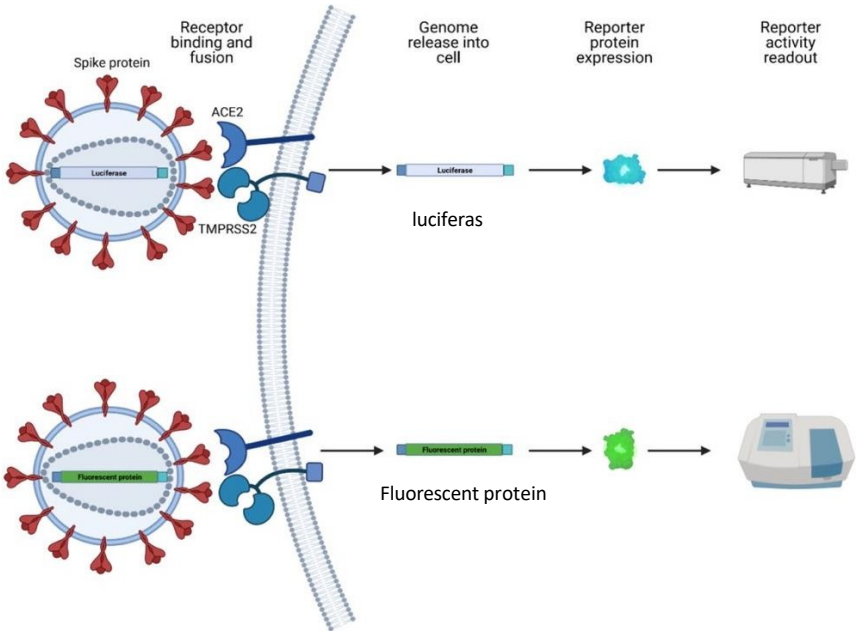
Pseudoviruses or pseudotyped particles are chimeric viruses consisting of a viral core (typically a lentiviral vector) surrounded by a lipid envelope with the surface glycoproteins of another virus (the virus of interest). Pseudoviruses have a conformational structure of surface proteins that closely resembles that of the native virus of interest, and they have the same ability to enter cells using the same mechanisms and receptors as the virus of interest, but they are much safer to handle than the virus from which they originated, in that by using a vector that cannot replicate they are not pathogenic. This allows them to be handled safely in biosafety level (BSL) 2 laboratories, which typically work with agents that pose a moderate risk to human health. This is a great advantage, since pathogenic viruses such as SARS-CoV-2 require BSL-3 laboratories, which are much less common than BSL-2s.
In the case of SARS-CoV-2, the pseudovirus must express S-glycoprotein, which mediates entry into host cells by binding to human angiotensin-converting enzyme 2 (ACE2). In addition to the surface glycoproteins of the virus of interest, the pseudotyped virus contains the gene for a luciferase or fluorescent protein, which is expressed only after entering the cell. The more pseudoviruses enter the cells, the more luciferase or fluorescent protein is expressed and the greater the intensity of light or fluorescence emitted. (fig.1)
The pseudovirus neutralization assay involves incubating the cells and pseudovirus in the presence of different concentrations of an antibody of interest and measuring light emission using a plate luminometer or multimodal reader when luciferase is used, or a fluorometer if a fluorescent protein is used as a marker.
If the antibody is effective in neutralizing surface glycoproteins and blocking entry into cells, a significant reduction in light or fluorescence emission will be measured. The effect of the antibody is visualized using one of the inhibitory concentrations (IC_{50}, IC_{80} or others). (Fig.2) [193]

[192] Pseudovirus neutralization assay in SARS-COV-2 research
https://www.berthold.com/it-it/bioanalitica/soluzioni-ricerca-sars-cov-2-covid-19/pseudovirus-neutralization-assay-nella-ricerca-sulla-sars-cov-2/

Millet JK, Tang T, Nathan L, Jaimes JA, Hsu HL, Daniel S, Whittaker GR.
Production of Pseudotyped Particles to Study Highly Pathogenic Coronaviruses in a Biosafety Level 2 Setting.
J Vis Exp. 2019 Mar 1;(145):10.3791/59010. doi: 10.3791/59010.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6677141/

Condor Capcha JM, Lambert G, Dykxhoorn DM, Salerno AG, Hare JM, Whitt MA, Pahwa S, Jayaweera DT, Shehadeh LA.
Generation of SARS-CoV-2 Spike Pseudotyped Virus for Viral Entry and Neutralization Assays: A 1-Week Protocol.
Front Cardiovasc Med. 2021 Jan 15;7:618651. doi: 10.3389/fcvm.2020.618651.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7843445/

[193] Neerukonda SN, Vassell R, Herrup R, Liu S, Wang T, Takeda K, Yang Y, Lin TL, Wang W, Weiss CD.
Establishment of a well-characterized SARS-CoV-2 lentiviral pseudovirus neutralization assay using 293T cells with stable expression of ACE2 and TMPRSS2.
PLoS One. 2021 Mar 10;16(3):e0248348. doi: 10.1371/journal.pone.0248348.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7946320/



https://www.technologynetworks.com/immunology/articles/leveraging-pseudoviruses-in-the-face-of-the-covid-19-pandemic-347021

Figure 1: Pseudoviruses expressing the SARS-CoV-2 S glycoprotein bind the ACE2 receptor on the host cell surface where the TMPRSS2 protease cleaves S resulting in conformational change and fusion of viral and host cell membranes. Upon fusion, the genome is released into the cell cytoplasm and the genome-encoded reporter is expressed, the activity of which is quantified by means of an instrument.

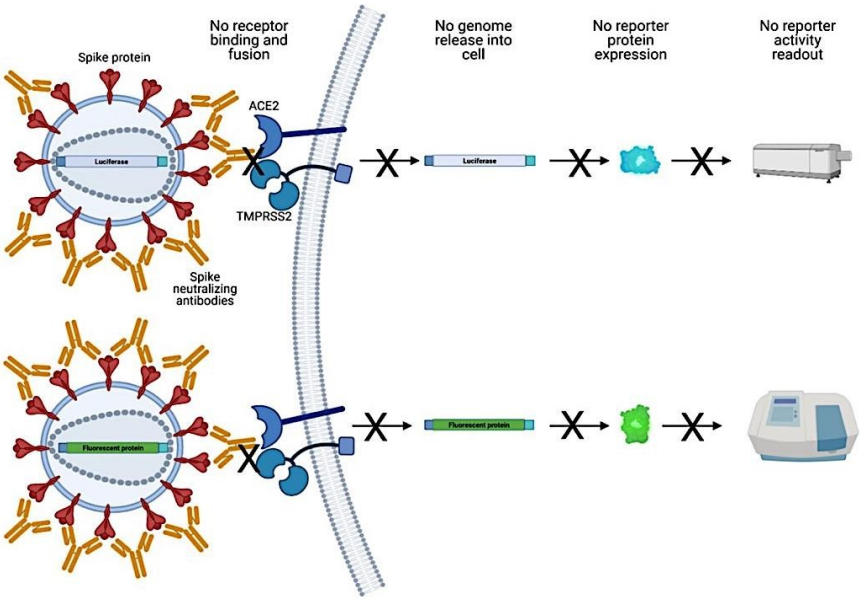


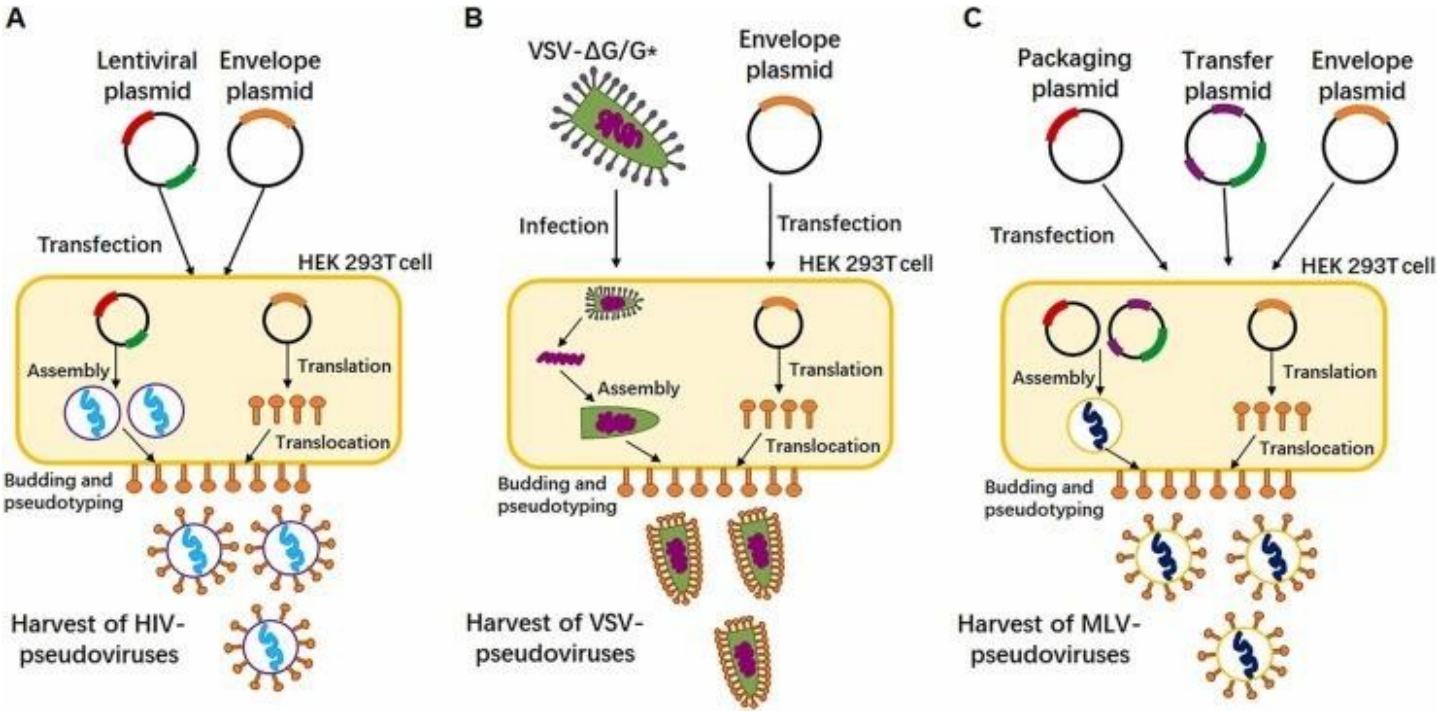
Figure 2: Neutralization assay employing pseudoviruses expressing SARS-CoV-2 S glycoprotein. Neutralizing antibodies that attack the S glycoprotein prevent binding to the ACE2 receptor and, thus, the subsequent steps of fusion, genome release and reporter gene expression.

Nath Neerukonda S, Vassell R, Weiss CD.
Neutralizing Antibodies Targeting the Conserved Stem Region of Influenza Hemagglutinin.
Vaccines (Basel). 2020 Jul 12;8(3):382. doi: 10.3390/vaccines8030382.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7563823/

Millet JK, Whittaker GR.
Murine Leukemia Virus (MLV)-based Coronavirus Spike-pseudotyped Particle Production and Infection.
Bio Protoc. 2016 Dec 5;6(23):e2035. doi: 10.21769/BioProtoc.2035.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5181643/

Wang P, et al
Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7.
Nature. 2021 May;593(7857):130-135. doi: 10.1038/s41586-021-03398-2.
https://pubmed.ncbi.nlm.nih.gov/33684923/

Construction of pseudoviruses



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8848573/

The schematic diagram of the acquisition of different pseudotyped viruses based on different assembly systems. **(A)** HEK 293 T cells were transfected with a plasmid encoding the lentiviral backbone and an envelope protein expressing the plasmid. The transfected cells produced recombined pseudoviruses, and these viral particles could be secreted into the extracellular environment before harvesting. **(B)** HEK 293 T cells were initially transfected with an envelope protein expression plasmid; twenty-four hours after transfection, the cells were infected with VSV* ΔG encoding firefly luciferase or GFP. Pseudotypic particles were collected 20 h a f t e r inoculation. **(C)** HEK 293 T cells were co-transfected with a plasmid encoding for envelope protein, an MLV Gag-Pol packaging plasmid and MLV transfer vector encoding for a luciferase reporter. The transfected cells produced pseudotypic MLV particles like HIV systems. The red bar in the plasmid represents packaging elements such as gag and pol; the green bar in the plasmid represents reporter genes such as GFP and luciferase; the orange bar in the plasmid represents the envelope protein gene; and the purple bar in the plasmid represents the packaging signals, 3'LTR and 5'LTR.

VSV platform

To create pseudoviruses using a VSV platform, initially a VSVΔG-G* pseudovirus must be produced by transfecting two plasmids, one coding for the VSV core genome lacking the native glycoprotein and containing the reporter gene (VSVΔG), and the other coding for the VSV envelope glycoprotein (G*), into HEK293T cells amenable to transfection.

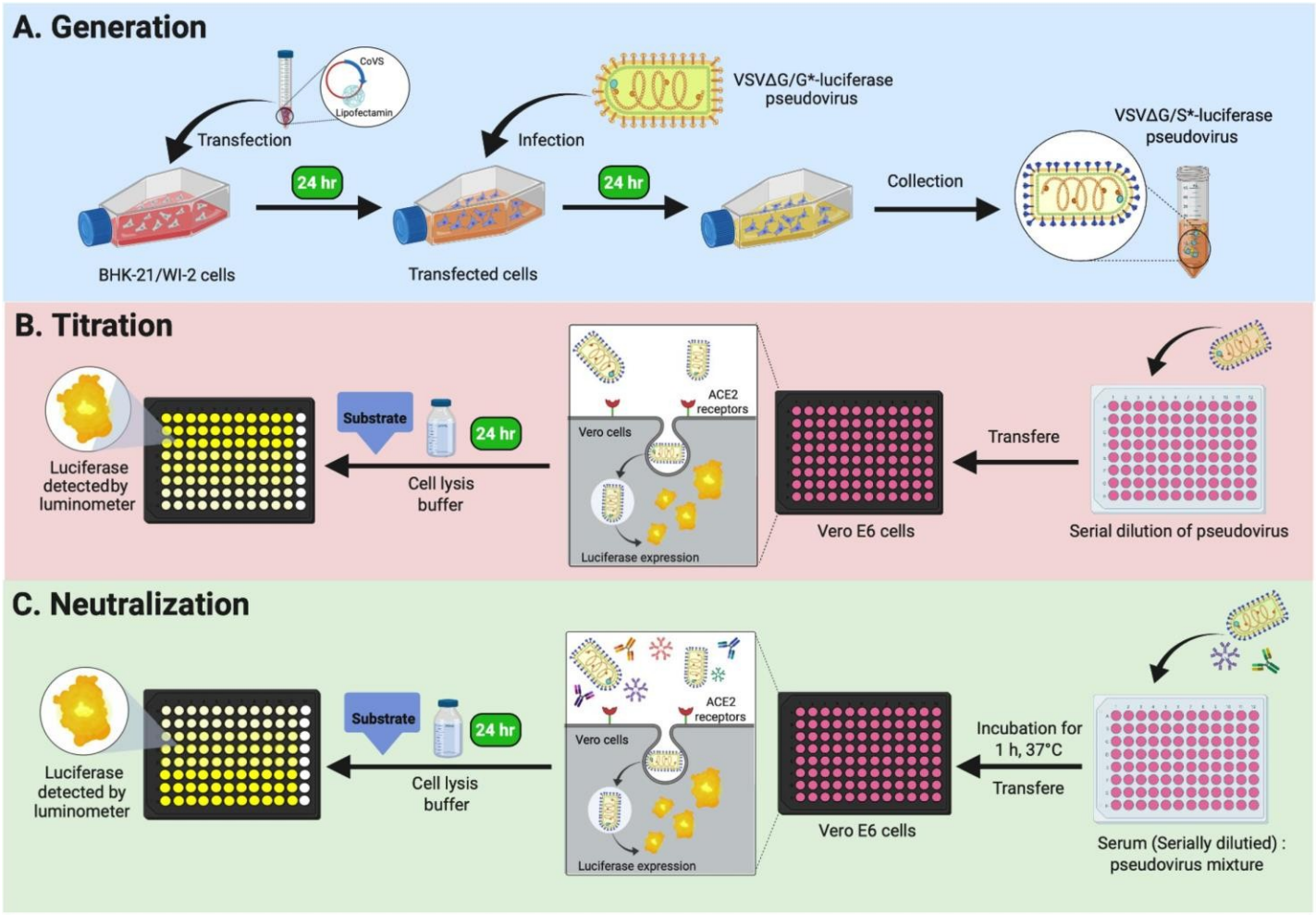
This leads to the production of VSVΔG-G* pseudoviruses that can then be used to infect HEK293T cells previously transfected with a plasmid expressing the SARS-CoV-2 S glycoprotein.

The VSVΔG-G* pseudovirus genome contains all the major components required for replication and a reporter gene, but lacks the envelope glycoprotein required for pseudovirus assembly and budding.

The separately expressed spike glycoprotein on the plasma membrane thus facilitates the assembly and budding of the VSVΔG -S pseudovirus. Any transfer of G* from the VSVΔG-G* pseudovirus used for infection is prevented by the addition of a neutralizing antibody directed against G*.

VSVΔG-S pseudoviruses can then be used directly for infection of HEK293T cells expressing the ACE2 receptor (HEK293T-ACE2) or Vero cells that allow S binding and entry.[194]

[194] Zettl F, Meister TL, Vollmer T, Fischer B, Steinmann J, Krawczyk A, V'kovski P, Todt D, Steinmann E, Pfaender S, Zimmer G. Rapid Quantification of SARS-CoV-2-Neutralizing Antibodies Using Propagation-Defective Vesicular Stomatitis Virus Pseudotypes. Vaccines (Basel). 2020 Jul 15;8(3):386. doi: 10.3390/vaccines8030386. PMID: 32679691; PMCID: PMC7563800. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7563800/



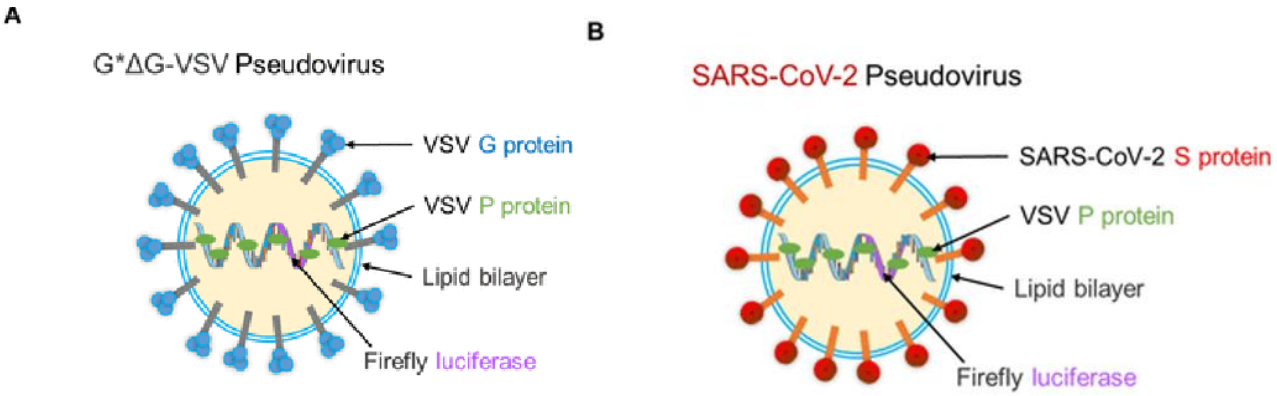
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7498578/
Graphical overview of protocols 1, 2 and 3. **(A)** Protocol 1: generation of VSV pseudoviruses carrying CoV S protein. **(B)** Protocol 2: titration assay of generated VSV pseudoviruses. **(C)** Protocol 3: neutralization assay to determine CoV-specific nAb titers in serum samples. VSV, vesicular stomatitis virus; CoV, coronavirus; nAb, neutralizing antibody.

Zhao MM, Yang WL, Yang FY, Zhang L, Huang WJ, Hou W, Fan CF, Jin RH, Feng YM, Wang YC, Yang JK.
Cathepsin L plays a key role in SARS-CoV-2 infection in humans and humanized mice and is a promising target for new drug development.
Signal Transduct Target Ther. 2021 Mar 27;6(1):134. doi: 10.1038/s41392-021-00558-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7997800/

Salazar-García M, Acosta-Contreras S, Rodríguez-Martínez G, Cruz-Rangel A, Flores-Alanis A, Patiño-López G, Luna-Pineda VM.
Pseudotyped Vesicular Stomatitis Virus-Severe Acute Respiratory Syndrome-Coronavirus-2 Spike for the Study of Variants, Vaccines, and Therapeutics Against Coronavirus Disease 2019.
Front Microbiol. 2022 Jan 14;12:817200. doi: 10.3389/fmicb.2021.817200.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8795712/

Tani H, et al
Evaluation of SARS-CoV-2 neutralizing antibodies using a vesicular stomatitis virus possessing SARS-CoV-2 spike protein.
Virol J. 2021 Jan 12;18(1):16. doi: 10.1186/s12985-021-01490-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7801864/

Almahboub SA, Algaissi A, Alfaleh MA, ElAssouli MZ, Hashem AM.
Evaluation of Neutralizing Antibodies Against Highly Pathogenic Coronaviruses: A Detailed Protocol for a Rapid Evaluation of Neutralizing Antibodies Using Vesicular Stomatitis Virus Pseudovirus-Based Assay.
Front Microbiol. 2020 Sep 4;11:2020. doi: 10.3389/fmicb.2020.02020.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7498578/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7997800/
Schematic diagram of pseudovirus structure.
(A) G*ΔG-VSV pseudovirus was generated using a recombinant VSV in which the glycoprotein gene (VSV-G) was deleted and replaced with genes encoding for firefly luciferase. When VSV-G is transiently expressed in cells infected with these recombinants, pseudotype VSV particles are produced.
(B) VSV virions do not specifically select the type of membrane protein that can be incorporated into the viral envelope, and VSV particles can bud in the absence of G protein. The SARS-CoV-2 pseudovirus was generated by incorporating the SARS-CoV-2 S protein into the recombinant VSV shown above.

Platform of Murine Leukemia Virus (MLV) and HIV

The MLV and HIV platforms employ three plasmids for transfection into HEK293T cells:[195]
- A plasmid encoding for MLV/HIV core, gag and pol genes, but lacking the MLV/HIV envelope glycoprotein env gene
- A transfer vector plasmid encoding the firefly luciferase or green fluorescent protein (GFP) reporter gene, the Ψ-RNA assembly signal along with LTR (long terminal repeat) flanking regions in 5'- and 3'
- A plasmid encoding for the envelope glycoprotein of interest, in this case SARS-CoV-2 S.

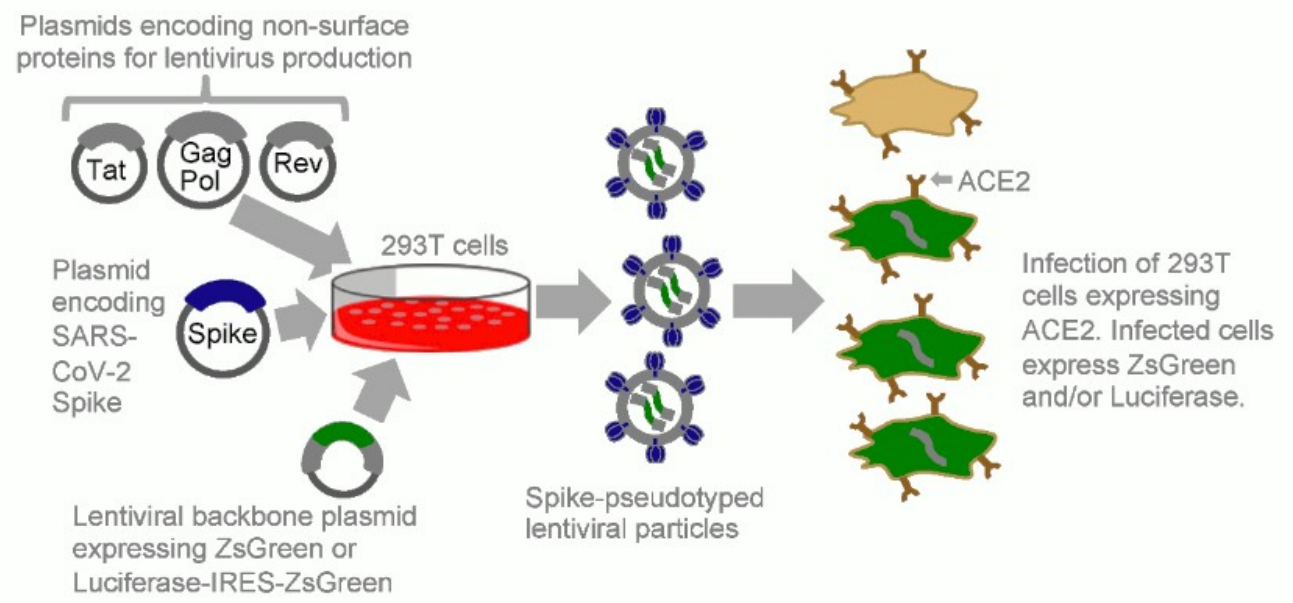
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291041/
General approach to lentiviral pseudotyping. 293T cells are transfected with a plasmid encoding for a lentiviral backbone (genome) expressing a marker protein, a plasmid expressing Spike, and plasmids expressing the other HIV proteins required for virion formation (Tat, Gag-Pol, and Rev). Transfected cells produce lentiviral particles with Spike on their surface. These viral particles can infect cells expressing the ACE2 receptor.

[195] Zheng Y, Larragoite ET, Williams ESCP, Lama J, Cisneros I, Delgado JC, Slev P, Rychert J, Innis EA, Coiras M, Rondina MT, Spivak AM, Planelles V.
Neutralization assay with SARS-CoV-1 and SARS-CoV-2 spike pseudotyped murine leukemia virions.
Virol J. 2021 Jan 4;18(1):1. doi: 10.1186/s12985-020-01472-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7780907/

Crawford KHD, et al
Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays.
Viruses. 2020 May 6;12(5):513. doi: 10.3390/v12050513.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7291041/

Schmidt F, et al
Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses.
bioRxiv [Preprint]. 2020 Jun 9:2020.06.08.140871. doi: 10.1101/2020.06.08.140871. Update in: J Exp Med. 2020 Nov 2;217(11):
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7302213/

Schmidt F, et al
Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses.
J Exp Med. 2020 Nov 2;217(11):e20201181. doi: 10.1084/jem.20201181.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7372514/



In vitro ADE testing

The ADE assay consists of pre-incubating a serial dilution of serum/antibody prepared on a plate with a fixed concentration of virus. This mixture is then incubated with effector cells carrying the Fc receptor and cells with the ACE2 receptor as control.
ADE is measured by comparing the percentage of infected cells at different serum concentrations with untreated/unboosted controls.[196]

Below is an example of the ADE test based on rSARS-CoV-2 Spike Luciferase pseudotyped:

Effector cells:
Fc receptor-bearing cells (Raji cells, K562 cells, primary B cells, etc.).
293T/ACE2 cells (293T cells with ACE2 overexpression) as a positive control of the system

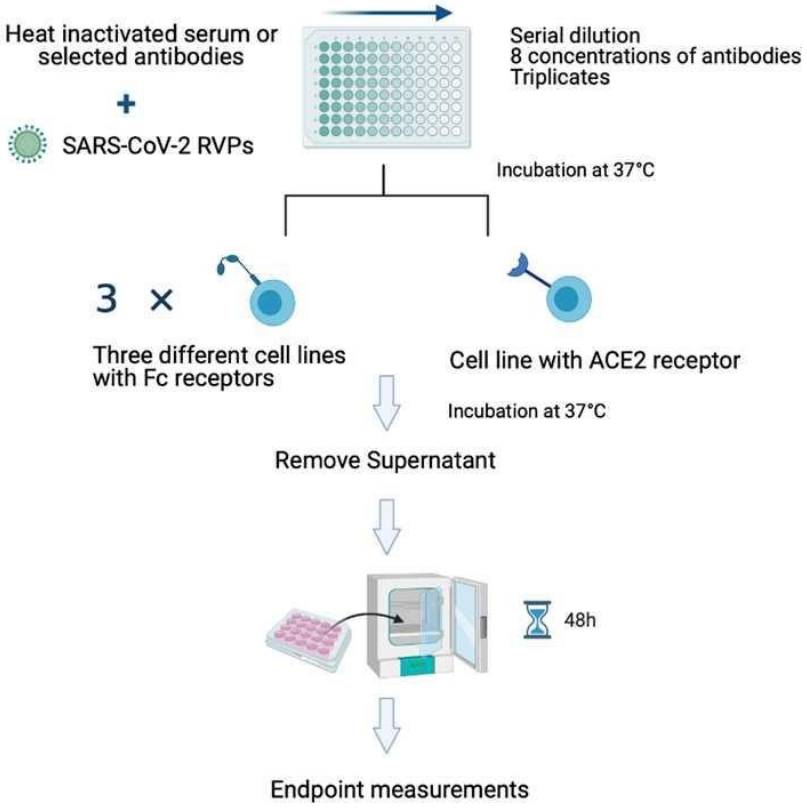
Detection method
Infectious SARS-CoV-2 or pseudotyped SARS-CoV-2 infects effector cells after incubation with serial dilution of antibody or test serum.
ADE is measured by comparing the percentage of infected cells at different serum/antibody concentrations with untreated/nonenhancing controls.
In the live virus-based ADE test (BLS3), infection is quantified by immunostaining and flow cytometry analysis.

[196] Shuang Wang, et al
An antibody-dependent enhancement (ADE) activity eliminated neutralizing antibody with potent prophylactic and therapeutic efficacy against SARS-CoV-2 in rhesus monkeys
bioRxiv 2020.07.26.222257; doi: https://doi.org/10.1101/2020.07.26.222257
https://www.biorxiv.org/content/10.1101/2020.07.26.222257v1

Yip MS, Cheung CY, Li PH, Bruzzone R, Peiris JSM, Jaume M.
Investigation of Antibody-Dependent Enhancement (ADE) of SARS coronavirus infection and its role in pathogenesis of SARS.
BMC Proc. 2011 Jan 10;5(Suppl 1):P80. PMCID:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3019510/

Luan N, Li T, Wang Y, Cao H, Yin X, Lin K, Liu C.
Th2-Oriented Immune Serum After SARS-CoV-2 Vaccination Does Not Enhance Infection In Vitro.
Front Immunol. 2022 Apr 8;13:882856. doi: 10.3389/fimmu.2022.882856.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9024142/

In the viral particle-based (RVP) Reporter (e.g., GFP or Luciferase) ADE assay, infection is quantified by flow cytometry analysis or luciferase assay system.
Untreated and uninfected controls are included in each plate to control assay variability between plates. The assay can be controlled internally using known concentrations of purified enhancing and isotypic antibodies.
The assay is optimized in a 96-well plate format to reduce the volume of test material required, which in turn allows for parallel neutralization tests and/or multiple replicates/repeats for increased robustness.



https://www.creative-diagnostics.com/sars-cov-2-ade-assay.htm

Criticisms and manipulations of preclinical studies on ADEs

The preclinical study of ADE is a key safety requirement from regulatory agencies for pharmaceutical companies, already at the fast-track approval stage. [197]
The first company to carry out the preclinical study was Astrazeneca, as discussed in the paper "COVID-19 The Vaccine" in the update from p. 93:

van Doremalen N, et al.
ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques.
bioRxiv [Preprint]. 2020 May 13:2020.05.13.093195. doi: 10.1101/2020.05.13.093195. Update in: Nature. 2020 Jul 30
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7241103/

[197] https://www.ema.europa.eu/en/documents/other/consideration-core-requirements-rmps-covid-19-vaccines_en.pdf

https://epi.tghn.org/articles/consensus-considerations-assessment-risk-disease-enhancement-covid-19-vaccines/

https://www.chop.edu/centers-programs/vaccine-education-center/vaccine-safety/antibody-dependent-enhancement-and-vaccines

Analyzing the results of this study in detail, from the comparison of the sequence of the Spike protein used to produce the vaccine antigen (YP_009724390.1) and the infectious virus (nCoV-WA1-2020 (MN985325.1)) inoculated for the challenge test, it can be shown that the overlap is 100%.
It follows that this study does not allow assessment of the risk of ADE in vaccinees, contrary to the stated in the title and abstract.

Equally, in the preclinical study for the Pfizer vaccine:

DiPiazza AT,et al
COVID-19 vaccine mRNA-1273 elicits a protective immune profile in mice that is not associated with vaccine-enhanced disease upon SARS- CoV-2 challenge.
Immunity. 2021 Jul 2:S1074-7613(21)00262-4. doi: 10.1016/j.immuni.2021.06.018.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8249710/

By comparing the sequences of the vaccine construct (Wuhan-Hu-1) and the virus used for the challenge test and to study disease enhancement, it can be seen that, again, the sequence is the same: comparison-Pfizer

THE CONFORMATIONS OF THE SPIKE AND ADE

Structure of protein S [198]

With a size of 180-200 kDa, the S protein consists of an extracellular N-terminal, a transmembrane domain (TM) anchored in the viral membrane and a short intracellular C-terminal segment.[199]
The S protein normally exists in a metastable prefusion conformation; once the virus interacts with the host cell, extensive structural rearrangement of the S protein occurs, allowing the virus to fuse with the host cell membrane.
The clubs (spikes) are coated with polysaccharide molecules to camouflage them and evade surveillance by the host's immune system during entry.[200]
The total length of SARS-CoV-2 S is 1273 aa and consists of a signal peptide (amino acids 1-13) located at the N-terminus, the S1 subunit (14-685 residues) and the S2 subunit (686- 1273 residues); the last two regions are responsible for receptor binding and membrane fusion, respectively.
In the S1 subunit, there is an N-terminal domain (14-305 residues) and a receptor binding domain (RBD, 319-541 residues).
The fusion peptide (FP) (788-806 residues), heptapeptide repeat sequence 1 (HR1) (912-984 residues), HR2 (1163-1213 residues), TM domain (1213-1237 residues) and cytoplasm domain (1237- 1273 residues) constitute the S2 subunit.[201]

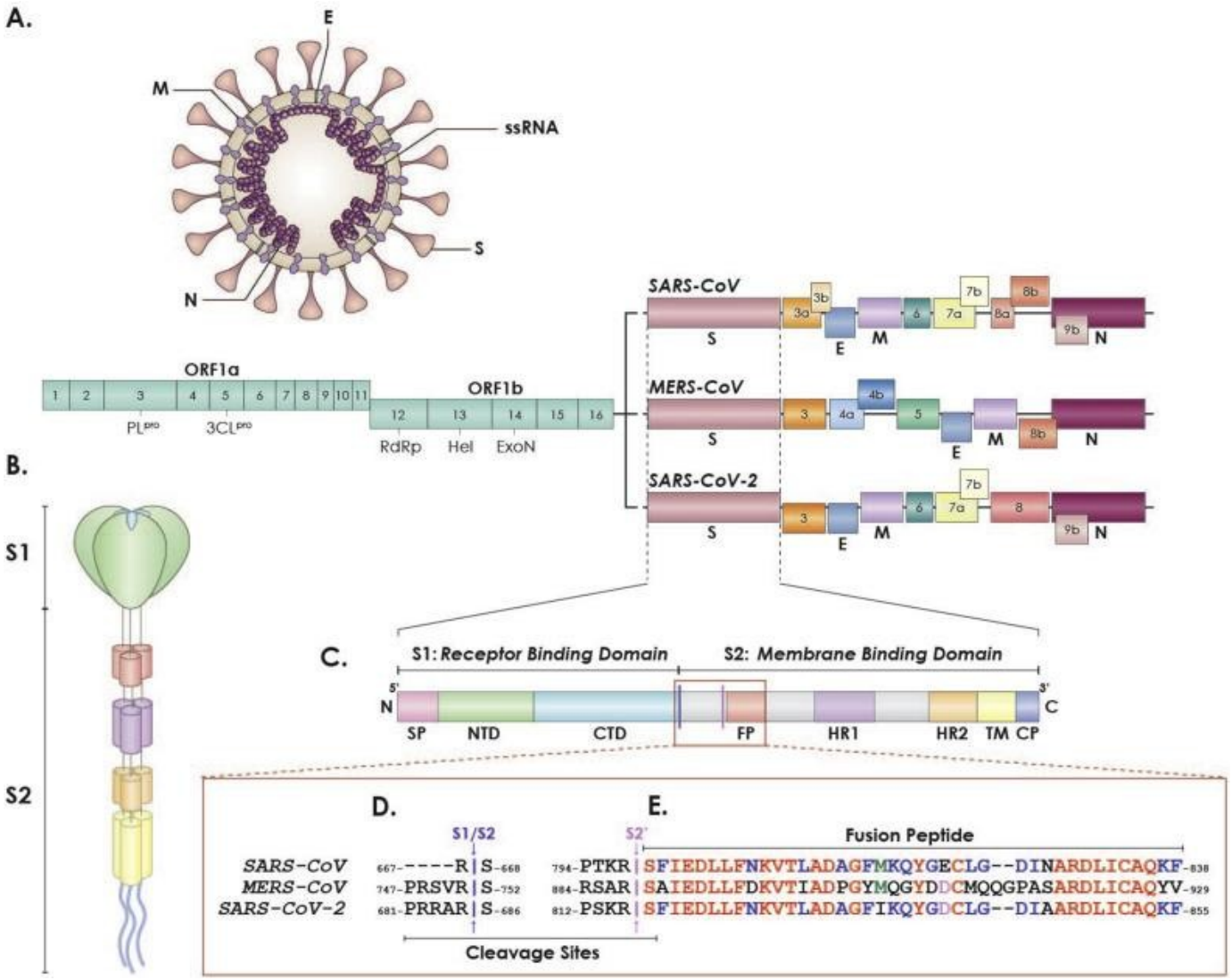
[198] Huang, Y., Yang, C., Xu, Xf. et al.
Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19.
Acta Pharmacol Sin 41, 1141-1149 (2020). https://doi.org/10.1038/s41401-020-0485-4
https://www.nature.com/articles/s41401-020-0485-4.pdf

[199] Bosch BJ, van der Zee R, de Haan CA, Rottier PJ.
The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex.
J Virol. 2003 Aug;77(16):8801-11. doi: 10.1128/jvi.77.16.8801-8811.2003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC167208/

[200] Watanabe Y, Allen JD, Wrapp D, McLellan JS, Crispin
M. Site-specific glycan analysis of the SARS-CoV-2 spike.
Science. 2020 Jul 17;369(6501):330-333. doi: 10.1126/science.abb9983.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7199903/

[201] Xia S, Zhu Y, Liu M, Lan Q, Xu W, Wu Y, Ying T, Liu S, Shi Z, Jiang S, Lu L.
Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein.
Cell Mol Immunol. 2020 Jul;17(7):765-767. doi: 10.1038/s41423-020-0374-2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7075278/

S protein trimers visually form a characteristic bulbous crown-shaped halo surrounding the viral particle. Based on the structure of coronavirus S protein monomers, S1 and S2 subunits form the bulbous head and stem region.[202]



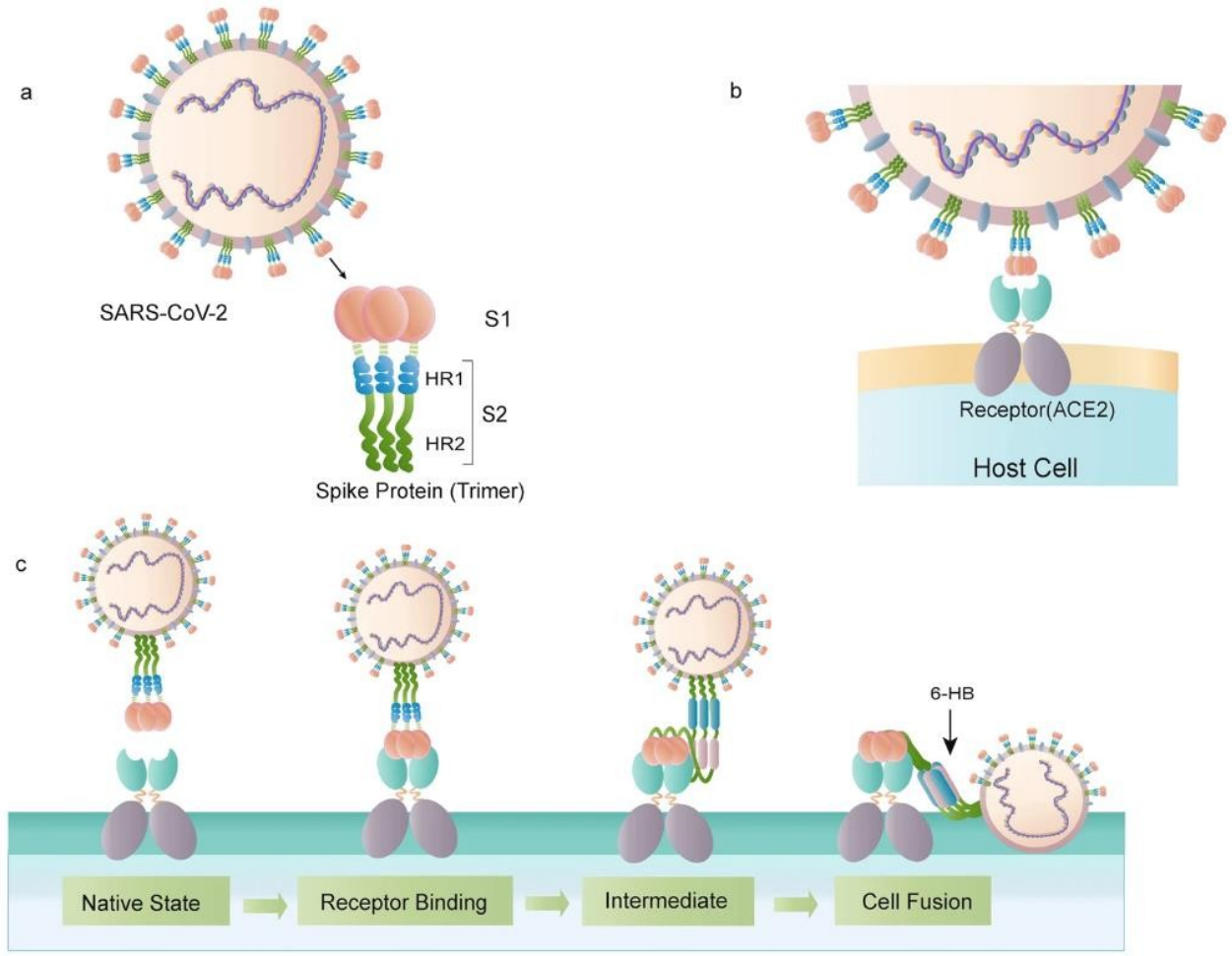
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194977/

Coronavirus spike protein (S). **A.** Figure of CoV particle (top) and complete CoV viral genome (bottom). CoVs have a lipid envelope with three structural transmembrane proteins: spike (S), membrane (M) and envelope (E). The interior of the virus contains the viral genome encapsulated by the nucleocapsid protein (N). The single-stranded genome of CoV encodes for 16 nonstructural proteins, including papain-like protease (PLpro), 3C-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp), helicase (Hel) and exonuclease (ExoN) . Subgenomic RNAs encode for four structural proteins: spike (S; dark pink), envelope (E; dark blue), membrane (M; purple) and nucleocapsid (N; magenta) and a number of accessory proteins. **B.** Figure of the CoV S protein trimer. C. The CoV S gene denoting the functional components of the protein. The CoV S protein is composed of the two subunits: S1 and S2, which comprise the main functional components: SP (signal peptide; pink); NTD (N-terminal domain; green), CTD (C-terminal domain; light blue), FP (fusion peptide; red), HR1 (heptade repeat 1; purple), HR2 (heptade repeat 2; orange), TM (transmembrane ; yellow) and CP (cytoplasmic; dark blue). The S protein has two cleavage sites indicated by dark purple (S1/S2) and pink (S2') arrows.
D. Sequence alignment of S1/S2 cleavage site (dark purple arrow) and S2' cleavage site (pink) among MERS-CoV, SARS-CoV and SARS-CoV-2.
E. Within the genome, the fusion peptide, denoting MERS-CoV FP and SARS-CoV FP sequences, is highlighted. Red denotes conserved residues between the MERS-CoV, SARS-CoV and SARS-CoV-2 FP sequences; blue denotes conserved SARS-CoV and SARS-CoV-2 FP residues; green denotes conserved SARS-CoV and MERS-CoV FP residues; and purple denotes conserved MERS-CoV and SARS-CoV-2 residues. The fusion peptide sequence of SARS-CoV-2 was determined by performing pairwise alignment with MUSCLE using Geneious (version 2020.0.5). The amino acid sequence of the

[202] Tang T, Bidon M, Jaimes JA, Whittaker GR, Daniel S.
Coronavirus membrane fusion mechanism offers a potential target for antiviral development.
Antiviral Res. 2020;178:104792. doi:10.1016/j.antiviral.2020.104792
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194977/

spike protein was obtained from NCBI Genbank based on the following: SARS-CoV-2 (MN908947.3), MERS-CoV (AFS88936.1), SARS-CoV (AAP13441.1).

The structure of the trimeric S-protein of SARS-CoV-2 was determined by cryoelectron microscopy at the atomic level, revealing different conformations of the S-domain RBD in the open and closed states and its corresponding functions, as will be discussed in more detail below.[203]



https://www.nature.com/articles/s41401-020-0485-4
(a) The schematic structure of protein S. (b) Protein S binds to the ACE2 receptor. (c) The process of virus-cell binding and fusion mediated by protein S.

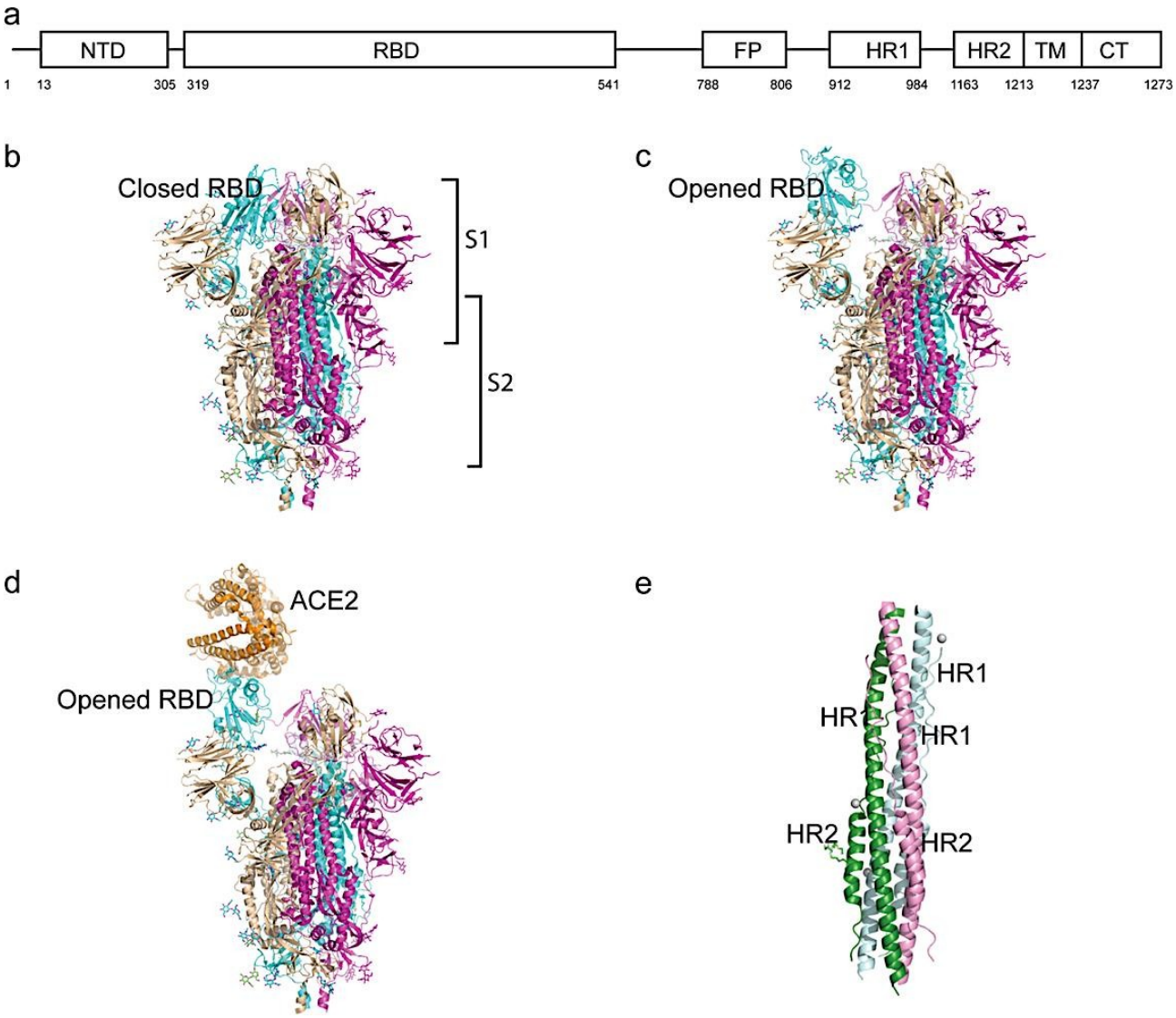
S1 subunit structure

Binding of viral particles to cell receptors on the host cell surface is the initiation of viral infection, so receptor recognition is an important determinant of viral entry and a target for drug design. Specifically, the RBD located in the S1 subunit binds to the cellular ACE2 receptor in the aminopeptidase N region. [204]

[203] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS.
Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science. 2020 Mar 13;367(6483):1260-1263. doi: 10.1126/science.abb2507.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/

Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D.
Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein [published correction appears in Cell. 2020 Dec 10;183(6):1735].
Cell. 2020;181(2):281-292.e6. doi:10.1016/j.cell.2020.02.058
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102599/

[204] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science. 2020 Mar 13;367(6483):1260-1263. doi: 10.1126/science.abb2507.



https://www.nature.com/articles/s41401-020-0485-4
a)Schematic representation of the SARS-CoV-2 spike**. b-c)** The S protein RBD is in closed and open position. **d)** The S protein binds to ACE2 with open RBD in the S1 subunit. **e)** The six-helix structure formed by HR1 and HR2 of the S2 subunit

The RBD region is a critical target for neutralizing antibodies (nAbs), but although SARS-CoV-2 and SARS-CoV RBD are ~73%-76% similar in sequence, studies with monoclonal antibodies specific against

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/

Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X.
Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.
Nature. 2020 May;581(7807):215-220. doi: 10.1038/s41586-020-2180-5.
https://pubmed.ncbi.nlm.nih.gov/32225176/

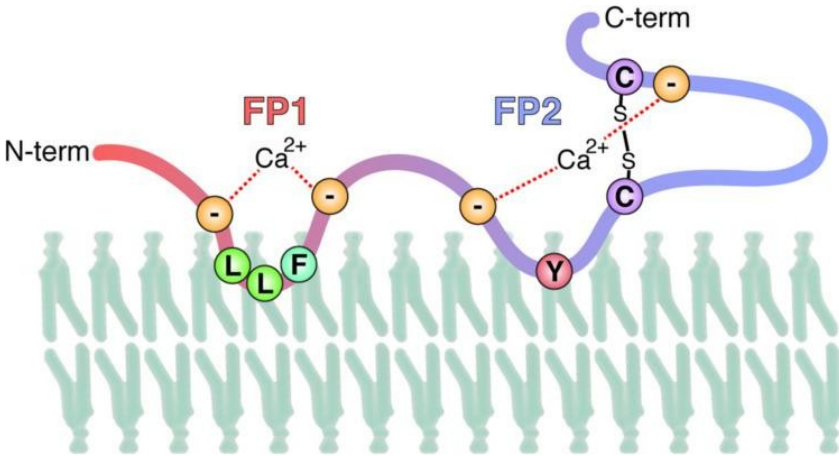
Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D.
Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein.
Cell. 2020 Apr 16;181(2):281-292.e6. doi: 10.1016/j.cell.2020.02.058. Epub 2020 Mar 9. Erratum in: Cell. 2020 Dec 10;183(6):1735.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102599/

Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY, Wang Q, Zhou H, Yan J, Qi J.
Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2.
Cell. 2020 May 14;181(4):894-904.e9. doi: 10.1016/j.cell.2020.03.045.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7144619/

the RBD S1 suggest that it may not be an ideal drug target because of the excessive mutability.[205]

S2 subunit structure

The S2 subunit, composed of a FP (fusion peptide) domain, HR1, HR2 (HR: heptad repeats), TM (transmembrane domain) and CP (cytoplasmin domain), is responsible for fusion and viral entry.
FP is a short segment of 15-20 conserved amino acids of the viral family, composed mainly of hydrophobic residues, such as glycine (G) or alanine (A), that anchor to the target membrane when the S protein adopts the pre-turning conformation. FP has been shown to play an essential role in mediating membrane fusion by disrupting and bridging the lipid bilayers of the host cell membrane.[206]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7112017/
Predicted model of the CoV fusion peptide region, along with its interaction with the lipid bilayer. The conserved negatively charged and hydrophobic residues and a proposed position for Ca ions are shown^{2+} .

HR1 and HR2 are composed of a repetitive heptapeptide: HPPHCPC, where H is a hydrophobic or traditionally bulky residue, P is a polar or hydrophilic residue, and C is another charged residue.[207]
HR1 and HR2 form the six-helical bundle (6-HB) (Fig. e, p. 67), which is essential for viral fusion and the entry function of the S2 subunit.[208]

[205] Xia S, Yan L, Xu W, et al.
A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike.
Sci Adv. 2019;5(4):eaav4580. Published 2019 Apr 10. doi:10.1126/sciadv.aav4580
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6457931/

[206] Millet JK, Whittaker GR.
Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells.
Virology. 2018 Apr;517:3-8. doi: 10.1016/j.virol.2017.12.015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7112017/

[207] Chambers P, Pringle CR, Easton AJ.
Heptad repeat sequences are located adjacent to hydrophobic regions in several types of virus fusion glycoproteins.
J Gen Virol. 1990 Dec;71(Pt 12):3075-80. doi: 10.1099/0022-1317-71-12-3075.
https://pubmed.ncbi.nlm.nih.gov/2177097/

[208] Xia S, Zhu Y, Liu M, Lan Q, Xu W, Wu Y, Ying T, Liu S, Shi Z, Jiang S, Lu L.
Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein.
Cell Mol Immunol. 2020 Jul;17(7):765-767. doi: 10.1038/s41423-020-0374-2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7075278/

HR1 is located at the C-terminus of a hydrophobic FP and HR2 is located at the N-terminus of the TM domain.[209] The downstream TM domain anchors the S protein to the viral membrane, and the S2 subunit terminates in a CT tail.[210]
The RBD binds to ACE2 and S2 changes conformation by inserting FP into the target cell membrane, exposing the coiled coil of the HR1 domain and triggering the interaction between the HR2 domain and the HR1 trimer to form 6-HB, which thus brings the viral envelope and cell membrane into close proximity for viral fusion and entry, as will be discussed in more detail later.[211]
HR1 forms a homotrimeric complex in which three highly conserved hydrophobic grooves are exposed on the surface that bind to HR2.
The HR2 domain forms both a rigid helix and a flexible loop to interact with the HR1 domain.
In the CoV postfusion fork conformation, there are many strong interactions between the HR1 and HR2 domains within the helical region, which is called the "fusion core region" (HR1core and HR2core regions, respectively).

While the S1 RBD domain, as already seen, is part of a highly mutable region and is not an ideal target site for the development of broad-spectrum antiviral inhibitors[212] , the HR region of the S2 subunit plays an essential role in HCoV infections and is conserved among HCoVs, as is the mode of interaction between HR1 and HR2, and thus the repetitive heptapeptide (HR) has attracted the most interest in the study of therapeutic drugs. [213]

Mechanism of action of protein S

Spike protein is a trimeric class I* transmembrane glycoprotein responsible for viral entry, and is present in all types of HCoV, as well as in other viruses such as HIV (HIV glycoprotein 160, Env), influenza virus (influenza hemagglutinin, HA), Paramyxovirus (parainfluenza viruses, mumps virus, measles virus, and human respiratory syncytial virus belong to the Paramyxoviridae family) and

[209] Robson B.
Computers and viral diseases. Preliminary bioinformatics studies on the design of a synthetic vaccine and a preventive peptidomimetic antagonist against the SARS-CoV-2 (2019-nCoV, COVID-19) coronavirus.
Comput Biol Med. 2020 Apr;119:103670. doi: 10.1016/j.compbiomed.2020.103670.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7094376/

[210] Tang T, Bidon M, Jaimes JA, Whittaker GR, Daniel S.
Coronavirus membrane fusion mechanism offers a potential target for antiviral development.
Antiviral Res. 2020 Jun;178:104792. doi: 10.1016/j.antiviral.2020.104792.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194977/

[211] Xia S, Xu W, Wang Q, Wang C, Hua C, Li W, Lu L, Jiang S.
Peptide-Based Membrane Fusion Inhibitors Targeting HCoV-229E Spike Protein HR1 and HR2 Domains.
International Journal of Molecular Sciences. 2018; 19(2):487. https://doi.org/10.3390/ijms19020487
https://www.mdpi.com/1422-0067/19/2/487/htm

Wang X, Xia S, Wang Q, Xu W, Li W, Lu L, Jiang S.
Broad-Spectrum Coronavirus Fusion Inhibitors to Combat COVID-19 and Other Emerging Coronavirus Diseases.
International Journal of Molecular Sciences. 2020; 21(11):3843. https://doi.org/10.3390/ijms21113843
https://www.mdpi.com/1422-0067/21/11/3843/htm

[212] Lu G, Wang Q, Gao GF.
Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond.
Trends Microbiol. 2015 Aug;23(8):468-78. doi: 10.1016/j.tim.2015.06.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7125587/

[213] Liu S, Xiao G, Chen Y, He Y, Niu J, Escalante CR, Xiong H, Farmar J, Debnath AK, Tien P, Jiang S.
Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: implications for virus fusogenic mechanism and identification of fusion inhibitors.
Lancet. 2004 Mar 20;363(9413):938-47. doi: 10.1016/S0140-6736(04)15788-7.
https://pubmed.ncbi.nlm.nih.gov/15043961/

Ebola (Ebola virus glycoprotein). [214] Similar to other coronaviruses, the S protein of SARS-CoV-2 mediates receptor recognition, cell attachment, and fusion during viral infection.[215]

** S protein is a trimer that follows a fusion mechanism defined as class I: it has, in fact, a metastable pre-fusion conformation and undergoes substantial structural rearrangement to fuse the viral membrane with the host membrane. The prefusion state is termed "down" and is that of non-accessibility to the ACE receptor. In it, one of the three RBD domains of the S1 subunit faces upward. When the S1 subunit binds to the receptor, it makes a hinge movement, which exposes or hides the other two RBDs and causes the S2 subunit to assume an even less stable postfusion conformation. This state is termed "up" because the viral membrane can fuse with the cell. [216]*

Receptor binding

As seen previously, the S protein binds to ACE2 through the RBD region of the S1 subunit, mediating viral attachment to host cells in the form of trimers. SARS-CoV-2 S binds to human ACE2 with a dissociation constant (KD) of 14.7 nM, while that of SARS-CoV S is 325.8 nM, indicating that SARS-CoV-2 S is more ACE2-related than SARS-CoV S. [217]

Viral fusion

Viral fusion refers to the fusion of the viral membrane with the host cell membrane, and the subsequent release of the viral genome into the host cell.

The SARS-Cov-2 spike, like various other SARS-CoV spike proteins, is cut by cellular proteases at the boundary between S1 and S2 subunits, generating two separate regions that remain noncovalently bound in the so-called "pre-fusion conformation."[218]

---

[214] Weissenhorn W, Dessen A, Calder LJ, Harrison SC, Skehel JJ, Wiley DC.
Structural basis for membrane fusion by enveloped viruses.
Mol Membr Biol. 1999 Jan-Mar;16(1):3-9. doi: 10.1080/096876899294706.
https://pubmed.ncbi.nlm.nih.gov/10332732/

[215] Gui M, Song W, Zhou H, Xu J, Chen S, Xiang Y, Wang X.
Cryo-electron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding.
Cell Res. 2017 Jan;27(1):119-129. doi: 10.1038/cr.2016.152.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5223232/

Hulswit RJ, de Haan CA, Bosch BJ.
Coronavirus Spike Protein and Tropism Changes.
Adv Virus Res. 2016;96:29-57. doi: 10.1016/bs.aivir.2016.08.004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7112277/

Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q.
Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2.
Science. 2020 Mar 27;367(6485):1444-1448. doi: 10.1126/science.abb2762
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164635/

[216] Anti-Covid 19 vaccine, focus on Spike Protein
https://www.microbiologiaitalia.it/immunologia/vaccino-anti-covid-19-focus-sulle-spike-protein/

Zhang DY, Wang J, Dokholyan NV.
Prefusion spike protein stabilization through computational mutagenesis.
Proteins. 2021 Apr;89(4):399-408. DOI: 10.1002/prot.26025.
https://europepmc.org/article/MED/33231324

[217] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS.
Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science. 2020 Mar 13;367(6483):1260-1263. doi: 10.1126/science.abb2507.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/

[218] Tortorici MA, Walls AC, Lang Y, Wang C, Li Z, Koerhuis D, Boons GJ, Bosch BJ, Rey FA, de Groot RJ, Veesler D.
Structural basis for human coronavirus attachment to sialic acid receptors.
Nat Struct Mol Biol. 2019 Jun;26(6):481-489. doi: 10.1038/s41594-019-0233-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6554059/

https://sibbm.zanichelli.it/italiano/2020/08/23/infezione-biologia-strutturale/

One of the cellular proteases involved in the spike entry of SARS-CoV-2 into host cells is Transmembrane Serine Protease 2 (TMPRSS2), which is also required for SARS-CoV infection. In fact, inhibition of TMPRS22 blocks the entry of SARS-CoV and CoV-2 into cells. It is interesting that CoV-2-S possesses a four-amino acid insertion (PRRAR polybasic site) at the boundary between S1 and S2 compared with SARS-CoV protein S, in which it is absent. [219]

These four additional amino acids constitute the cutting site for a specific human protease called furin. The presence of this peculiar cutting site for furin in SARS-CoV-2-S, given the virtually ubiquitous expression of furin-like proteases, is responsible for the broader cellular and tissue tropism of SARS-CoV-2 compared with SARS-CoV, as well as for the increase in its transmissibility and pathogenicity[220] .

It has recently been shown that, like TMPRSS2, furin is essential for CoV-2 entry into host cells. Specifically, SARS-CoV-2 entry requires sequential cleavage of the spike glycoprotein at the S1/S2 and S2' cleavage sites to mediate membrane fusion.[221]

In addition, cathepsin D, a protease typical of lysosomes, is also required for efficient entry of CoV-2.

---

[219] Coutard B, Valle C, de Lamballerie X, Canard B, Seidah NG, Decroly E.
The spike glycoprotein of the new 2019-nCoV coronavirus contains a furin-like cleavage site absent in CoV of the same clade.
Antiviral Res. 2020;176:104742. doi:10.1016/j.antiviral.2020.104742
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114094/

Rabaan AA, Al-Ahmed SH, Haque S, Sah R, Tiwari R, Malik YS, Dhama K, Yatoo MI, Bonilla-Aldana DK, Rodriguez-Morales AJ.
SARS-CoV-2, SARS-CoV, and MERS-COV: A comparative overview.
Infez Med. 2020 Ahead Of Print Jun 1;28(2):174-184. PMID: 32275259.
https://www.infezmed.it/media/journal/Vol_28_2_2020_7.pdf

[220] Anwarul Hasan, et al.
A review on the cleavage priming of the spike protein on coronavirus by angiotensin-converting enzyme-2 and furin,
Journal of Biomolecular Structure and Dynamics, (2021) 39:8, 3025-3033, DOI: 10.1080/07391102.2020.1754293
https://doi.org/10.1080/07391102.2020.1754293

Millet JK, Whittaker GR.
Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis.
Virus Res. 2015;202:120-134. doi:10.1016/j.virusres.2014.11.021
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4465284/

[221] Peacock, T.P., Goldhill, D.H., Zhou, J. et al.
The furin cleavage site in the SARS-CoV-2 spike protein is required for transmission in ferrets.
Nat Microbiol 6, 899-909 (2021). https://doi.org/10.1038/s41564-021-00908-w
https://www.nature.com/articles/s41564-021-00908-w

Xia, S., Lan, Q., Su, S. et al.
The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin.
Sig Transduct Target Ther 5, 92 (2020). https://doi.org/10.1038/s41392-020-0184-0
https://www.nature.com/articles/s41392-020-0184-0

Wu Y, Zhao S.
Furin cleavage sites naturally occur in coronaviruses
[published online ahead of print, 2020 Dec 9]. Stem Cell Res. 2020;50:102115. doi:10.1016/j.scr.2020.102115
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7836551/

Yujia Alina Chan, Shing Hei Zhan,
The Emergence of the Spike Furin Cleavage Site in SARS-CoV-2,
Molecular Biology and Evolution, Volume 39, Issue 1, January 2022, msab327, https://doi.org/10.1093/molbev/msab327
https://academic.oup.com/mbe/article/39/1/msab327/6426085

Johnson BA, Xie X, Kalveram B, et al.
Furin Cleavage Site Is Key to SARS-CoV-2 Pathogenesis.
Preprint. bioRxiv. 2020;2020.08.26.268854. Published 2020 Aug 26. doi:10.1101/2020.08.26.268854
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7457603/

Whittaker GR.
SARS-CoV-2 spike and its adaptable furin cleavage site.
Lancet Microbe. 2021;2(10):e488-e489. doi:10.1016/S2666-5247(21)00174-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8346238/

Variation around the viral envelope glycoprotein cleavage site has been shown to play a role in cell trophism and pathogenesis.
For example, the pathogenesis of some CoVs has previously been related to the presence of a furin-like cleavage site in the sequence of protein S. Specifically, the inclusion of a similar cleavage site in the S protein of infectious bronchitis virus (IBV) results in increased pathogenicity, pronounced neural symptoms, and neurotropism in infected chickens.[222]
Similarly, in the case of influenza virus, the low-pathogenic forms contain a single basic residue at the cleavage site, which is cleaved by trypsin-like proteases, and the tissue distribution of the activating protease typically limits infections to respiratory and/or intestinal organs[223] .
In contrast, the highly pathogenic forms of influenza have a furin-like cleavage site on which several cellular proteases, including furin, expressed in a wide variety of cell types act, enabling an enlargement of the cellular trophism of the virus[224] .
In addition, the insertion of a multibasic **RERRRKKR↓GL** motif into the HA hemagglutinin cleavage site of H5N1 may have been responsible for the hypervirulence of the virus during the Hong Kong outbreak in 1997.[225]
This motif shows Arginine at P1 and basic residues at P2 and P4, P6 and P8 and an aliphatic Leucine at P2' positions (Schechter and Berger nomenclature)[226] , typical of a furin-like cleavage specificity. [227]

---

[222] Cheng J, Zhao Y, Xu G, et al.
The S2 Subunit of QX-type Infectious Bronchitis Coronavirus Spike Protein Is an Essential Determinant of Neurotropism.
Viruses. 2019;11(10):972. Published 2019 Oct 22. doi:10.3390/v11100972
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6832359/

[223] Sun X, Tse LV, Ferguson AD, Whittaker GR.
Modifications to the hemagglutinin cleavage site control the virulence of a neurotropic H1N1 influenza virus.
J Virol. 2010;84(17):8683-8690. doi:10.1128/JVI.00797-10
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2919019/

[224] Kido H, Okumura Y, Takahashi E, Pan HY, Wang S, Yao D, Yao M, Chida J, Yano M.
Role of host cellular proteases in the pathogenesis of influenza and influenza-induced multiple organ failure.
Biochim Biophys Acta. 2012 Jan;1824(1):186-94. doi: 10.1016/j.bbapap.2011.07.001.
https://doi.org/10.1016/j.bbapap.2011.07.001

[225] Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, Krauss S, Shortridge KF, Webster RG.
Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus.
Lancet. 1998 Feb 14;351(9101):472-7. doi: 10.1016/S0140-6736(97)11212-0. Erratum in: Lancet 1998 Apr 25;351(9111):1292.
https://pubmed.ncbi.nlm.nih.gov/9482438/

Kido H, Okumura Y, Takahashi E, Pan HY, Wang S, Yao D, Yao M, Chida J, Yano M.
Role of host cellular proteases in the pathogenesis of influenza and influenza-induced multiple organ failure.
Biochim Biophys Acta. 2012 Jan;1824(1):186-94. doi: 10.1016/j.bbapap.2011.07.001.
https://doi.org/10.1016/j.bbapap.2011.07.001

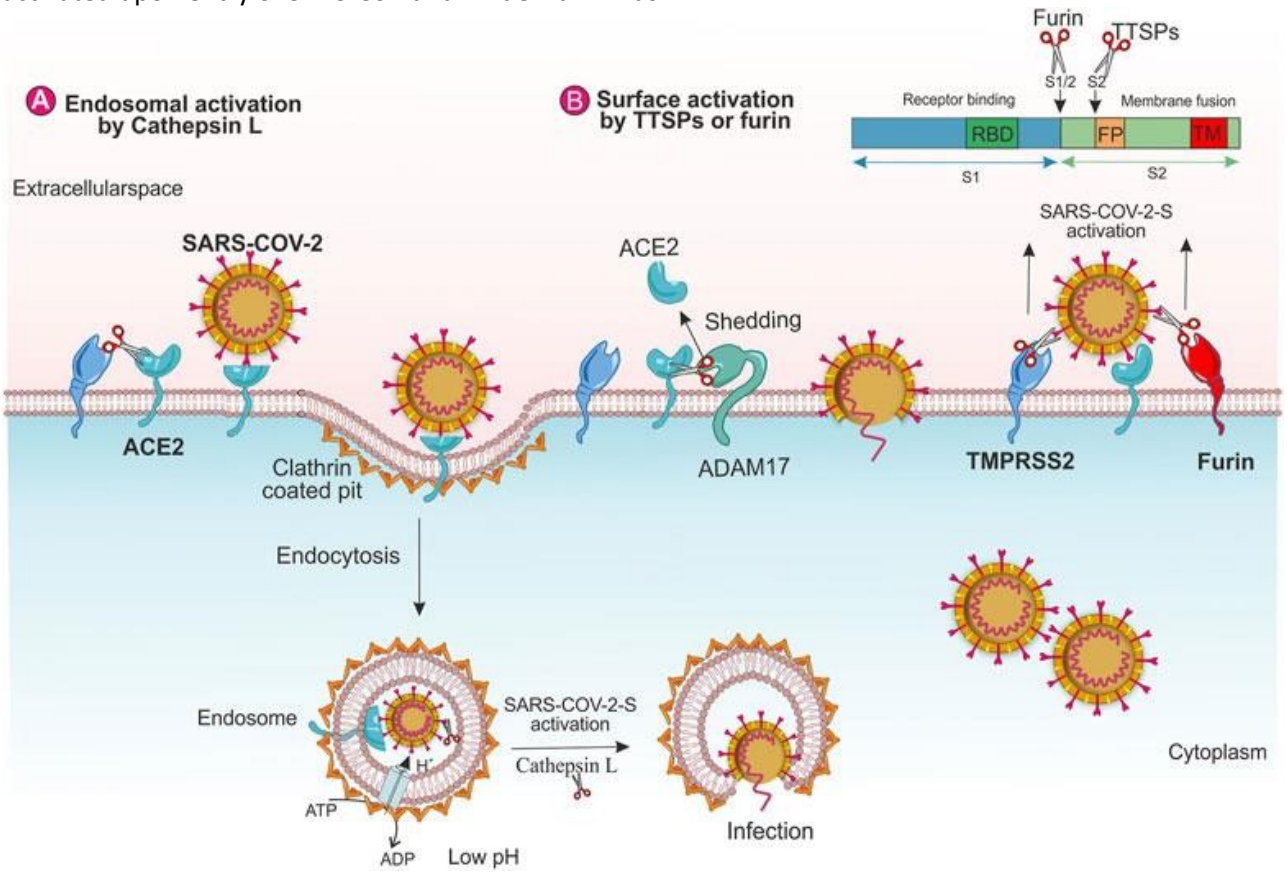
[226] Schechter I, Berger A.
On the active site of proteases. Mapping the active site of papain; specific peptide inhibitors of papain 3.
Biochem Biophys Res Commun. 1968 Sep 6;32(5):898-902. doi: 10.1016/0006-291x(68)90326-4.
https://pubmed.ncbi.nlm.nih.gov/5682314/

[227] Braun E, Sauter D.
Furin-mediated protein processing in infectious diseases and cancer.
Clin Transl Immunology. 2019;8(8):e1073. Published 2019 Aug 5. doi:10.1002/cti2.1073
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6682551/

Izaguirre G.
The Proteolytic Regulation of Virus Cell Entry by Furin and Other Proprotein Convertases.
Viruses. 2019;11(9):837. Published 2019 Sep 9. doi:10.3390/v11090837
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6784293/

Seidah NG, Prat A.
The biology and therapeutic targeting of the proprotein convertases.
Nat Rev Drug Discov. 2012 May;11(5):367-83. doi: 10.1038/nrd3699.
https://doi.org/10.1038/nrd3699

Host cell proteases such as TMPRSS2 are essential for S protein priming and have been shown to be activated upon entry of SARS-CoV and influenza A virus.[228]



https://www.frontiersin.org/articles/10.3389/fmolb.2021.725528/full
The role of human host proteases on the entry of SARS-CoV-2. Virus entry through (A) endosomal pathway and (B) TMPRSS2 and furin.

Insight

The furin cleavage site of SARS-Cov-2

As already discussed, there are two cleavage events associated with spike-mediated membrane fusion, in which the S1 domain is the binding receptor, while the S2 domain contains the apparatus for fusion to the membrane.
The first is initiation cleavage that occurs at the S1/S2 region interface for some coronaviruses, and the second is obligatory activation cleavage that occurs within the S2 region (S2').
Trigger cleavage generally converts the spike protein to a fusion-competent form, allowing the spike to bind better to receptors or expose hidden cleavage sites.

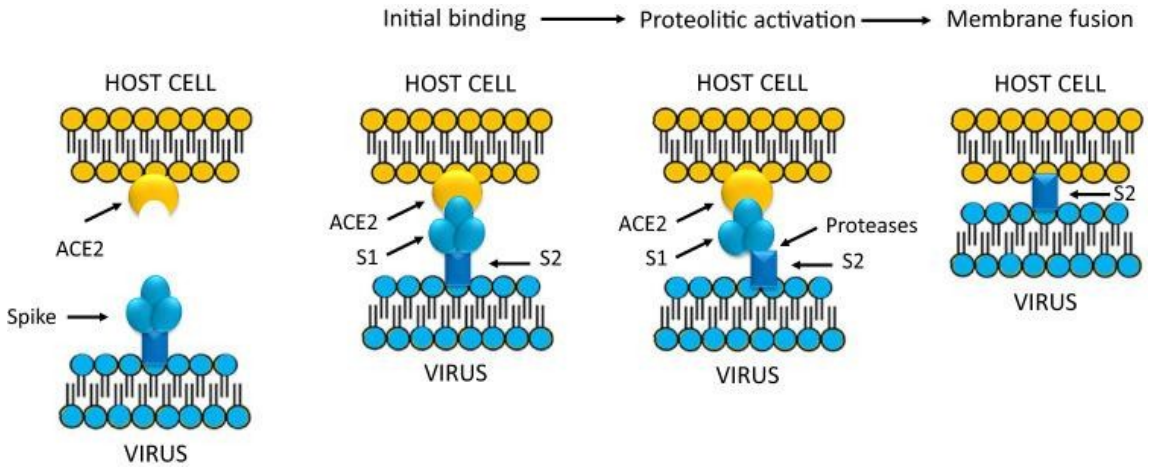
[228] Hoffmann M, Kleine-Weber H, Schroeder S, et al.
SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.
Cell. 2020;181(2):271-280.e8. doi:10.1016/j.cell.2020.02.052
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102627/

Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pöhlmann S.
TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein.
J Virol. 2014;88(2):1293-1307. doi:10.1128/JVI.02202-13
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911672/

Limburg H, Harbig A, Bestle D, et al.
TMPRSS2 Is the Major Activating Protease of Influenza A Virus in Primary Human Airway Cells and Influenza B Virus in Human Type II Pneumocytes.
J Virol. 2019;93(21):e00649-19. Published 2019 Oct 15. doi:10.1128/JVI.00649-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6803253/

Activation cleavage initiates a series of conformational changes that allow the S protein to attach to the host membrane for membrane fusion. [229]

** Recall again that the prefusion conformation is the conformation of the viral fusion protein that appears on the viral envelope after initiation but before fusion activation, whereas the postfusion conformation is the conformation of the fusion protein/subunit after the fusion reaction has occurred.*



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7393809/
After initial ACE2 receptor binding, the SARS-CoV spike is proteolytically activated and enzymatically cleaved at the S1/S2 level. S1 dissociating from S2 and the truncated subunit 2 of the spike protein facilitates fusion of cell and viral membranes.

There are a variety of proteases that can trigger and activate coronavirus (CoV) spike proteins.
Depending on the available protease (furin, trypsin, cathepsin protease) the CoV may fuse with the plasma membrane or with the endosomal membrane.[230]
The pro-protein convertase (PC) family of enzymes encompasses a group of nine proteases with a wide range of activity on the substrate. [231]

---

[229] White JM, Whittaker GR.
Fusion of Enveloped Viruses in Endosomes.
Traffic. 2016 Jun;17(6):593-614. doi: 10.1111/tra.12389.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4866878/

Zhu G, Zhu C, Zhu Y, Sun F.
Minireview of progress in the structural study of SARS-CoV-2 proteins.
Curr Res Microb Sci. 2020 Sep;1:53-61. doi: 10.1016/j.crmicr.2020.06.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323663/

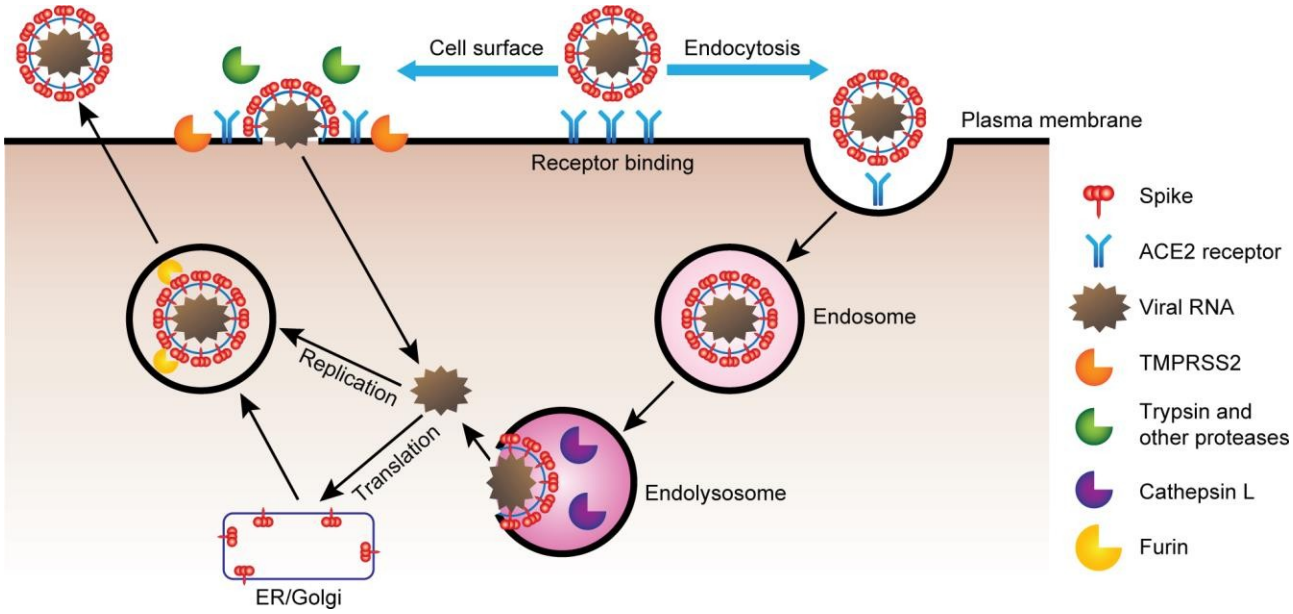
Belouzard S, Millet JK, Licitra BN, Whittaker GR.
Mechanisms of coronavirus cell entry mediated by the viral spike protein.
Viruses. 2012 Jun;4(6):1011-33. doi: 10.3390/v4061011. Epub 2012 Jun 20.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3397359/

Millet JK, Whittaker GR.
Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis.
Virus Res. 2015 Apr 16;202:120-34. doi: 10.1016/j.virusres.2014.11.021. Epub 2014 Nov 22.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4465284/

[230] Heald-Sargent T, Gallagher T.
Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence.
Viruses. 2012 Apr;4(4):557-80. doi: 10.3390/v4040557. Epub 2012 Apr 12.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347323/

[231] Jaimes JA, Millet JK, Whittaker GR.
Proteolytic Cleavage of the SARS-CoV-2 Spike Protein and the Role of the Novel S1/S2 Site.
iScience. 2020 Jun 26;23(6):101212. doi: 10.1016/j.isci.2020.101212. Epub 2020 May 28
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7255728/

In this group, furin is a dibasic endopeptidase and is ubiquitously expressed in the Golgi apparatus of all cells[232] . Trypsin is a serine endopeptidase that is commonly expressed in digestive and respiratory cells and has been described to be highly active as a digestive enzyme in the small intestine.
Cathepsins are a heterogeneous group of proteases that are typically found in endosomes and lysosomes and are involved in numerous processes of antigen degradation and presentation.
Finally, type II transmembrane serine proteases, or TTSPs, are another group of enzymes with different cellular functions that participate in viral activation. This group includes enzymes such as transmembrane serine protease 2 (TMPRSS2) and matriptase.[233]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7888651/
Mechanisms of entry of SARS-CoV-2.
The viral spike protein binds to ACE2 and, in some cases, perhaps NRP1, reactive cells. The viral spike protein is processed by TMPRSS2 and other serine proteases that facilitate entry onto the cell surface or endocytosed into endosomes where the spike is processed by CTSL in the lysosome. Viral RNA released from TMPRSS2-mediated entry or endosome release is replicated as partial and complete genome copies and translated into the ER to form new SARS-CoV-2 virions. Processing of the spike protein by furin occurs before the release of new viruses into the extracellular environment.

The results support the observations that the S1/S2 site of SARS-CoV-2 serves to expand viral trophism to lung cells. [234]

---

[232] Thomas G.
Furin at the cutting edge: from protein traffic to embryogenesis and disease.
Nat Rev Mol Cell Biol. 2002;3(10):753-766. doi:10.1038/nrm934
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1964754/

[233] Jana Koch, Zina M Uckeley, Patricio Doldan, Megan Stanifer, Steeve Boulant, Pierre-Yves Lozach
Host Cell Proteases Drive Early or Late SARS-CoV-2 Penetration
bioRxiv 2020.12.22.423906; doi: https://doi.org/10.1101/2020.12.22.423906
https://www.biorxiv.org/content/10.1101/2020.12.22.423906v1.full.pdf

Murgolo N, Therien AG, Howell B, et al.
SARS-CoV-2 tropism, entry, replication, and propagation: Considerations for drug discovery and development.
PLoS Pathog. 2021;17(2):e1009225. Published 2021 Feb 17. doi:10.1371/journal.ppat.1009225
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7888651/

[234] Hoffmann M, Kleine-Weber H, Pöhlmann S.
A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells.
Mol Cell. 2020 May 21;78(4):779-784.e5. doi: 10.1016/j.molcel.2020.04.022.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194065/

Thomas P. Peacock, et al.

Without pre-cleavage in S1/S2, SARS-CoV-2 would be endocytosed and, due to the low expression of cathepsin L in respiratory endosomes[235] associated with the expression of antiviral restriction factors in endosomes, SARS-CoV-2 would not effectively infect via the respiratory endosome.
Early studies on SARS-CoV-2 showed that the S1/S2 junction contained an additional insert, PRRA (R - arginine, A-alanine), which was not present in SARS-CoV or in the closest bat ancestor CoVs[236] . This insert forms a P-R-R-A-R sequence, and although it contains the minimal furin recognition motif, R-X-X-R, it is unusual and diverges from the preferred and canonical R-X-K/R-R motif.
Compared with the canonical motif, the residues at position P2 and P3 for SARS-CoV-2 S1/S2 are reversed, with R at position P3 instead of position P2 and an A at position P2 instead of position P3.[237]
The presence of an arginine at the third P3 position before the FCS (furin cleavage site) increases its efficiency tenfold[238] . Its presence is also rare and occurs in only 5 of 132 known FCSs. [239]

https://www.biorxiv.org/content/10.1101/2020.10.04.325522v1.full.pdf
Furin cleavage score analysis of CoV S1/S2 cleavage sites. CoV S sequences were analyzed using the ProP1 1.0 and PiTou2 3.0 furin prediction algorithm, generating a score with green numbers indicating predicted furin cleavage and red numbers indicating no predicted furin cleavage. Purple lines indicate the location of the predicted S1/S2 cleavage site. The basic residues of arginine
(R) and lysine (K) are highlighted in blue. Sequence numbers refer to the position of amino acids within the protein spike.
* For Bat-RmYN02, sequence number was determined by S alignment with SARS-CoV-2 S using Geneious. Sequences corresponding to the S1/S2 region of HCoV-HKU1 (AAT98580.1), SARS-CoV (AAT74874.1), SARS-CoV-2 (QHD43416.1), Bat-CoVRaTG13 (QHR63300.2), Bat-SL -CoVZC45 (AVP78031.1), Bat-SL-CoVZXC21 (AVP78042.1), MERS-CoV (AFS88936.1), BatCoV-HKU4 (YP_001039953.1), BatCoV-HKU5 (YP_001039962.1), and Influenza A/Chicken/Hong Kong/822.1/01/H5N1 (AF509026.2) were obtained from GenBank. Sequences corresponding to S1 / S2 and S2 regions of RmYN02 (EPI_ISL_412977) were obtained from GISAID.

The furin cleavage site of SARS-CoV-2 spike protein is a key determinant for transmission due to enhanced replication in airway cells
bioRxiv 2020.09.30.318311; doi: https://doi.org/10.1101/2020.09.30.318311
https://www.biorxiv.org/content/10.1101/2020.09.30.318311v1

[235] Park JE, Li K, Barlan A, Fehr AR, Perlman S, McCray PB Jr, Gallagher T.
Proteolytic processing of Middle East respiratory syndrome coronavirus spikes expands virus tropism.
Proc Natl Acad Sci U S A. 2016 Oct 25;113(43):12262-12267. doi: 10.1073/pnas.1608147113.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5086990/

[236] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS.
Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science. 2020 Mar 13;367(6483):1260-1263. doi: 10.1126/science.abb2507.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/

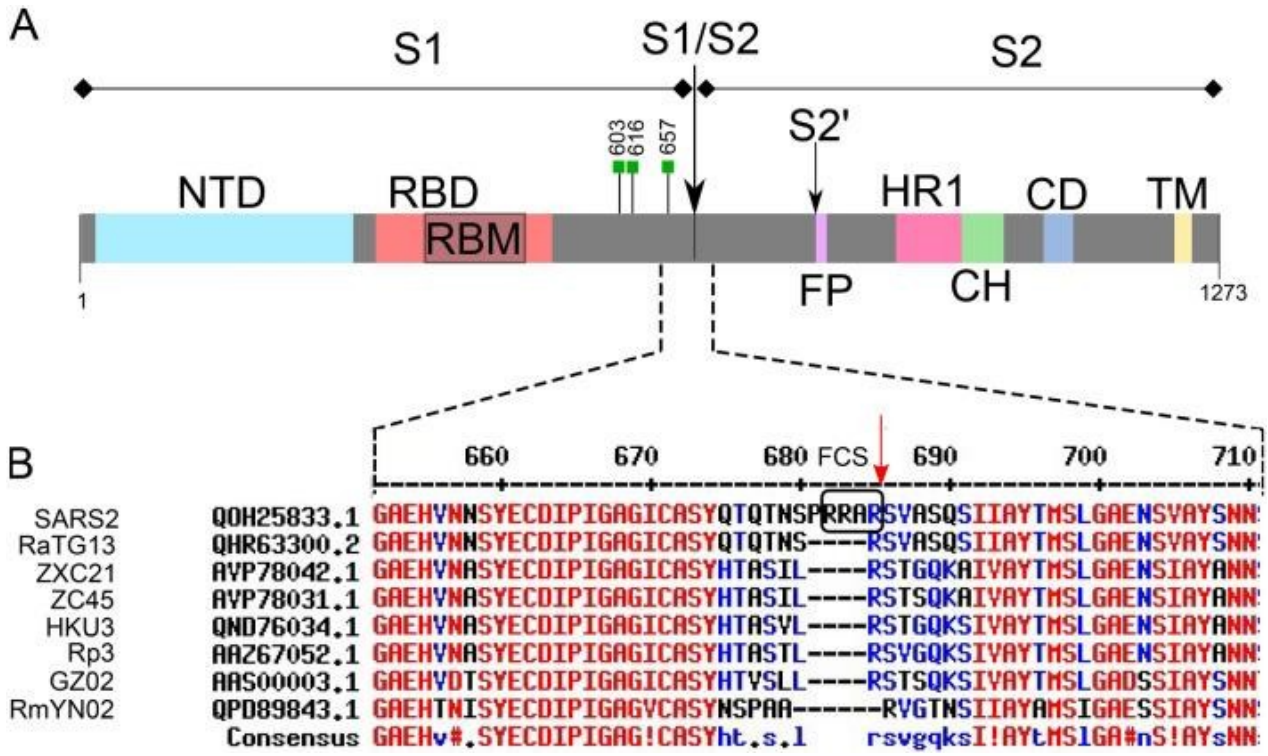
Jaimes JA, André NM, Chappie JS, Millet JK, Whittaker GR.
Phylogenetic Analysis and Structural Modeling of SARS-CoV-2 Spike Protein Reveals an Evolutionary Distinct and Proteolytically Sensitive Activation Loop.
J Mol Biol. 2020 May 1;432(10):3309-3325. doi: 10.1016/j.jmb.2020.04.009.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7166309/

[237] Henrich S, Lindberg I, Bode W, Than ME.
Proprotein convertase models based on the crystal structures of furin and kexin: explanation of their specificity.
J Mol Biol. 2005 Jan 14;345(2):211-27. doi: 10.1016/j.jmb.2004.10.050.
https://pubmed.ncbi.nlm.nih.gov/15571716/

238 Henrich S, Cameron A, Bourenkov GP, Kiefersauer R, Huber R, Lindberg I, Bode W, Than ME.
The crystal structure of the proprotein processing proteinase furin explains its stringent specificity.
Nat Struct Biol. 2003 Jul;10(7):520-6. doi: 10.1038/nsb941. Erratum in: Nat Struct Biol. 2003 Aug;10(8):669.
https://pubmed.ncbi.nlm.nih.gov/12794637/

[239] Lemmin T, Kalbermatter D, Harder D, Plattet P, Fotiadis D.
Structures and dynamics of the novel S1/S2 protease cleavage site loop of the SARS-CoV-2 spike glycoprotein.
J Struct Biol X. 2020;4:100038. doi: 10.1016/j.yjsbx.2020.100038.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7534663/

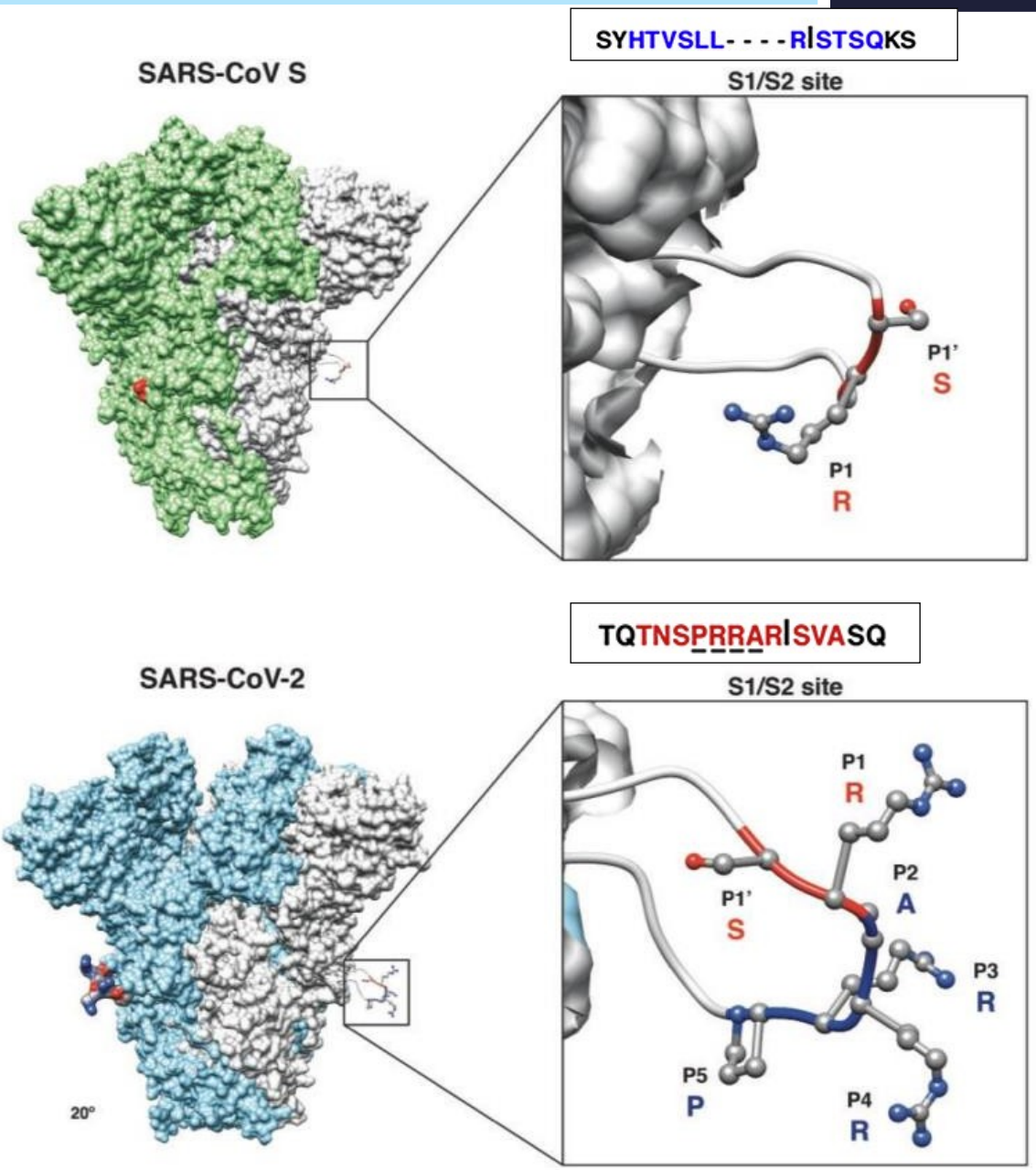
Virus	S1/S2 Sequence	Furin Score PiTou	Furin Score ProP
Betacoronavirus			
Lineage A			
HCoV-HKU1	747-YNSPSSSSSRRKRR|SISASY-766	**+14.634**	**0.918**
Lineage B			
SARS-CoV	654-AGICASYHTVSLLR|STSQKS-673	-5.167	0.123
SARS-CoV-2	672-ASYQTQTNSPRRAR|SVASQS-691	**+9.196**	**0.620**
Bat-RmYN02*	667-GAGVCASYNSPAAR|VGTNSI-686	-5.085	0.111
Bat-CoV RaTG13	668-AGICASYQTQTNSR|SVASQS-687	-4.672	0.151
Bat-CoV ZC45	645-AGICASYHTASILR|STSQKA-664	-5.333	0.153
Bat-CoV ZXC21	644-AGICASYHTASILR|STGQKA-663	-5.333	0.170
Lineage C			
MERS-CoV	738-LPDTPSTLTPRSVR|SVPGEM-757	**+5.155**	**0.563**
BatCoV-HKU4	736-GQSLCAVPPVSTFR|SYSASQ-755	-5.14	0.229
BatCoV-HKU5	732-LCAIPPTTSSRVRR|ATSGAS-751	**+10.259**	**0.822**
Influenza A			
H5N1	322-LRNTPQRERRRKKR|GLFGAI-341	**+13.59**	**0.808**



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7993900/

SARS-CoV-2 spike protein showing subunits and domains as well as local sequence alignments with other beta-coronaviruses. **(A)** Spike domains: N-terminal domain (NTD), receptor binding domain (RBD), receptor binding motif (RBM), fusion peptide (FP), hetapad repeat 1 (HR1), central helix (CH), connector domain (CD) , transmembrane domain (TM), multibasic furin cleavage site (FCS) indicated by a black box. S1 ↓ S2 and S2 ′ cleavage sites are indicated by arrows. Green boxes indicate the location of N-glycosylated residues proximal to FCS. **(B)** MultAlin alignments using reference to the sequence numbering of the SARS-CoV-2 spike protein (Corpet *1988*) (*(*http://www.sacs.ucsf.edu/cgi-bin/multalin.py). Definition and accession numbers as follows: SARS2: SARS-CoV-2 Wuhan-Hu-1 (*QOH25833.1*); RaTG13 (*QHR63300.2*); ZXC21: bat-SL-CoVZXC21 (*AVP78042.1*); ZC45: bat-SL-CoVZC45 (*AVP78031.1*); HKU3: Bat SARS coronavirus HKU3 (*QND76034.1*); Rp3: Rp3 / 2004 (*AAZ67052.1*); GZ02: SARS coronavirus GZ02 (*AAS00003.1*); RmYN02: Bat coronavirus RmYN02 (*QPD89843.1*).

Sequence indices related to SARS-CoV-2 are shown.



https://www.biorxiv.org/content/10.1101/2020.10.04.325522v1.full.pdf
Predicted structural model of SARS-CoV and SARS-CoV-2 S proteins. (Box) Enlargement of S1/S2 site with conserved R and S residues (red ribbon) and insertion of four unique P-R-R-A amino acids for SARS-CoV-2 (blue ribbon) are shown. The P's denote the location of that amino acid from the S1/S2 cleavage site, with P1-P5 referring to amino acids before the cleavage site and P1 'referring to amino acids after the cleavage site.

In addition, the "RRAR" motif creates a C-terminal motif (CendR) with a binding site for neuropilin membrane receptors (NRP1 and NRP2)[240,] which are more widely expressed than ACE2.
It has been shown that NRP1 can act as an alternative pathway for virus entry, particularly into the olfactory epithelial cells of the nasal cavity.
NRP1 requires a substrate cleaved by furin to facilitate virus entry, and mRNA expression of NRP1 is elevated in SARS-CoV-2-infected cells in COVID-19 patients. [241]

[240] Teesalu T, Sugahara KN, Kotamraju VR, Ruoslahti E.
C-end rule peptides mediate neuropilin-1-dependent cell, vascular, and tissue penetration.
Proc Natl Acad Sci U S A. 2009;106(38):16157-16162. doi:10.1073/pnas.0908201106
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2752543/

[241] Cantuti-Castelvetri L, Ojha R, Pedro LD, et al.
Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity.

NRP-1 and SARS-Cov-2

NRP-1 is a cell surface receptor that plays an essential role in angiogenesis[242] , regulation of vascular permeability[243] , development of the nervous system (axonal guidance and synapse formation of the GABA-ergic circuit and retinal vasculature)[244] , and immunological synapse formation between dendritic cells and T cells[245] .

Science. 2020;370(6518):856-860. doi:10.1126/science.abd2985
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857391/

Daly JL, et al
Neuropilin-1 is a host factor for SARS-CoV-2 infection.
Science. 2020 Nov 13;370(6518):861-865. doi: 10.1126/science.abd3072.
https://science.sciencemag.org/content/370/6518/861/tab-pdf

[242] Plein A, Fantin A, Ruhrberg C.
Neuropilin regulation of angiogenesis, arteriogenesis, and vascular permeability.
Microcirculation. 2014 May;21(4):315-23. doi: 10.1111/micc.12124.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4230468/

Guo HF, Vander Kooi CW.
Neuropilin Functions as an Essential Cell Surface Receptor.
J Biol Chem. 2015 Dec 4;290(49):29120-6. doi: 10.1074/jbc.R115.687327.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4705917/

[243] Becker PM, Waltenberger J, Yachechko R, Mirzapoiazova T, Sham JS, Lee CG, Elias JA, Verin AD. Neuropilin-1 regulates vascular endothelial growth factor-mediated endothelial permeability.
Circ Res. 2005 Jun 24;96(12):1257-65. doi: 10.1161/01.RES.0000171756.13554.49.
https://www.ahajournals.org/doi/epub/10.1161/01.RES.0000171756.13554.49

Acevedo LM, Barillas S, Weis SM, Göthert JR, Cheresh DA.
Semaphorin 3A suppresses VEGF-mediated angiogenesis yet acts as a vascular permeability factor.
Blood. 2008 Mar 1;111(5):2674-80. doi: 10.1182/blood-2007-08-110205.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2254547/

Mamluk R, Klagsbrun M, Detmar M, Bielenberg DR.
Soluble neuropilin targeted to the skin inhibits vascular permeability.
Angiogenesis. 2005;8(3):217-27. doi: 10.1007/s10456-005-9009-6.
https://pubmed.ncbi.nlm.nih.gov/16328161/

[244] Telley L, Cadilhac C, Cioni JM, Saywell V, Jahannault-Talignani C, Huettl RE, Sarrailh-Faivre C, Dayer A, Huber AB, Ango F.
Dual Function of NRP1 in Axon Guidance and Subcellular Target Recognition in Cerebellum.
Neuron. 2016 Sep 21;91(6):1276-1291. doi: 10.1016/j.neuron.2016.08.015.
https://www.cell.com/neuron/pdfExtended/S0896-6273(16)30507-4

Erskine L, François U, Denti L, et al.
VEGF-A and neuropilin 1 (NRP1) shape axon projections in the developing CNS via dual roles in neurons and blood vessels.
Development. 2017;144(13):2504-2516. doi:10.1242/dev.151621
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5536872/

Ango F.
NRP1 and synapse formation.
Oncotarget. 2016 Dec 13;7(50):81975-81976. doi: 10.18632/oncotarget.13462.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5347665/

[245] Romeo PH, Lemarchandel V, Tordjman R.
Neuropilin-1 in the immune system. Adv Exp Med Biol. 2002;515:49-54. doi: 10.1007/978-1-4615-0119-0_4.
https://pubmed.ncbi.nlm.nih.gov/12613542/

Mayi BS, Leibowitz JA, Woods AT, Ammon KA, Liu AE, Raja A.
The role of neuropilin-1 in COVID-19.
PLoS Pathog. 2021 Jan 4;17(1):e1009153. doi: 10.1371/journal.ppat.1009153.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7781380/

Roy S, Bag AK, Singh RK, Talmadge JE, Batra SK, Datta K.
Multifaceted Role of Neuropilins in the Immune System: Potential Targets for Immunotherapy.
Front Immunol. 2017 Oct 10;8:1228. doi: 10.3389/fimmu.2017.01228.

Tordjman R, Lepelletier Y, Lemarchandel V, Cambot M, Gaulard P, Hermine O, Roméo PH.

VEGF-A165 and other NRP-1 ligands possess a CendR C-terminal sequence that interacts with the b1 domain of NRP-1 and causes cellular internalization and vascular leakage. One of the pathways of VEGF-mediated vascular extravasation involves a transcellular pathway through a labyrinth of conduits called vesicular organelles[246] , and it is possible that CendR peptides also activate this pathway.
Proteolytic cleavage of viral envelope proteins by exposure of CendR elements seems to be a recurrent theme in tissue dissemination and infectivity of many viruses, in addition to SARS-Cov-2 (some examples are given in Table S1).
Pathological vascular edema is associated with many disease states, such as those occurring in hemorrhagic virus infections, sepsis, and organ-specific vascular extravasation syndromes. [247]

Table S1. Examples of viruses with surface CendR elements

Virus	Protein	Sequence [*: cleavage site]	Reference
Human cytomegalovirus	Envelope glycoprotein B (UL55)	LNITH**RTRR***STSDN	(1)
Measles virus	Fusion protein	SVASSR**RHKR***FAGVV	(2)
Tick-born encephalitis virus	PreM protein	KQEGS**RTRR***SVLIP	(3)
Respiratory syncytial virus	Fusion protein	PATNN**RARR***ELPRF	(4)
Influenza A virus (H5N1)	Hemagglutinin	PQRERR**RKKR***GLFGA	(5)
HIV-1	Envelope precursor gp160	RRVVQ**REKR***AVGIG	(6)
Zaire ebolavirus	Virion spike glycoprotein precursor	LITGG**RRTR***REAIV	(7)
Mumps virus	Fusion protein	PSSGSR**RHKR***FAGIA	(8)
Yellow fever virus	PreM protein	CDSAG**RSRR***SRRAI	(9)
Human herpesvirus 4	BALF4 (glycoprotein B)	AAVL**RRRR***RDAGN	(10)
Human metapneumo-virus	Fusion glycoprotein precursor	QIENP**RQSR***FVLGA	(11)
Human T-lymphotropic virus-2	Env propeptide	PPPAT**RRRR***AVPIA	(12)
Crimean-Congo hemorrhagic fever virus	Glycoprotein precursor	PSPTN**RSKR***NLKME	(13)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2752543/ [248]

A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response.
Nat Immunol. 2002 May;3(5):477-82. doi: 10.1038/ni789. Epub 2002 Apr 15. Erratum in: Nat Immunol. 2003 Apr;4(4):394.
https://pubmed.ncbi.nlm.nih.gov/11953749/

[246] Dvorak AM, Feng D.
The vesiculo-vacuolar organelle (VVO). A new endothelial cell permeability organelle.
J Histochem Cytochem. 2001 Apr;49(4):419-32. doi: 10.1177/002215540104900401.
https://journals.sagepub.com/doi/pdf/10.1177/002215540104900401

Ono S, Egawa G, Kabashima K.
Regulation of blood vascular permeability in the skin.
Inflamm Regen. 2017 Jul 10;37:11. doi: 10.1186/s41232-017-0042-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5725833/

Dvorak AM, Feng D.
The vesiculo-vacuolar organelle (VVO). A new endothelial cell permeability organelle.
J Histochem Cytochem. 2001 Apr;49(4):419-32. doi: 10.1177/002215540104900401
https://journals.sagepub.com/doi/epub/10.1177/002215540104900401

Feng D, Nagy JA, Hipp J, Dvorak HF, Dvorak AM.
Vesiculo-vacuolar organelles and the regulation of venule permeability to macromolecules by vascular permeability factor, histamine, and serotonin. J Exp Med. 1996 May 1;183(5):1981-6. doi: 10.1084/jem.183.5.1981.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2192559/pdf/je18351981.pdf

[247] Basu A, Chaturvedi UC.
Vascular endothelium: the battlefield of dengue viruses.
FEMS Immunol Med Microbiol. 2008 Aug;53(3):287-99. doi: 10.1111/j.1574-695X.2008.00420.x. Epub 2008 Jul 3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7110366/

Weis MS.
Vascular permeability in cardiovascular disease and cancer.
Curr Opin Hematol. 2008 May;15(3):243-9. doi: 10.1097/MOH.0b013e3282f97d86.
https://pubmed.ncbi.nlm.nih.gov/18391792/

[248] References shown in the table

1. Vey M, et al. (1995) Proteolytic processing of human cytomegalovirus glycoprotein B (gpUL55) is mediated by the human endoprotease furin. *Virology* 206:746 -749.
2. Varsanyi TM, Jornval lH, NorrbyE(1985) Isolation and characterization of the measles virus F1 polypeptide: comparison with other paramyxovirus fusion proteins.*Virology147*:110-117.

Preclinical studies have suggested that neuropilin-1 (NRP1) shows high expression in the respiratory and olfactory epithelium, and may be implicated in the neurological manifestations of COVID-19 by promoting the entry of SARS- CoV-2 into the brain through the olfactory epithelium.
Davies et al showed that NRP1 is also expressed in the CNS, including olfactory-related regions such as olfactory tubercles and paraolfactory gyri.[249]
Variability in expression by age, race, or sex may explain the differing morbidity of infection and the inverse association between anosmia and severity; with higher expression there may be a greater risk of impaired olfactory function, but also a greater activation of regulatory T lymphocytes that could suppress the cytokine storm.[250]
Since both protein S and VEGF-A, a pro-nociceptive and angiogenic agent, engage NRP-1 in a common binding domain, it is worth examining the potential of protein S to inhibit VEGF-A/NRP-1 signaling.
Given the higher levels of VEGF-A observed in COVID-19 patients, complaints related to increased pain were expected; instead, it was found that S protein blocks VEGF-A/NRP-1 signaling, which promotes nociception, inducing analgesia. [251] This mechanism could promote asymptomatic presentation of the disease or failure to perceive the disease state.

3. Chambers TJ, et al. (1990) Evidence that the N-terminal domain of nonstructural protein NS3 from yellow fever virus is a serine protease responsible for site-specific cleavages in the viral polyprotein. *Proc Natl Acad Sci USA* 87:8898-8902.
4. González-Reyes L, et al. (2001) Cleavage of the human respiratory syncytial virus fusion protein at two distinct sites is required for activation of membrane fusion. *Proc Natl Acad Sci USA* 98:9859-9864.
5. Steinhauer DA (1999) Role of hemagglutinin cleavage for the pathogenicity of influenza virus. *Virology* 258:1-20.
6. Moulard M, Decroly E (2000) Maturation of HIV envelope glycoprotein precursors by cellular endoproteases. *Biochim Biophys Acta* 1469:121-132.
7. Wool-Lewis RJ, Bates P (1999) Endoproteolytic processing of the ebola virus envelope glycoprotein: Cleavage is not required for function. *J Virol* 73:1419 -1426.
8. Elango N, Varsanyi TM, Kövamees J, Norrby E (1989) The mumps virus fusion protein mRNA sequence and homology among the paramyxoviridae proteins. *J Gen Virol* 70:801- 807.
9. Ruiz-Linares A, Cahour A, Després P, Girard M, Bouloy M (1989) Processing of yellow fever virus polyprotein: Role of cellular proteases in maturation of the structural proteins. *J Virol* 63:4199 - 4209.
10. Johannsen E, et al. (2004) Proteins of purified Epstein-Barr virus. *Proc Natl Acad Sci USA* 101:16286-16291.
11. Biacchesi S, et al. (2006) Modification of the trypsin-dependent cleavage activation site of the human metapneumovirus fusion protein to be trypsin independent does not increase replication or spread in rodents or nonhuman primates. *J Virol* 80:5798 -5806.
12. Sjöberg M, Wallin M,Lindqvist B,Garoff H (2006) Furin cleavage potentiates the membrane fusion-controlling inter subunit disulfide bond isomerization activity of leukemia virus Env. *J Virol* 80:5540-5551.
13. Sanchez AJ, Vincent MJ, Erickson BR, Nichol ST (2006) Crimean-congo hemorrhagic fever virus glycoprotein precursor is cleaved by Furin-like and SKI-1 proteases to generate a novel 38-kilodalton glycoprotein. *J Virol* 80:514-525.

[249] Davies J, Randeva HS, Chatha K, et al.
Neuropilin-1 as a new potential SARS-CoV-2 infection mediator implicated in the neurologic features and central nervous system involvement of COVID-19.
Mol Med Rep. 2020;22(5):4221-4226. doi:10.3892/mmr.2020.11510
https://pubmed.ncbi.nlm.nih.gov/33000221/

Ludovico Cantuti-Castelvetri, et al.
Neuropilin-1 facilitates SARS-CoV-2 cell entry and provides a possible pathway into the central nervous system
bioRxiv 2020.06.07.137802; doi: https://doi.org/10.1101/2020.06.07.137802
https://www.biorxiv.org/content/10.1101/2020.06.07.137802v3

Cantuti-Castelvetri L, et al
Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity.
Science. 2020 Nov 13;370(6518):856-860. doi: 10.1126/science.abd2985.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857391/

[250] Hopkins C, Lechien JR, Saussez S.
More that ACE2? NRP1 may play a central role in the underlying pathophysiological mechanism of olfactory dysfunction in COVID-19 and its association with enhanced survival.
Med Hypotheses. 2021 Jan;146:110406. doi: 10.1016/j.mehy.2020.110406.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7678428/

[251] Moutal A, Martin LF, Boinon L, et al.
SARS-CoV-2 spike protein co-opts VEGF-A/neuropilin-1 receptor signaling to induce analgesia.
Pain. 2021;162(1):243-252. doi:10.1097/j.pain.0000000000002097

Mechanism of viral fusion

After cleavage of the S protein, the FP of SARS-CoV-2 is exposed and triggers viral fusion. [252] The distance between the viral membrane and the host cell membrane is shortened, and the HR1 domain of the S protein is close to the host cell membrane, while the HR2 domain is closer to the side of the viral membrane.

Then, HR2 folds over HR1, the two HR domains form a six-helix structure in an antiparallel fusion core format, the viral membrane is pulled toward the host cell membrane and binds tightly to it until the two membranes fuse.[253]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7737878/

Jobe A, Vijayan R.
Neuropilins: C-end rule peptides and their association with nociception and COVID-19
[published online ahead of print, 2021 Mar 26]. Comput Struct Biotechnol J. 2021;10.1016/j.csbj.2021.03.025. doi:10.1016/j.csbj.2021.03.025
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7997051/

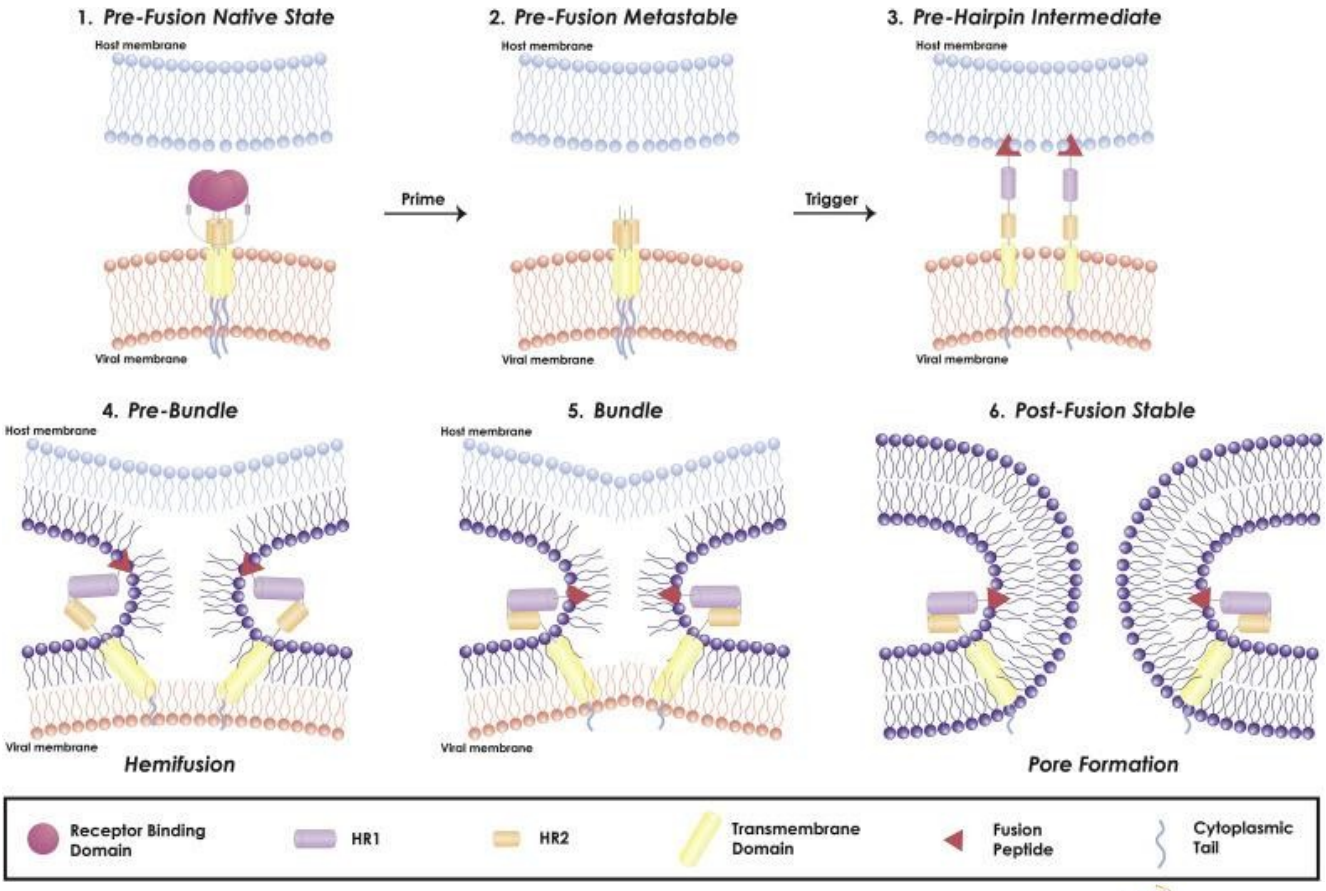
Cantuti-Castelvetri L, Ojha R, Pedro LD, et al.
Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity.
Science. 2020;370(6518):856-860. doi:10.1126/science.abd2985
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857391/

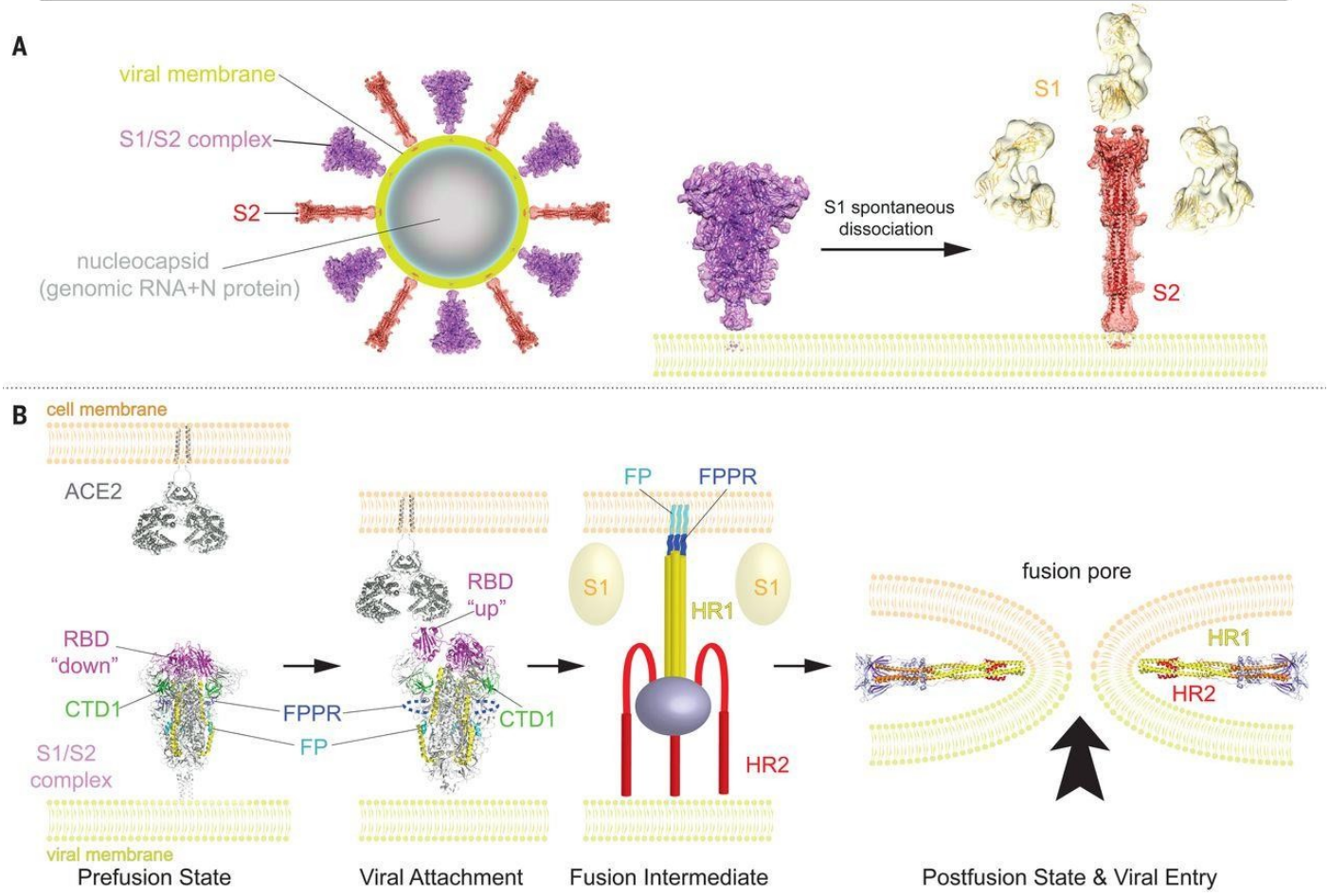
Kyrou I, Randeva HS, Spandidos DA, Karteris E.
Not only ACE2-the quest for additional host cell mediators of SARS-CoV-2 infection: Neuropilin-1 (NRP1) as a novel SARS-CoV-2 host cell entry mediator implicated in COVID-19.
Signal Transduct Target Ther. 2021 Jan 18;6(1):21. doi: 10.1038/s41392-020-00460-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7812344/

https://www.asbmb.org/asbmb-today/science/110820/a-second-pathway-into-cells-for-sars-cov-2

[252] Kawase M, Kataoka M, Shirato K, Matsuyama S.
Biochemical Analysis of Coronavirus Spike Glycoprotein Conformational Intermediates during Membrane Fusion.
J Virol. 2019;93(19):e00785-19. Published 2019 Sep 12. doi:10.1128/JVI.00785-19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6744234/

[253] Eckert DM, Kim PS.
Mechanisms of viral membrane fusion and its inhibition.
Annu Rev Biochem. 2001;70:777-810. doi: 10.1146/annurev.biochem.70.1.777.
https://pubmed.ncbi.nlm.nih.gov/11395423/





https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464562/

A model for structural rearrangements of the SARS-Cov-2 S protein.
(A) Structural changes independent of a target cell. We suggest that both prefusion and postfusion spikes are present on the surface of the mature virion, and the ratio between them may vary (virion diagram). Postfusion spikes on the virion are formed by S2 after S1 dissociates in the absence of ACE2.
(B) ACE2-dependent structural rearrangements. The structural transition from the prefusion conformation to the postfusion conformation that induces membrane fusion probably proceeds in steps as follows:
1) FPPR blocks the RBD through CTD1 in the S trimester of the prefusion (this study), but occasionally flips from position and allows an RBD to present the "up" conformation (PDB ID: 6vyb).
2) Binding of RBD with ACE2 (PBD ID: 6m17) creates a flexible FPPR that allows exposure of the S2' cleavage site immediately upstream of the adjacent fusion peptide (FP). Cleavage at the S2 site, and perhaps also at the S1 / S2 site, releases structural constraints on the fusion peptide and initiates a cascade of folding events in S2, likely accompanied by complete dissociation of S1.
3) Formation of the central spiral coil with three long filaments and folding of HR2.
4) Formation of the postfusion structure of S2 (this study) that joins the two membranes, facilitating t h e formation of a fusion pore and viral entry.

Video
Viral Cell Entry of the SARS-CoV-2 virus
Model of Membrane Fusion by SARS CoV-2 Spike Protein

The "open" and "closed" conformations of the Spike

The S protein undergoes several significant transformations during maturation and for its function, accompanied by conformational changes. These changes in various coronaviruses have been the subject of more than a decade of research[254] .
As detailed earlier, the S protein of SARS-CoV-2 virus functions as a trimer and consists of three identical molecules, which are encoded by the same gene. [255]
The S1 subunit can be in two conformations: *open* and *closed*, and consequently the RBD domain can be in "up" or "down" positions.
It has been shown that the RBD domain of the S protein of SARS-CoV-2 virus is mainly in position "down"[256] and that the closed conformation form of the protein is weakly immunogenic[257] .

[254] Tripet B, Howard MW, Jobling M, Holmes RK, Holmes KV, Hodges RS.
Structural characterization of the SARS-coronavirus spike S fusion protein core.
J Biol Chem. 2004 May 14;279(20):20836-49. doi: 10.1074/jbc.M400759200.
https://jbc.org/retrieve/pii/S0021925820669368

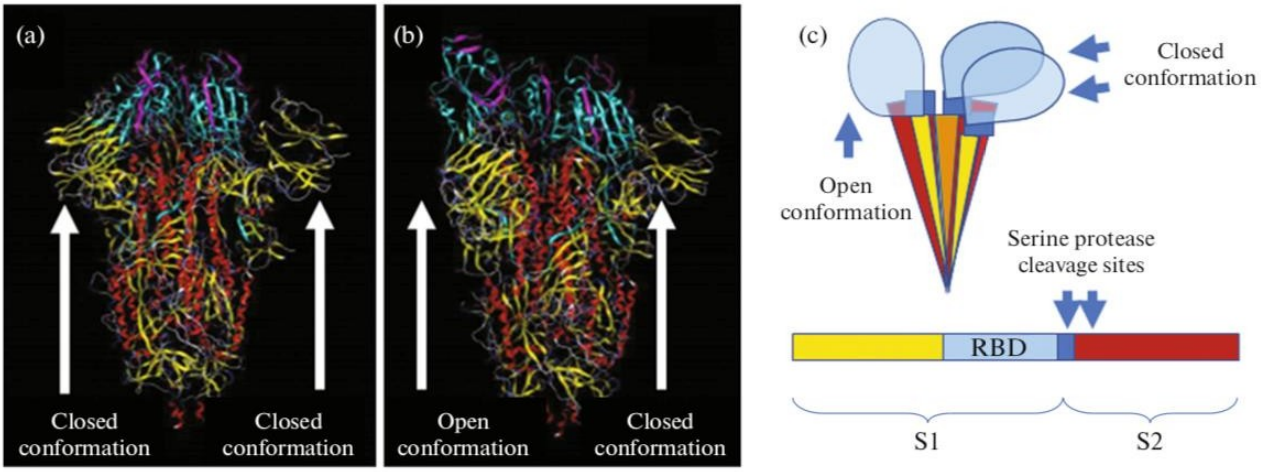
Walls AC, Tortorici MA, Snijder J, et al.
Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion.
Proc Natl Acad Sci U S A. 2017;114(42):11157-11162. doi:10.1073/pnas.1708727114
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5651768/

Li F, Berardi M, Li W, Farzan M, Dormitzer PR, Harrison SC.
Conformational states of the severe acute respiratory syndrome coronavirus spike protein ectodomain.
J Virol. 2006;80(14):6794-6800. doi:10.1128/JVI.02744-05
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1489032/

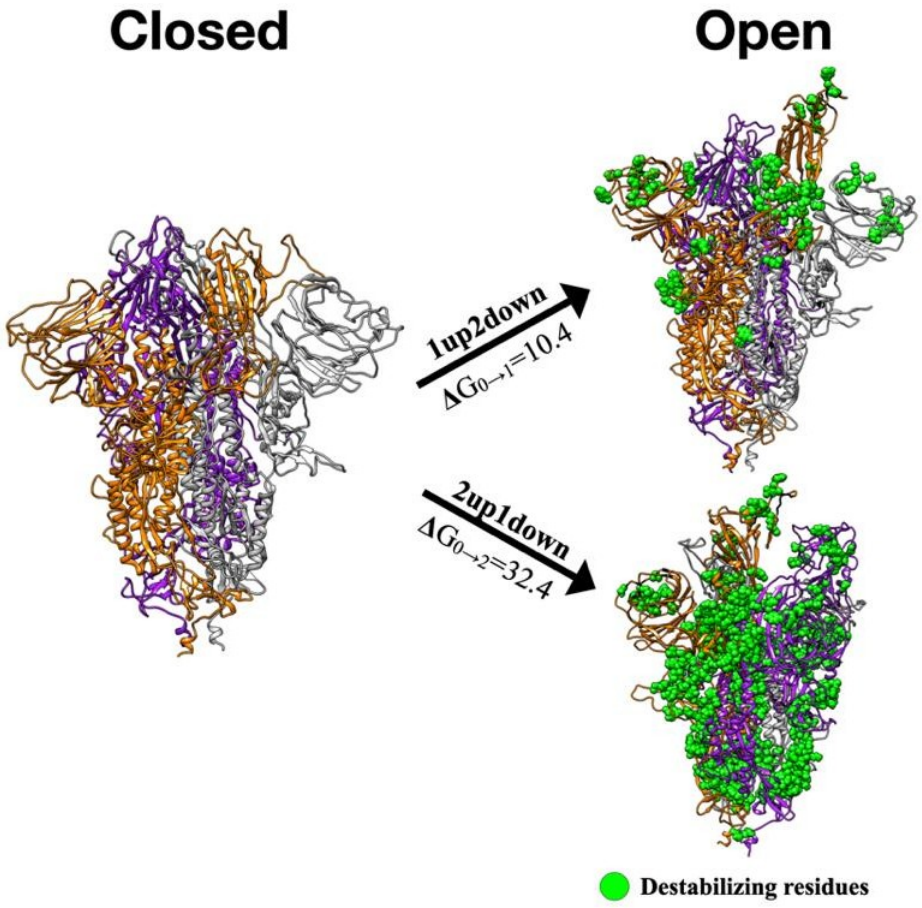
[255] Lan, J., Ge, J., Yu, J. et al.
Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.
Nature 581, 215-220 (2020). https://doi.org/10.1038/s41586-020-2180-5
https://www.nature.com/articles/s41586-020-2180-5

[256] Shang J, Wan Y, Luo C, et al.
Cell entry mechanisms of SARS-CoV-2.
Proc Natl Acad Sci U S A. 2020;117(21):11727-11734. doi:10.1073/pnas.2003138117
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7260975/

[257] Yong Jia, et al
Analysis of the mutation dynamics of SARS-CoV-2 reveals the spread history and emergence of RBD mutant with lower ACE2 binding affinity
bioRxiv 2020.04.09.034942; doi: https://doi.org/10.1101/2020.04.09.034942
https://www.biorxiv.org/content/10.1101/2020.04.09.034942v1.full.pdf

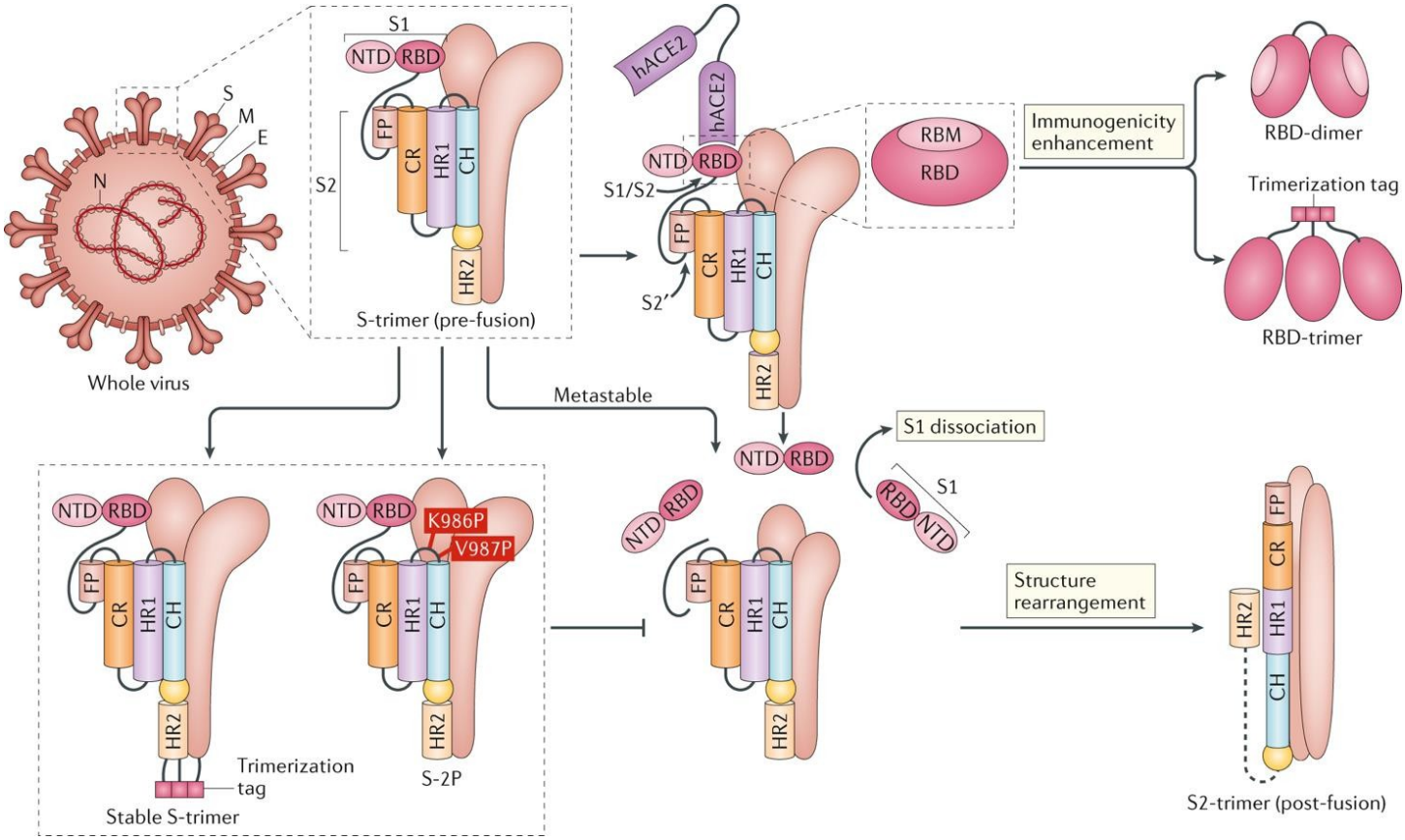


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7473411/
S protein conformations in the homotrimers. **(a)** All S1 subunits are in a closed conformation. **(b)** One subunit is in an open conformation and one is in a closed conformation. **(c)** The conformations of protein S in the trimer and the structure of the protein domain are shown schematically. The receptor binding domain (RBD, blue), together with the N-terminal domain (yellow), is part of the S1 subunit. Within the S1 subunit (blue) is a proteolytic cleavage site for furin, and within the S2 subunit (brown) is a TMPRSS2 protease cleavage site [258]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7730245/
The transition in the S protein of SARS-Cov-2 occurs at the cost of a destabilization of some residues and a ΔG in energy

[258] Hoffmann M, Kleine-Weber H, Pöhlmann S.
A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells.
Mol Cell. 2020;78(4):779-784.e5. doi:10.1016/j.molcel.2020.04.022
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194065/



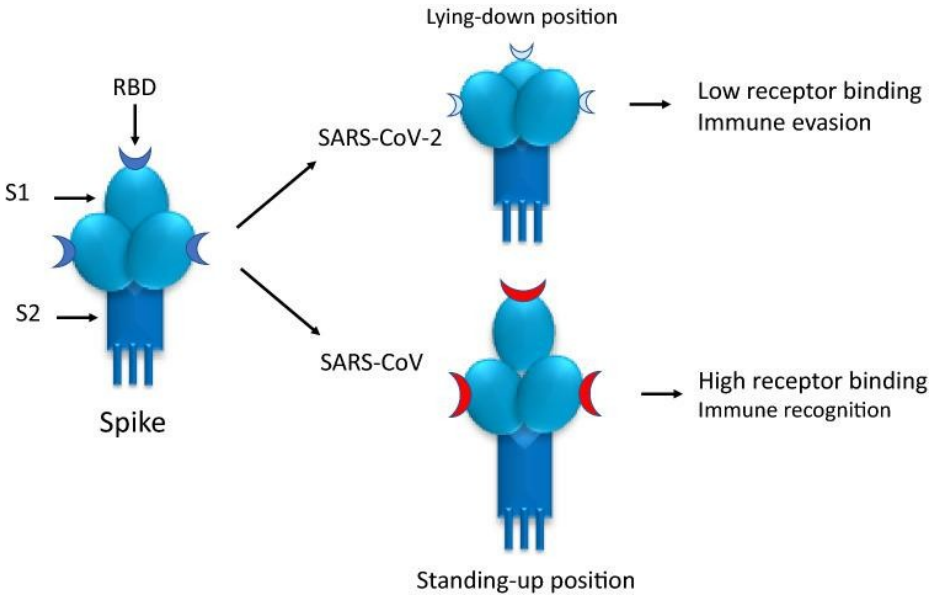
https://www.nature.com/articles/s41577-020-00480-0/

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) contains four main structure proteins: spike (S) proteins, membrane (M) and envelope (E) proteins, which are embedded on the surface of the virion, and nucleocapsid (N) proteins, which binds viral RNA within the virion. The trimer of the S protein in its pre-fusion conformation is shown. The S protein includes the S1 subunit (which includes the N-terminal domain (NTD) and receptor binding domain (RBD)) (the receptor binding motif (RBM) within the RBD is also labeled) and the S2 subunit (which includes the fusion peptide (FP), the connection region (CR), the heptad repeat 1 (HR1), the heptad repeat (HR2) and the central helix (CH)). The SARS-CoV-2 S protein binds to its host receptor, the dimeric human angiotensin-converting enzyme 2 (hACE2), via the RBD and dissociates the S1 subunits.

Cleavage at both S1 - S2 and S2 ′ sites allows structural rearrangement of the S2 subunit required for virus-host membrane fusion. The S2 trimer in its post-fusion arrangement is shown. The RBD is an interesting vaccine target. Generation of an RBD-dimer or RBD-trimer has been shown to increase the immunogenicity of RBD-based vaccines. A stabilized S-trimer shown with a C-terminal trimer tag is a vaccine target. The pre-fusion S protein is generally metastable during in vitro preparations and tends to transform into its post-fusion conformation. Mutation of two residues (K986 and V987) to proline stabilizes protein S (S-2P) and prevents structural change from pre-fusion to post-fusion.

After approaching the ACE2 receptor, the RBD domain of SARS-CoV-2 is activated and binds to the receptor. This interaction is characterized by a higher binding constant than in SARS-CoV-1, and it was seen that the open conformation of the S protein is more characteristic for SARS-CoV-1 and the closed conformation for SARS-CoV-2. [259]

[259] Rossi GA, Sacco O, Mancino E, Cristiani L, Midulla F.
Differences and similarities between SARS-CoV and SARS-CoV-2: spike receptor-binding domain recognition and host cell infection with support of cellular serine proteases.
Infection. 2020 Oct;48(5):665-669. doi: 10.1007/s15010-020-01486-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7393809/



The receptor binding domain (RBD) of the S protein can constantly switch from a "lying down" (down or closed) to a "standing up" (up or open) position. In SARS-CoV-2, the RBD is mainly in the "lying down" position, a state associated not only with ineffective receptor binding but also with immune evasion. In SARS-CoV, the RBD is mostly in the "standing" position, a state associated not only with high effective receptor binding but also with immune recognition.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7393809/

Therefore, the S1 subunit of protein S is capable of significant conformational rearrangements and can exist in at least two conformational states. It should be noted that a biophysical study of the SARS-CoV-2 protein S and a structural analysis with a resolution of 3.5 Å showed that most frequently two of the three S1 subunits of the S protein trimer are in the closed conformation and one is open.[260]
If all three trimer chains are equivalent, four different conformations are possible for a trimer.

ADE epitope and mRNA vaccines

After vaccination, a cell may present the S protein produced (or its subunits/peptide fragments) to mobilize immune responses or be abolished by the immune system (e.g., by cytotoxic T cells). [261]

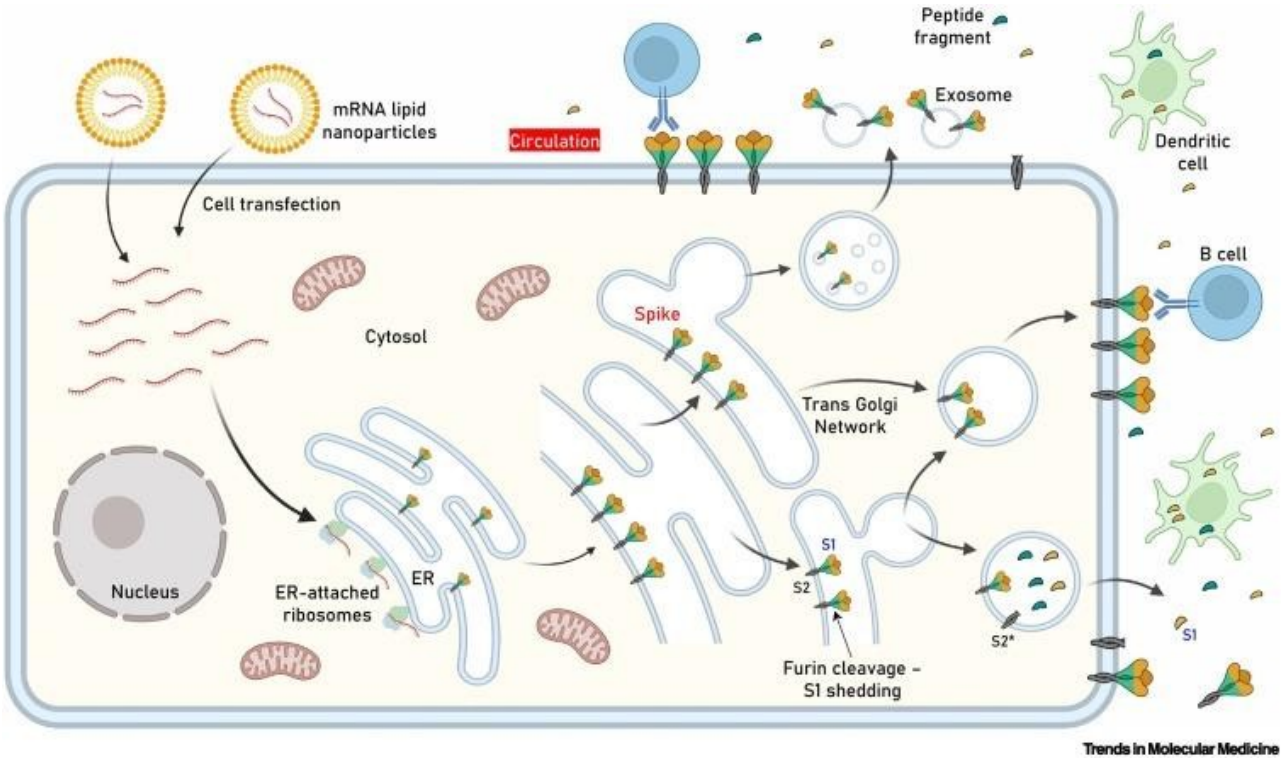
[260] Kirchdoerfer RN, Wang N, Pallesen J, et al.
Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis
Published correction in Sci Rep. 2018 Dec 10;8(1):17823. Sci Rep. 2018;8(1):15701. Published 2018 Oct 24. doi:10.1038/s41598-018-34171-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6200764/

Peters MH, Bastidas O, Kokron DS, Henze CE.
Static all-atom energetic mappings of the SARS-Cov-2 spike protein and dynamic stability analysis of "Up" versus "Down" protomer states. PLoS One. 2020 Nov 10;15(11):e0241168. doi: 10.1371/journal.pone.0241168.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7654774/

[261] Trougakos IP, Terpos E, Alexopoulos H, Politou M, Paraskevis D, Scorilas A, Kastritis E, Andreakos E, Dimopoulos MA.
Adverse effects of COVID-19 mRNA vaccines: the spike hypothesis.
Trends Mol Med. 2022 Jul;28(7):542-554. doi: 10.1016/j.molmed.2022.04.007.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9021367/

Chaudhary N, Weissman D, Whitehead KA.
mRNA vaccines for infectious diseases: principles, delivery and clinical translation.
Nat Rev Drug Discov. 2021 Nov;20(11):817-838. doi: 10.1038/s41573-021-00283-5. Epub 2021 Aug 25. Erratum in: Nat Rev Drug Discov. 2021 Sep 21
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8386155/

Consequently, the debris produced, or even the direct secretion (including "shedding", dispersion) of the antigen by the transfected cells, can release large amounts of the S protein or its subunits/peptide fragments into the circulation,[262] as also occurs during infection. [263]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9021367/

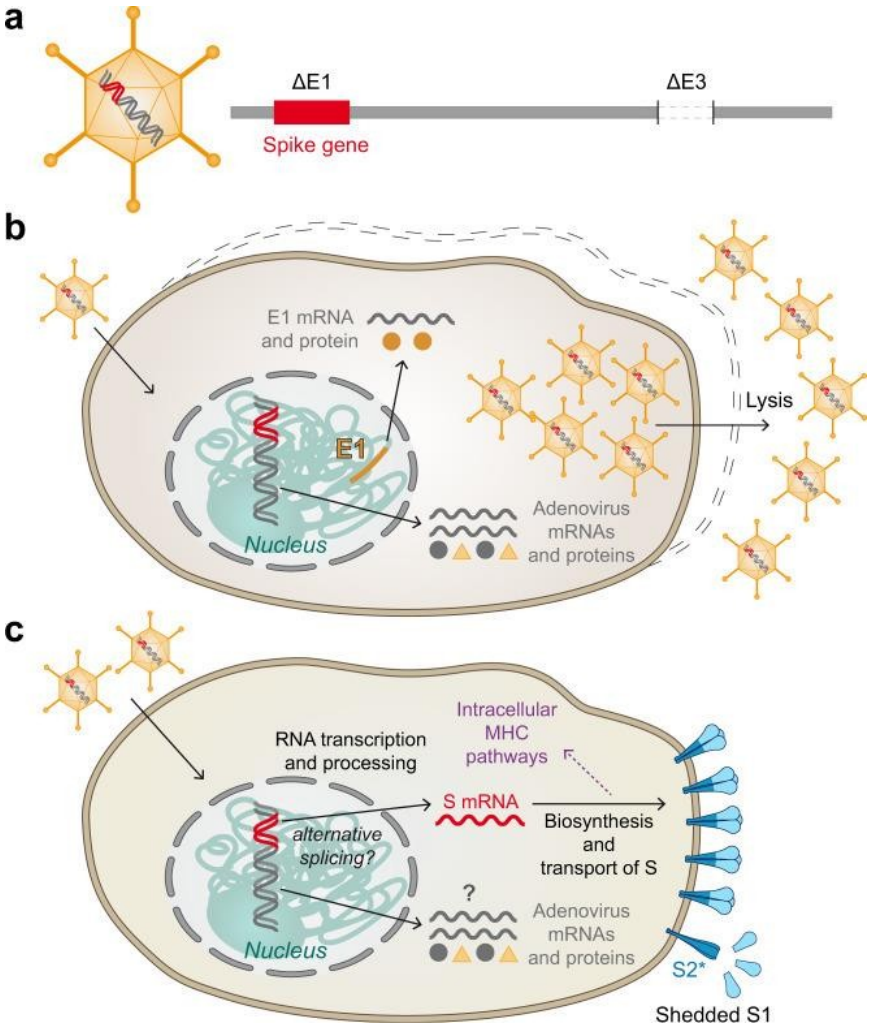
Key Figure. Antigen-localization expression after cell transfection with lipid nanoparticles (LNPs) containing spike protein (S) mRNA used in SARS-CoV-2 mRNA vaccines.

After LNP internalization and mRNA release, the authentic viral peptide-signal (as in Pfizer-BioNTech and Moderna vaccines) drives antigen production into the lumen of the endoplasmic reticulum (ER) where it adopts its natural transmembrane localization via subunit 2 (S2) anchoring. After sorting in the trans Golgi network (TGN), the S protein acquires its final position in the transfected human cell membrane, where S1 is exposed to the extracellular space (i.e., it can face the circulation). Although the extent of antigen expression per cell remains unknown, it is reasonable to assume that this process results in a rather extensive decoration of transfected cells with protein S. Furin-mediated proteolytic cleavage (as in SARS-CoV-2-infected cells) in the absence of a mutated S1/S2 furin cleavage site at the TGN may result in the shedding of cleaved S1 and conversion of S2 to its postfusion structure (S2*). Antigen sorting and trafficking can also induce the release of exosomes containing protein S. The events shown will occur in the apical and/or basolateral surfaces of polarized cells (e.g., epithelial). The Pfizer-BioNTech and Moderna constructs do not contain a mutated S1/S2 furin cleavage site. Further research will elucidate the impact of S1/S2 subunits stabilizing the D614G (or other) mutation or a mutated furin cleavage site in antigen distribution, vaccine immunogenicity, and induced adverse events (AEs). Dendritic cells (professional antigen-presenting cells, APCs) engulfing circulating antigens and B lymphocyte antibody-mediated binding to cell-anchored antigens are also shown.

[262] Jackson CB, Farzan M, Chen B, Choe H.
Mechanisms of SARS-CoV-2 entry into cells.
Nat Rev Mol Cell Biol. 2022 Jan;23(1):3-20. doi: 10.1038/s41580-021-00418-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8491763/

Heinz FX, Stiasny K.
Distinguishing features of current COVID-19 vaccines: knowns and unknowns of antigen presentation and modes of action.
NPJ Vaccines. 2021 Aug 16;6(1):104. doi: 10.1038/s41541-021-00369-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8368295/

[263] Letarov AV, Babenko VV, Kulikov EE.
Free SARS-CoV-2 Spike Protein S1 Particles May Play a Role in the Pathogenesis of COVID-19 Infection.
Biochemistry (Mosc). 2021 Mar;86(3):257-261. doi: 10.1134/S0006297921030032.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7772528/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8368295/
Vector vaccines principle for adenovirus.
(a) Schematic diagram of the non-replication competent adenoviral vector particle and its DNA. E1 and E3: early adenovirus genes 1 and 3, respectively. **b)** Formation of vaccine particles in the E1 complementary production cell line from the chromosomally integrated E1 gene. Release of newly produced vector particles through cell lysis. **c)** Spike expression in cells of vaccinated individuals. In the absence of stabilizing mutations, more or less shedding of S1 and conversion of S2 to its post-fusion structure (S2*) may occur.

Specifically, LNPs (liposomes) containing mRNA from the anti-SARS-CoV-2 vaccines (Pfizer and Moderna) are injected into the deltoid muscle and exert an effect on the muscle tissue itself, the lymphatic system, and the spleen, but they can also localize in the liver and other tissues[264] from where the S protein or its peptide subunits/fragments can enter the circulation and distribute throughout the body.

[264] https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf

Yang R, et al
A core-shell structured COVID-19 mRNA vaccine with favorable biodistribution pattern and promising immunity.
Signal Transduct Target Ther. 2021 May 31;6(1):213. doi: 10.1038/s41392-021-00634-z.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8165147/

Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, Madden TD, Hope MJ, Weissman D.
Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes.
J Control Release. 2015 Nov 10;217:345-51. doi: 10.1016/j.jconrel.2015.08.007.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4624045/

It is worth mentioning that hepatic localization of LNPs is not a general property, as specific changes in their chemistry can maintain immunogenicity with minimal liver involvement [265]. Consistent with a plausible systemic distribution of the antigen, protein S has been found to circulate in the plasma of subjects vaccinated with BNT162b2 or mRNA-1273 as early as day 1 after the first vaccine injection.[266]

According to the findings, antigen clearance is related to the production of antigen-specific immunoglobulins or may remain in circulation (e.g., in exosomes) for longer periods[267] .

Therefore, there is likely to be a wide range of expected interactions between protein S/subunit/fluctuating peptide fragments and circulating ACE2 in the blood (or lymph) or ACE2 expressed by cells in various tissues/organs [268].

This effect supports the mechanism of damage with adenoviral vector vaccines, as the S protein produced has similar functionality to the native virus spike (including the ability to release S1 subunits into circulation) and has a stabilized prefusion structure that promotes binding to its receptors .[269]

Vervaeke P, Borgos SE, Sanders NN, Combes F.
Regulatory guidelines and preclinical tools to study the biodistribution of RNA therapeutics.
Adv Drug Deliv Rev. 2022 May;184:114236. doi: 10.1016/j.addr.2022.114236.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8957368/

[265] Hassett KJ, et al
Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines.
Mol Ther Nucleic Acids. 2019 Apr 15;15:1-11. doi: 10.1016/j.omtn.2019.01.013.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6383180/

[266] Fertig TE, Chitoiu L, Marta DS, Ionescu VS, Cismasiu VB, Radu E, Angheluta G, Dobre M, Serbanescu A, Hinescu ME, Gherghiceanu M.
Vaccine mRNA Can Be Detected in Blood at 15 Days Post-Vaccination.
Biomedicines. 2022 Jun 28;10(7):1538. doi: 10.3390/biomedicines10071538.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9313234/

Ogata AF, Cheng CA, Desjardins M, Senussi Y, Sherman AC, Powell M, Novack L, Von S, Li X, Baden LR, Walt DR.
Circulating Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients.
Clin Infect Dis. 2022 Mar 1;74(4):715-718. doi: 10.1093/cid/ciab465.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8241425/

Naasani I.
Establishing the Pharmacokinetics of Genetic Vaccines is Essential for Maximising their Safety and Efficacy.
Clin Pharmacokinet. 2022 Jul;61(7):921-927. doi: 10.1007/s40262-022-01149-8.
https://link.springer.com/article/10.1007/s40262-022-01149-8

Palmer, Michael and Sucharit Bhakdi. "The Pfizer mRNA vaccine: pharmacokinetics and toxicity." (2021).
https://doctors4covidethics.org/wp-content/uploads/2021/07/Pfizer-pharmacokinetics-and-toxicity.pdf

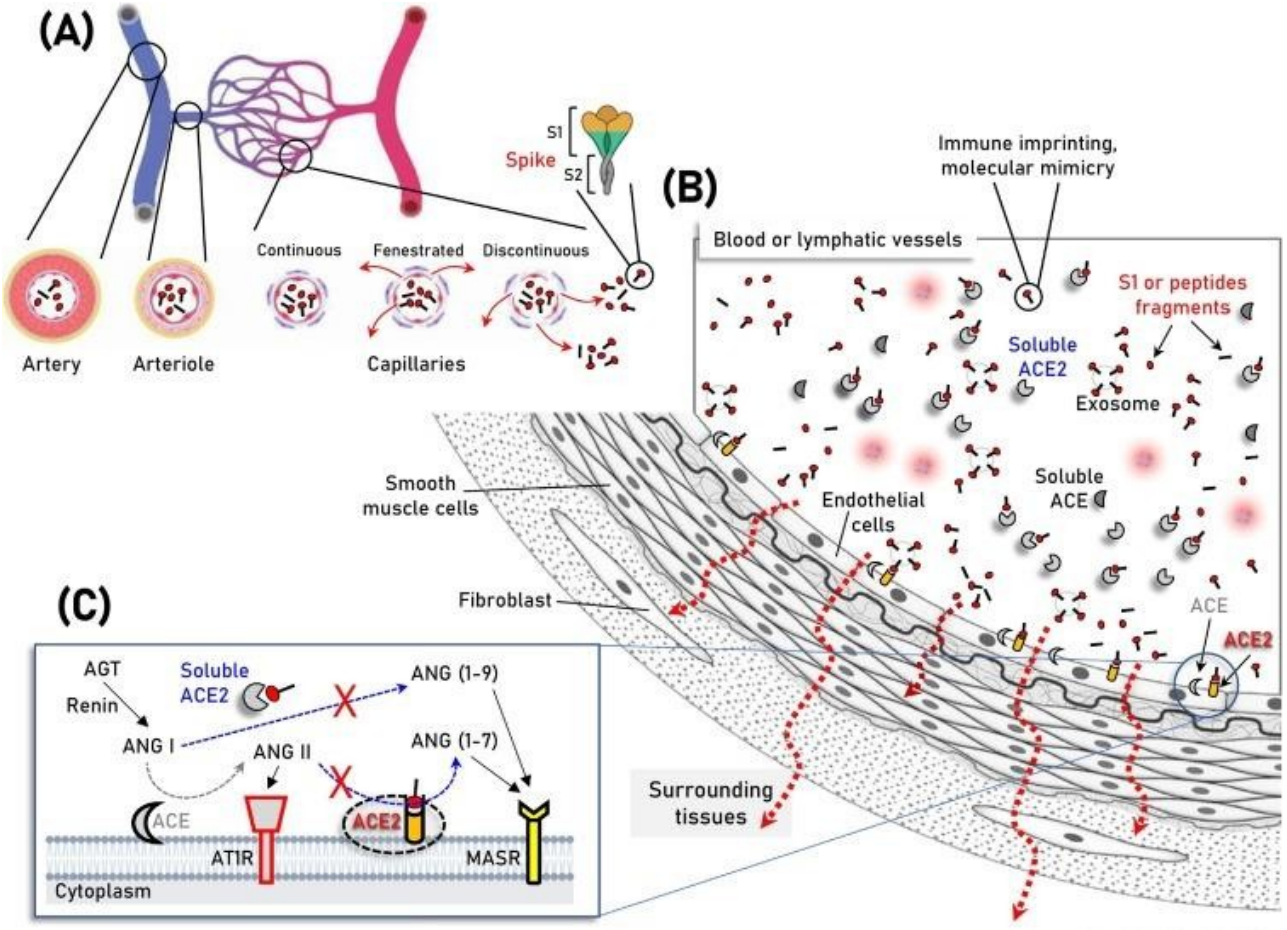
[267] Bansal S, Perincheri S, Fleming T, Poulson C, Tiffany B, Bremner RM, Mohanakumar T.
Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines.
J Immunol. 2021 Nov 15;207(10):2405-2410. doi: 10.4049/jimmunol.2100637.
https://www.jimmunol.org/content/207/10/2405.long

Cognetti JS, Miller BL.
Monitoring Serum Spike Protein with Disposable Photonic Biosensors Following SARS-CoV-2 Vaccination.
Sensors (Basel). 2021 Aug 31;21(17):5857. doi: 10.3390/s21175857.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8434114/

[268] Gkogkou E, Barnasas G, Vougas K, Trougakos IP.
Expression profiling meta-analysis of ACE2 and TMPRSS2, the putative anti-inflammatory receptor and priming protease of SARS-CoV-2 in human cells, and identification of putative modulators.
Redox Biol. 2020 Sep;36:101615. doi: 10.1016/j.redox.2020.101615.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7311357/

Ziegler CGK, et al HCA Lung Biological Network. Electronic address: lung-network@humancellatlas.org; HCA Lung Biological Network.
SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues.
Cell. 2020 May 28;181(5):1016-1035.e19. doi: 10.1016/j.cell.2020.04.035.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7252096/

[269] Watanabe Y, et al



Schematic diagram of vascular components showing S-protein/subunit/peptides fragments produced by vaccination in t h e circulation, as well as angiotensin-converting enzyme 2 (ACE2) bound to the membrane of soluble or endothelial cells.
(A,B) In parallel with immune activation, binding of circulating protein S/subunit/peptide fragments **(B)** to ACE2 may occur not only in ACE2-expressing endothelial cells but also in multiple cell types of the vascular system and surrounding tissues due to antigen dissemination (e.g., in fenestrated or discontinuous capillary beds) (A, red arrows). These series of molecular events are unlikely for any SARS-CoV-2-related antigen in the absence of severe coronavirus disease 2019 (COVID-19), in which SARS-CoV-2 is contained in the respiratory system. In **(C)** the two contrasting pathways of the renin-angiotensin system (RAS), namely the 'conventional' arm, which involves ACE generating angiotensin II (ANG II) from angiotensin I (ANG I), and the ACE2 arm that hydrolyzes Are depicted ANG II to generate angiotensin (1-7) [ANG (1-7)] or ANG I to generate angiotensin (1-9) [ANG (1-9)]. ANG II binding and activation of ANG II type 1 receptor (AT1R) p r o m o t e s inflammation, fibrotic remodeling, and vasoconstriction, while ANG (1-7) and ANG (1-9) peptides that bind to the MAS receptor (MASR) activate antifibrotic, anti-inflammatory pathways and vasodilation. Additional modules of the MASR (i.e., renin and angiotensinogen, AGT) are also shown. Abbreviation: AT1R, angiotensin II type 1 receptor.

In mRNA vaccines ("Pfizer" and "Modern" vaccines) mutation of S protein residues 986 and 987 to proline produces a stabilized S antigen in the pre-fusion conformation (P2 S)[270] .

Native-like SARS-CoV-2 Spike Glycoprotein Expressed by ChAdOx1 nCoV-19/AZD1222 Vaccine.
ACS Cent Sci. 2021 Apr 28;7(4):594-602. doi: 10.1021/acscentsci.1c00080.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8043200/

[270] Annette B. Vogel, et al
A prefusion SARS-CoV-2 spike RNA vaccine is highly immunogenic and prevents lung infection in non-human primates
bioRxiv 2020.09.08.280818; doi: https://doi.org/10.1101/2020.09.08.280818
https://www.biorxiv.org/content/10.1101/2020.09.08.280818v1.full.pdf

Kirchdoerfer RN, Wang N, Pallesen J, Wrapp D, Turner HL, Cottrell CA, Corbett KS, Graham BS, McLellan JS, Ward AB. Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. Sci Rep. 2018 Oct 24;8(1):15701. doi: 10.1038/s41598-018-34171-7. Erratum in: Sci Rep. 2018 Dec 10;8(1):17823.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6200764/

Pallesen J, et al
Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen.

Specifically, after expression of the BNT162b2 coding sequence in the cells, a portion of the molecules had one RBD in the "up" state (accessible for receptor binding), and two RBDs "down" (closed conformation), which accounted for 20.4 percent of the trimeric molecules.
The rest was in the all "down" conformation of the RBD. The RBD in the "up" conformation was less defined than in other parts of the structure, suggesting conformational flexibility and dynamic balance between the RBD "up" and RBD "down" states as also proposed in other studies [271].
Binding and structural analyses indicated that the BNT162b2 RNA sequence encodes for a recombinant S P2 protein that has the ACE2 binding site and other epitopes capable of binding to SARS-CoV-2 neutralizing antibodies.
The rigidity of this conformation (largely closed and thus conducive to the formation of low-affinity antibodies to RBD) and the sequence specificity of the vaccine antigen (obtained by chemical synthesis from the unique Wuhan-1 sequence) result in the formation of antibodies targeted against this antigen, which, in the presence of a circulating quasispecies, will induce selection of mutants that will bind weakly to vaccine antibodies, resulting in an increased risk of ADE.
It should also be considered that, as already discussed in the monograph devoted to *mRNA vaccines*, the *in vitro* transcription used to produce the vaccine construct and the *in vivo* translation introduce unpredictable mutations in both the mRNA and the final protein, with the generation of spikes with amino acid sequences and spatial conformations different from the designed one.
This could result in selective pressure with the formation of vaccine-resistant variants and a
Greater induction potential of ADE.

In a study published in 2016 by Whang et al[272] on the epitopes of the SARS-Cov-1 spike capable of inducing the formation of disease potentiating antibodies, they experimentally demonstrated the induction of immunopathology by ADE from inactivated vaccine both in vitro and in an in vivo nonhuman primate model. In addition, they found the association between the formation of disease potentiating antibodies and an epitope, (epitope-ADE) peptide $_{S597-603}$ (597-LYQDVNC-603), which was able to induce ADE antibodies both *in vitro* and *in vivo* using an epitope sequence-dependent (ESD) mechanism, with a high level of serological reactivity (64%).
It is relevant to vaccine safety to note that the 611-617 epitope found in the stick region of the spike protein of SARS-Cov-2 (recognized by antibody 43-3-14), corresponding to the 597- LYQDVNC-603 peptide of SARS-Cov-1, and an ADE epitope in the NTD of SARS-Cov-2 (recognized by antibody 1052), [273]

Proc Natl Acad Sci U S A. 2017 Aug 29;114(35):E7348-E7357. doi: 10.1073/pnas.1707304114. Epub 2017 Aug 14.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5584442/

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS.
Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science. 2020 Mar 13;367(6483):1260-1263. doi: 10.1126/science.abb2507. Epub 2020 Feb 19
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/

[271] Cai Y, Zhang J, Xiao T, et al.
Distinct conformational states of SARS-CoV-2 spike protein.
Science. 2020;369(6511):1586-1592. doi:10.1126/science.abd4251
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464562/

Henderson R, et al
Controlling the SARS-CoV-2 spike glycoprotein conformation.
Nat Struct Mol Biol. 2020 Oct;27(10):925-933. doi: 10.1038/s41594-020-0479-4. Epub 2020 Jul 22.
https://www.nature.com/articles/s41594-020-0479-4

[272] Wang Q, Zhang L, Kuwahara K, Li L, Liu Z, Li T, Zhu H, Liu J, Xu Y, Xie J, Morioka H, Sakaguchi N, Qin C, Liu G.
Immunodominant SARS Coronavirus Epitopes in Humans Elicited Both Enhancing and Neutralizing Effects on Infection in Non-human Primates.
ACS Infect Dis. 2016 May 13;2(5):361-76. doi: 10.1021/acsinfecdis.6b00006. Epub 2016 Apr 11. Erratum in: ACS Infect Dis. 2020 May 8;6(5):1284-1285.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7075522/

[273] Li D, et al
In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies.
Cell. 2021 Aug 5;184(16):4203-4219.e32. doi: 10.1016/j.cell.2021.06.021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8232969/

are both present on the spike protein generated from the mRNA vaccines, as the formulations are still based on the original Wuhan strain.[274]

SPIKE AND SYNCYTIAL FORMATION

Yuri Lazebnik in his article "*Cell fusion as a link between the SARS-CoV-2 spike protein, COVID-19 complications, and vaccine side effects*"[275] delves into the characteristic formation of syncytia in severe forms by COVID and the implications on vaccinees.
Syncytes are the fusion product between two or more cells and have been found in COVID patients with severe and extensive lung damage as infected multinucleated syncytial pneumocytes.[276]

[274] Xia X.
Guérin P, Yahi N, Azzaz F, Chahinian H, Sabatier JM, Fantini J.
Structural Dynamics of the SARS-CoV-2 Spike Protein: A 2-Year Retrospective Analysis of SARS-CoV-2 Variants (from Alpha to Omicron) Reveals an Early Divergence between Conserved and Variable Epitopes.
Molecules. 2022 Jun 15;27(12):3851. doi: 10.3390/molecules27123851.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9230616/

Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines.
Vaccines (Basel). 2021 Jul 3;9(7):734. doi: 10.3390/vaccines9070734.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8310186/

[275] Lazebnik Y.
Cell fusion as a link between the SARS-CoV-2 spike protein, COVID-19 complications, and vaccine side effects.
Oncotarget. 2021 Dec 7;12(25):2476-2488. doi: 10.18632/oncotarget.28088.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8664391/

[276] Buchrieser J, et al
Syncytia formation by SARS-CoV-2-infected cells.
EMBO J. 2020 Dec 1;39(23):e106267. doi: 10.15252/embj.2020106267. Epub 2020 Nov 4. Erratum in: EMBO J. 2021 Feb 1;40(3):e107405. PMID: 33051876;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7646020/

Bussani R, Schneider E, Zentilin L, Collesi C, Ali H, Braga L, Volpe MC, Colliva A, Zanconati F, Berlot G, Silvestri F, Zacchigna S, Giacca M.
Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology.
EBioMedicine. 2020 Nov;61:103104. doi: 10.1016/j.ebiom.2020.103104.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7677597/

Rajah MM, et al
SARS-CoV-2 Alpha, Beta, and Delta variants display enhanced Spike-mediated syncytia formation.
EMBO J. 2021 Dec 15;40(24):e108944. doi: 10.15252/embj.2021108944.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8646911/

Wang H, Guo S, Yang H.
Rapid quantitative monitoring of SARS-CoV-2 spike protein-mediated syncytia formation using split NanoLuc.
J Med Virol. 2022 Aug 8. doi: 10.1002/jmv.28053.
https://pubmed.ncbi.nlm.nih.gov/35940856/

Lin L, Li Q, Wang Y, Shi Y.
Syncytia formation during SARS-CoV-2 lung infection: a disastrous unity to eliminate lymphocytes.
Cell Death Differ. 2021 Jun;28(6):2019-2021. doi: 10.1038/s41418-021-00795-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8114657/

Ren H, Ma C, Peng H, Zhang B, Zhou L, Su Y, Gao X, Huang H.
Micronucleus production, activation of DNA damage response and cGAS-STING signaling in syncytia induced by SARS-CoV-2 infection.
Biol Direct. 2021 Oct 21;16(1):20. doi: 10.1186/s13062-021-00305-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8530504/

Asarnow D, et al
Structural insight into SARS-CoV-2 neutralizing antibodies and modulation of syncytia.
Cell. 2021 Jun 10;184(12):3192-3204.e16. doi: 10.1016/j.cell.2021.04.033.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8064868/

Video Cellular fusion

Nanolive imaging suggests that cell-cell fusion could play a key role in the SARS-CoV-2 infection process

https://vimeo.com/528310356

Definitions

Cell fusion *Process of fusing two or more cells into one by joining their plasma membranes.*

Fusogen *An agent, often a protein such as the SARS-CoV-2 spike, capable of fusing cell membranes. Viral fusogens fuse the viral envelope with the plasma membrane of the target cell and can fuse the plasma membranes of adjacent cells together.*

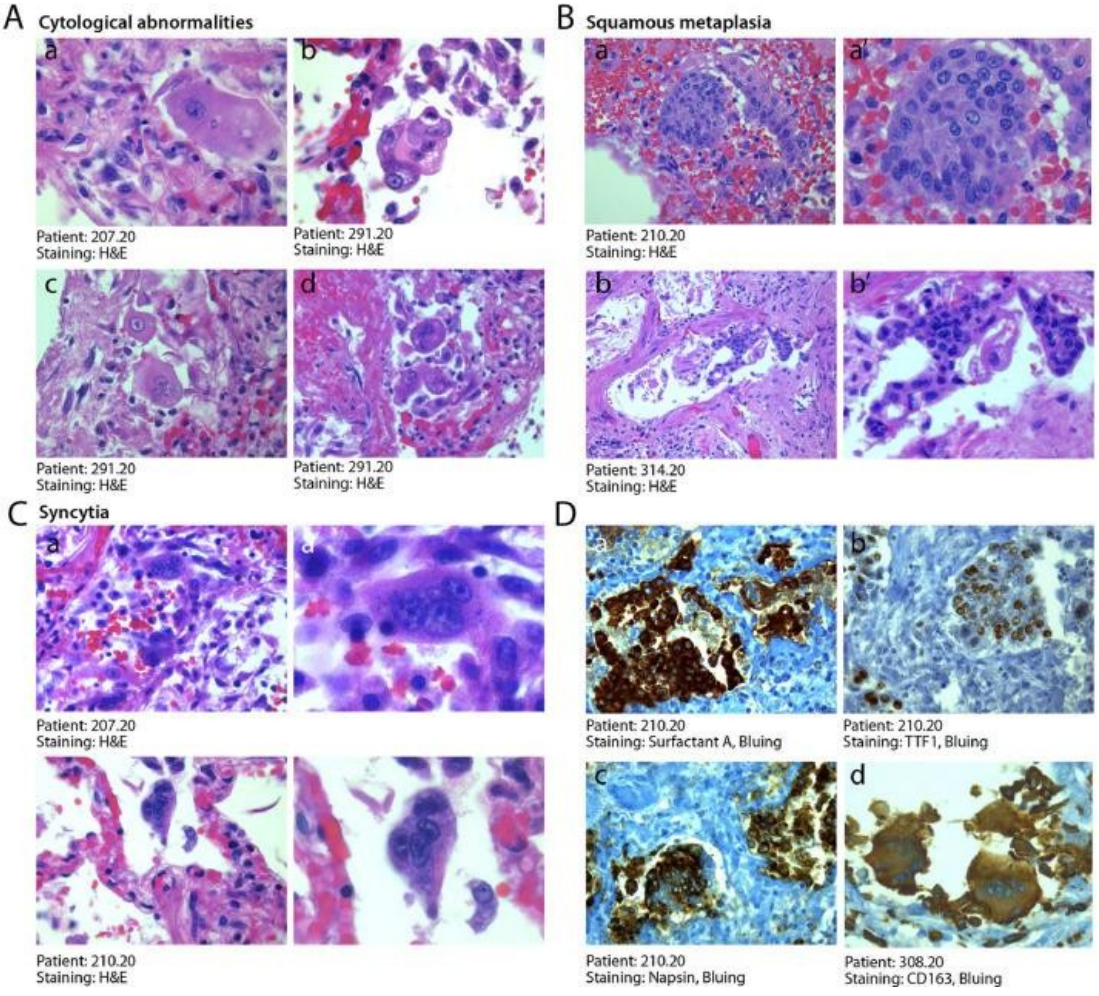
Syncytium or syncytium *(plural syncytia) A multinucleated cell produced by the fusion of two or more cells. The term comes from the Greek syn "together" and kytos "box, or cell."*

Heterokaryon *A syncytium produced by more than one cell type, such as a pneumocyte fused with an epithelial progenitor or a leukocyte.*

Homokaryon *A syncytium produced by cells of the same type, as would be the case with the fusion of two or more pneumocytes.*

Hybrid cell *Mononuclear progeny of syncytia, produced once a syncytium undergoes mitosis. For example, hybridomas are made by fusing leukocytes with plasmacytoma cells to obtain hybrids that produce monoclonal antibodies.*

PS *(phosphatidylserine) The most abundant anionic (negatively charged) membrane lipid. In living cells, PS is actively moved to the cytoplasmic side of the plasma membrane.*



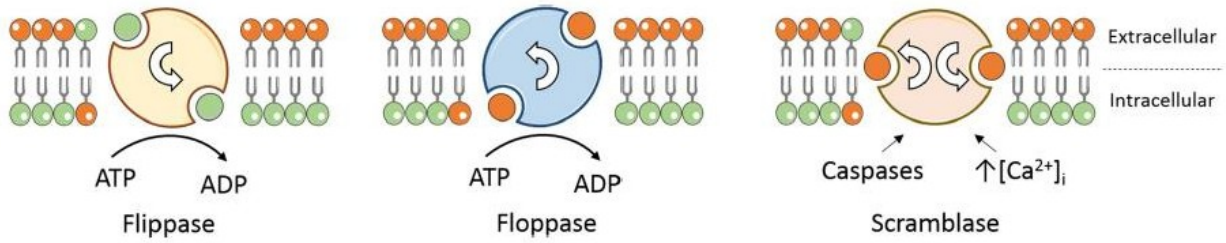
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7677597/
Abnormal pneumocytes and cell syncytia in COVID-19 lungs. A. Cytologic features of SARS-CoV-2-infected lungs. A consistent and typical feature in COVID-19 lungs was the presence of major cytologic abnormalities, including very large cells with dysmorphic phenotypes (a, x63; b, x40) that often showed bio-or multinucleation (c, d, x40). B. Squamous metaplasia (pseudosyncytia). A common finding was metaplasia of the alveolar epithelium, with a marked change in the morphology of pneumocytes and their aggregation to form pseudosyncytia (a, b, x20; a', b', x63). C. Syncytia. In addition to squamous metaplasia, true syncytial elements were observed in numerous lungs, showing extensive cytoplasm and nuclear aggregation (a, b, x20; a', b', x63). D. Origin of syncytial elements. Giant, multinucleated cells forming pseudo-syncytium or true syncytium scored positive for the pneumocyte markers Surfactant-A (a, x20), TTF1 (b, x20) and Napsin (c, x20), indicative of their epithelial origin. COVID-19 lungs also showed the more occasional presence of CD163-positive syncytia of histiocytic origin (d, x20). In AC, H&E: hematoxylin and eosin.

These syncytiums have been attributed to the ability of the spike to fuse the membrane of the host cell with the membrane of an adjacent cell if the latter cell also has a receptor for the spike.

Sinczi and Thrombosis

Given the finding that spike-induced cell fusion is associated with the activation of TMEM16F[277] , a scramblase that externalizes phosphatidylserine (PS)* , the author proposes that spike-externalized PS enables the formation of extrinsic tenase, the key trigger of the blood clotting cascade during viral infections.

** Scramblases (from the English "to scramble" meaning to mix) are proteins involved in moving phospholipids from one monolayer to another of the lipid bilayer within a cell's plasma membrane.*
This protein, unlike flippase is not selective toward lipids but "flips" them to the other side of the membrane in a random and nonspecific manner. It therefore causes phospholipids to be added not only on one side at the time of membrane formation or expansion, but also on the opposite side in contact with the extracellular environment. [278]



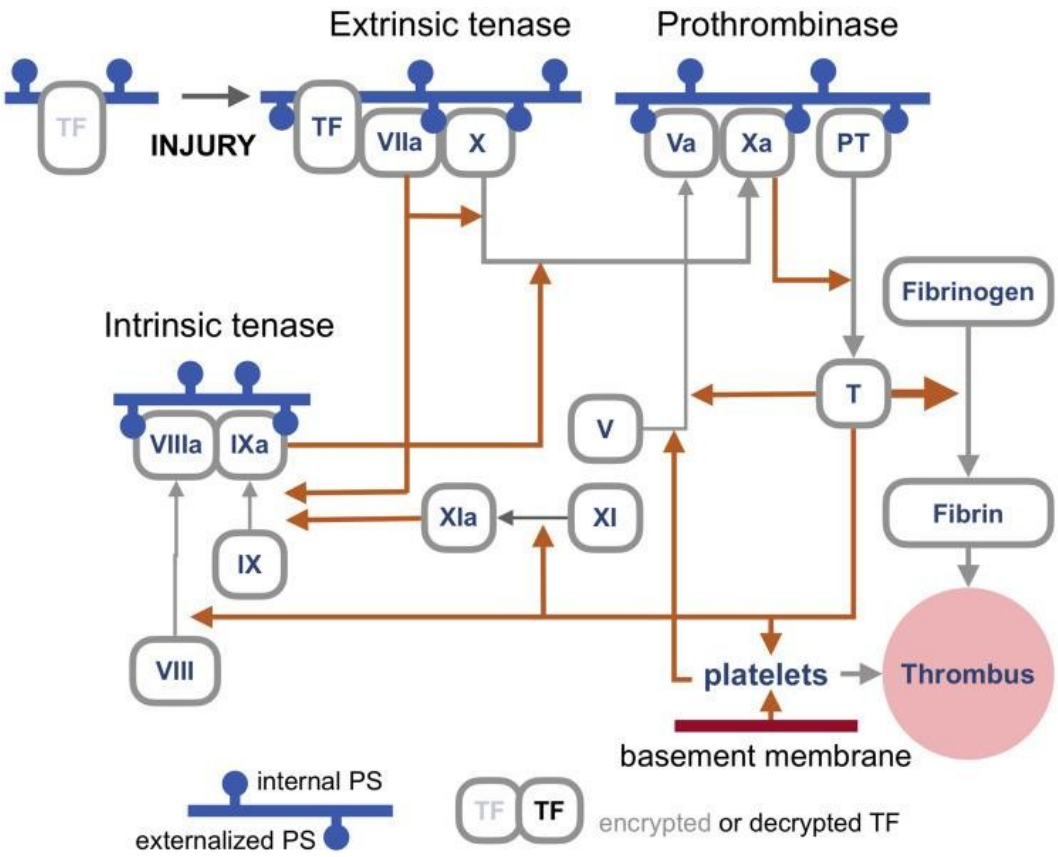
https://www.researchgate.net/publication/317660741_Phosphatidylethanolamine_targeting_for_cell_death_imaging_in_early_treatment_response_evaluation_and_disease_diagnosis

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8664391/
A diagram of the blood coagulation cascade.
The blood coagulation cascade is a network of proteases, their precursors, cofactors, cells, enzymes, feedbacks and feedforwards whose complexity and as yet unresolved issues make this necessarily rudimentary outline, with the main objective of illustrating where proteins that require binding to externalized PS (phosphatidylserine) for activation are in the network. Most of the proteins involved in coagulation are called factors and are labeled with Roman numerals, such as factor X or FX (so the enzymes that process FX are tenases). For simplicity, the letter F is omitted in this cartoon. Activated factors are labeled with an a, as in FXa. Orange arrows represent proteolytic activity, gray arrows show a transition between forms. Blue horizontal lines represent a cell membrane with the cell surface facing downward. Consequently, the pinheads of the externalized PS also face downward. Note that most of the PS is

[277] Braga L, et al
Drugs that inhibit TMEM16 proteins block SARS-CoV-2 spike-induced syncytia.
Nature. 2021 Jun;594(7861):88-93. doi: 10.1038/s41586-021-03491-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7611055/

[278] Elvas F, Stroobants S, Wyffels L.
Phosphatidylethanolamine targeting for cell death imaging in early treatment response evaluation and disease diagnosis.
Apoptosis. 2017 Aug;22(8):971-987. doi: 10.1007/s10495-017-1384-0.
https://pubmed.ncbi.nlm.nih.gov/28623512/

actively relocated to face the cytoplasm unless the cell dies or the distribution is randomized by lipid scramblases. As discussed in the text, the primary trigger of viral infection-induced coagulation is the extrinsic tenase (top left), which is a complex of TF (tissue factor) and FVIIa assembled on externalized PS in the presence of calcium ions. This tenase produces FXa to activate enough thrombin to generate the components of intrinsic tenase, which increases the production of FXa and, consequently, thrombin, which generates enough fibrin to form a thrombus, an entanglement of polymerized, cross-linked fibrin with trapped blood cells, mainly platelets, that is large and rigid enough to obstruct a blood vessel. Note that TF is encrypted and therefore unable to activate FVIIa, until it is decrypted by externalized PS



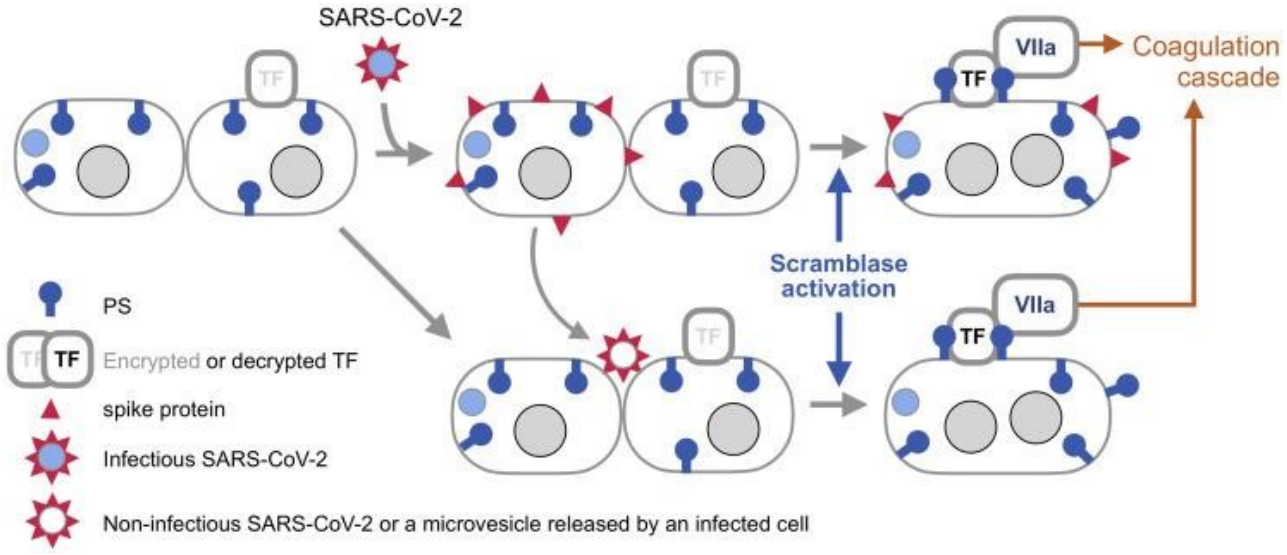
The SARS-CoV-2 spike can also fuse cells if the virus is not infectious, or even if the spike is embedded in membrane vesicles,[279] such as extracellular vesicles (exosomes) released from infected cells.
This mechanism is known as fusion from outside[280] , as the viral particle or a vesicle provides a bridge between membranes. Since the syncytia produced by this mechanism are not infected with SARS-CoV-2 in this case, their origin may be difficult to trace. This also means that extracellular vesicles produced in patients with COVID-19[281] may be able to form syncytia and thus cause thrombosis even in tissues that are not infected with the virus.

[279] Theuerkauf SA, Michels A, Riechert V, Maier TJ, Flory E, Cichutek K, Buchholz CJ.
Quantitative assays reveal cell fusion at minimal levels of SARS-CoV-2 spike protein and fusion from without.
iScience. 2021 Mar 19;24(3):102170. doi: 10.1016/j.isci.2021.102170.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7871100/

[280] Gallaher WR, Bratt MA.
Temperature-dependent inhibition of fusion from without.
J Virol. 1972 Jul;10(1):159-61. doi: 10.1128/JVI.10.1.159-161.1972.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC356440/pdf/jvirol00271-0169.pdf

[281] Rosell A, Havervall S, von Meijenfeldt F, Hisada Y, Aguilera K, Grover SP, Lisman T, Mackman N, Thålin C.
Patients With COVID-19 Have Elevated Levels of Circulating Extracellular Vesicle Tissue Factor Activity That Is Associated With Severity and Mortality-Brief Report.
Arterioscler Thromb Vasc Biol. 2021 Feb;41(2):878-882. doi: 10.1161/ATVBAHA.120.315547.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7837685/

Note that TF (Tissue Factor) is encrypted, which means it is unable to activate FVIIa, until it is decrypted by the externalized PS.[282]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8664391/

Large syncytia produced by viral fusions are prone to die, at least in cultured tissues. Thus, a second hypothesis of induction of thrombosis mediated by cell fusion suggests that the syncytium formed by cells lining a blood vessel could contribute to thrombosis simply by dying, because detaching would uncover a thrombogenic basement membrane stain with a surface area equal to that of many mononuclear cells.
Since even a single 20-micron collagen fiber, the main component of the basement membrane, is sufficient to trigger platelet-dependent coagulation,[283] the plaque exposed by the dying syncytium, composed of more than several cells, could be large enough to produce a thrombus by platelet activation (Figure "Coagulation cascade," bottom right).
Because even one thrombus can cause problems or even death, the potential contribution of the syncytium to thrombosis by COVID-19 via tenase activation or by uncovering a basement membrane patch could be clinically relevant.
SARS-CoV-2 may be able to activate both mechanisms either locally, by fusing infected cells, or remotely via exosomes carrying the spike.

Sincizi and NeuroCovid

Another damage caused by syncytium formation concerns the neurological manifestations of COVID complications, including pain[284] . While it is likely that SARS-CoV-2 contributes to these symptoms in multiple ways, the

---

[282] Grover SP, Mackman N.
Tissue Factor: An Essential Mediator of Hemostasis and Trigger of Thrombosis.
Arterioscler Thromb Vasc Biol. 2018 Apr;38(4):709-725. doi: 10.1161/ATVBAHA.117.309846.
https://pubmed.ncbi.nlm.nih.gov/29437578/

[283] Zhu S, Tomaiuolo M, Diamond SL.
Minimum wound size for clotting: flowing blood coagulates on a single collagen fiber presenting tissue factor and von Willebrand factor.
Integr Biol (Camb). 2016 Aug 8;8(8):813-20. doi: 10.1039/c6ib00077k.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4980166/

[284] Solomon T.
Neurological infection with SARS-CoV-2 - the story so far.
Nat Rev Neurol. 2021 Feb;17(2):65-66. doi: 10.1038/s41582-020-00453-w.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7789883/

short-circuiting neuronal networks by fusing neurons may explain not only how some neurological symptoms emerge, but also why they last after the infection is cleared.
The fact that neuron fusion can cause neurological problems has been considered in other virus-induced diseases.
In animals, the severe neurological symptoms of pseudorabies have been linked to the ability of pseudorabies virus to electrically couple the activity of neurons by fusing their axons. [285]
How such coupling may also contribute to loss of olfaction, a common symptom of COVID-19, can be understood from experiments in the nematode *C. elegans* in which the fusion of two functionally different chemosensory neurons altered chemosensing.[286]
In humans, fusion between neurons and glial cells surrounding neuronal bodies has been proposed to explain the origin and persistence of neuropathic pain that can last for months after the acute phase of shingles (shingles). [287]
This fusion was detected in a patient with shingles,[288] confirmed in a human xenograft model of this disease,[289] and accidentally discovered in an unrelated mouse model in which cortical neurons had been infected with a retrovirus pseudotyped with VSV-G , the fusogen of vesicular stomatitis virus[290] .
Whether human endogenous retrovirus (HERV) fusogens, whose expression has been associated with various neurological disorders, are able to function as pathogens of these diseases by fusing cells, as has been suggested,[291] has yet to be confirmed[292] . However, these observations mean that the

[285] Granstedt AE, Bosse JB, Thiberge SY, Enquist LW.
In vivo imaging of alphaherpesvirus infection reveals synchronized activity dependent on axonal sorting of viral proteins.
Proc Natl Acad Sci U S A. 2013 Sep 10;110(37):E3516-25. doi: 10.1073/pnas.1311062110.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3773797/

McCarthy KM, Tank DW, Enquist LW.
Pseudorabies virus infection alters neuronal activity and connectivity in vitro.
PLoS Pathog. 2009 Oct;5(10):e1000640. doi: 10.1371/journal.ppat.1000640.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2763221/

[286] Giordano-Santini R, Kaulich E, Galbraith KM, Ritchie FK, Wang W, Li Z, Hilliard MA.
Fusogen-mediated neuron-neuron fusion disrupts neural circuit connectivity and alters animal behavior.
Proc Natl Acad Sci U S A. 2020 Sep 15;117(37):23054-23065. doi: 10.1073/pnas.1919063117.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7502713/

[287] Zerboni L, Sen N, Oliver SL, Arvin AM.
Molecular mechanisms of varicella zoster virus pathogenesis.
Nat Rev Microbiol. 2014 Mar;12(3):197-210. doi: 10.1038/nrmicro3215.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4066823/

[288] Esiri MM, Tomlinson AH.
Herpes Zoster. Demonstration of virus in trigeminal nerve and ganglion by immunofluorescence and electron microscopy. J Neurol Sci. 1972;15(1):35-48. doi: 10.1016/0022-510x(72)90120-7.
https://pubmed.ncbi.nlm.nih.gov/4332851/

[289] Reichelt M, Zerboni L, Arvin AM.
Mechanisms of varicella-zoster virus neuropathogenesis in human dorsal root ganglia.
J Virol. 2008 Apr;82(8):3971-83. doi: 10.1128/JVI.02592-07.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2292995/

[290] Ackman JB, Siddiqi F, Walikonis RS, LoTurco JJ.
Fusion of microglia with pyramidal neurons after retroviral infection.
J Neurosci. 2006 Nov 1;26(44):11413-22. doi: 10.1523/JNEUROSCI.3340-06.2006.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6674527/

[291] Duelli D, Lazebnik Y.
Cell fusion: a hidden enemy?
Cancer Cell. 2003 May;3(5):445-8. doi: 10.1016/s1535-6108(03)00114-4.
https://doi.org/10.1016/S1535-6108(03)00114-4

[292] Geis FK, Goff SP.
Silencing and Transcriptional Regulation of Endogenous Retroviruses: An Overview.
Viruses. 2020 Aug 13;12(8):884. doi: 10.3390/v12080884.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7472088/

abnormal neuronal fusion induced by viral proteins is not restricted to a particular fusogen or to specific neurons.
In the case of SARS-Cov-2, the spike has been detected in the brains of deceased patients with COVID-19,[293] and considering the efficiency with which the spike fuses cells[270] and how intricate the neuronal networks are, the possibility that the spike could disrupt them by fusing some of their components is not negligible, as also demonstrated in a recent study in brain organoids.[294]
If the spike maintains this activity in the brain over time, it is not difficult to imagine how the neuronal anastomoses created by cell fusion might contribute to the cognitive impairments associated with COVID-19 and its long-term consequences.[275]
In fact, such short circuits may last for some time after the viral infection is over, because the mechanisms that can repair them by "disconnecting" anastomosed neurons or replacing them may be inefficient or nonexistent.

Sincizi and Tumors

Another potential concern stems from a long-standing model that cell fusion, particularly virus-induced fusion, contributes to cancer development, progression, metastasis, recurrence, dormancy, and acquired drug resistance[295] . This model has been supported by recent reports of cell hybrids in human cancers,[296] by multiple observations in animal models[297] ,

---

Giménez-Orenga K, Oltra E.
Human Endogenous Retroviruses as Therapeutic Targets in Neurologic Disease. Pharmaceuticals (Basel). 2021 May 24;14(6):495. doi: 10.3390/ph14060495.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8225122/

[293] Song E, et al
Neuroinvasion of SARS-CoV-2 in human and mouse brain.
J Exp Med. 2021 Mar 1;218(3):e20202135. doi: 10.1084/jem.20202135.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7808299/

[294] Martinez-Marmol R, Giordano-Santini R, Kaulich E, Cho AN, Riyadh MA, Robinson E, Balistreri G, Meunier FA, Ke YD, Ittner LM, Hilliard MA. The SARS-CoV-2 spike (S) and the orthoreovirus p15 cause neuronal and glial fusion.
bioRxiv. 2021; 2021.09.01.458544.
https://www.biorxiv.org/content/10.1101/2021.09.01.458544v1.

[295] Shabo I, Svanvik J, Lindström A, Lechertier T, Trabulo S, Hulit J, Sparey T, Pawelek J.
Roles of cell fusion, hybridization and polyploid cell formation in cancer metastasis.
World J Clin Oncol. 2020 Mar 24;11(3):121-135. doi: 10.5306/wjco.v11.i3.121.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7103524/

Parris GE.
Historical perspective of cell-cell fusion in cancer initiation and progression.
Crit Rev Oncog. 2013;18(1-2):1-18. doi: 10.1615/critrevoncog.v18.i1-2.20.
https://pubmed.ncbi.nlm.nih.gov/23237550/

Sieler M, Weiler J, Dittmar T.
Cell-Cell Fusion and the Roads to Novel Properties of Tumor Hybrid Cells.
Cells. 2021 Jun 11;10(6):1465. doi: 10.3390/cells10061465.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8230653/

[296] Gast CE, et al
Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival.
Sci Adv. 2018 Sep 12;4(9):eaat7828. doi: 10.1126/sciadv.aat7828.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6135550/

Laberge GS, Duvall E, Haedicke K, Pawelek J.
Leukocyte-Cancer Cell Fusion-Genesis of a Deadly Journey.
Cells. 2019 Feb 18;8(2):170. doi: 10.3390/cells8020170.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6406780/

[297] Pawelek JM, Chakraborty AK.
Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis.
Nat Rev Cancer. 2008 May;8(5):377-86. doi: 10.1038/nrc2371.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6406780/

by findings that human cells can be made cancerous through cell fusion,[298] and comparing the evolution of tumors and cell hybrids[299] .
However, it is yet to be determined whether any neoplastic hybrids found in humans are produced by viral fusogens, as has been suggested[300] , but as a precaution, it is important to continuously monitor the incidence and progression of neoplastic lesions in COVID-19 patients.[301]

It should be kept in mind that cell fusion, in addition to producing large multinucleated giant cell syncytia, may produce binuclear or trinuclear cells, which are often more abundant in experimental systems than large syncytia, but may go unnoticed in human tissues, or even if noticed, may not be attributed to cell fusion, because reliably distinguishing them from binuclear cells produced by failed mitosis in human tissues may be difficult or impossible with available instruments.[302]
A syncytium, especially if it has only two or three nuclei, may enter mitosis to produce mononuclear daughter cells. These mitoses are commonly multipolar and thus are prone to produce aneuploid cells with chromosomal aberrations, adding another layer of abnormal features to the progeny of the cell fusion[303] .

Noubissi FK, Ogle BM.
Cancer Cell Fusion: Mechanisms Slowly Unravel.
Int J Mol Sci. 2016 Sep 21;17(9):1587. doi: 10.3390/ijms17091587.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5037852/

[298] Delespaul L, et al
Fusion-mediated chromosomal instability promotes aneuploidy patterns that resemble human tumors.
Oncogene. 2019 Aug;38(33):6083-6094. doi: 10.1038/s41388-019-0859-6.
https://pubmed.ncbi.nlm.nih.gov/31270395/

Lartigue L, Merle C, Lagarde P, Delespaul L, Lesluyes T, Le Guellec S, Pérot G, Leroy L, Coindre JM, Chibon F.
Genome remodeling upon mesenchymal tumor cell fusion contributes to tumor progression and metastatic spread. Oncogene. 2020 May;39(21):4198-4211. doi: 10.1038/s41388-020-1276-6.
https://pubmed.ncbi.nlm.nih.gov/32242148/

[299] Miroshnychenko D, Baratchart E, Ferrall-Fairbanks MC, Velde RV, Laurie MA, Bui MM, Tan AC, Altrock PM, Basanta D, Marusyk A.
Spontaneous cell fusions as a mechanism of parasexual recombination in tumour cell populations.
Nat Ecol Evol. 2021 Mar;5(3):379-391. doi: 10.1038/s41559-020-01367-y.
https://pubmed.ncbi.nlm.nih.gov/33462489/

[300] Duelli DM, Hearn S, Myers MP, Lazebnik Y.
A primate virus generates transformed human cells by fusion.
J Cell Biol. 2005 Nov 7;171(3):493-503. doi: 10.1083/jcb.200507069.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2171256/

Parris GE.
The role of viruses in cell fusion and its importance to evolution, invasion and metastasis of cancer clones.
Med Hypotheses. 2005;64(5):1011-4. doi: 10.1016/j.mehy.2004.11.012.
https://pubmed.ncbi.nlm.nih.gov/15780502/

Duelli D, Lazebnik Y.
Cell-to-cell fusion as a link between viruses and cancer.
Nat Rev Cancer. 2007 Dec;7(12):968-76. doi: 10.1038/nrc2272.
https://pubmed.ncbi.nlm.nih.gov/18034186/

[301] Saini G, Aneja R.
Cancer as a prospective sequela of long COVID-19.
Bioessays. 2021 Jun;43(6):e2000331. doi: 10.1002/bies.202000331.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8206711/

[302] Gast CE, et al
Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival.
Sci Adv. 2018 Sep 12;4(9):eaat7828. doi: 10.1126/sciadv.aat7828.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6135550/

[303] Duelli DM, Padilla-Nash HM, Berman D, Murphy KM, Ried T, Lazebnik Y.
A virus causes cancer by inducing massive chromosomal instability through cell fusion.

Such abnormalities may be particularly significant for COVID-19 patients with existing neoplastic lesions, because chromosomal aberrations contribute to tumor progression[304] , as do epigenetic abnormalities found in cell fusion products.[305]

Sincizi and lymphopenia by COVID-19

Zhang et al, in the article "*SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte* elimination,"[306] report the results of research devoted to studying the mechanism of syncytium formation by the SARS-Cov-2 spike.

Specifically, the researchers found that SARS-CoV-2 infection in cultured cells led to the production of multinucleated syncytium, which could easily internalize multiple lymphocyte lineages and form typical cell-within-cell structures. These structures caused death of the internalized cells, potentially contributing to lymphopenia and pathogenesis in COVID-19 patients.

In vitro co-culture assay showed that the Vero cell line ($ACE2^+$), after expressing the SARS-CoV-2 spike protein, could form homologous syncytia or fuse with other cell lines as long as the ACE2 receptor was present.

Interestingly, when Vero cells were transfected with the spike protein from SARS-CoV-1, no syncytium formation was observed. Therefore, the key element responsible for SARS-CoV-2-mediated syncytium is absent in the spike protein from SARS-CoV-1.

Driven by this hypothesis, the authors compared the spike protein from SARS-CoV-2 and SARS-CoV1 and found that the four-amino acid insert (PRRA) of furin before the S1/S2 cleavage site present only in the SARS-CoV-2 spike is responsible for the ability to fuse cells.

Consistently, the spike protein of SARS-CoV1 effectively induced syncytium once the "PRRA" sequence was inserted before the S1/S2 cleavage site of the SARS-CoV-1 genome.

In addition, production of the S2 fusion fragment is triggered by a bi-arginine motif containing R682 and R685, and the authors showed that 6-D-Arg, a furin inhibitor, was able to significantly suppress the processing of the S glycoprotein into S2 and inhibit membrane fusion.

Curr Biol. 2007 Mar 6;17(5):431-7. doi: 10.1016/j.cub.2007.01.049.
https://doi.org/10.1016/j.cub.2007.01.049

Godinho SA, Kwon M, Pellman D.
Centrosomes and cancer: how cancer cells divide with too many centrosomes.
Cancer Metastasis Rev. 2009 Jun;28(1-2):85-98. doi: 10.1007/s10555-008-9163-6.
https://pubmed.ncbi.nlm.nih.gov/19156503/

[304] Gemoll T, Auer G, Ried T, Habermann JK.
Genetic Instability and Disease Prognostication.
Recent Results Cancer Res. 2015;200:81-94. doi: 10.1007/978-3-319-20291-4_4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7737009/

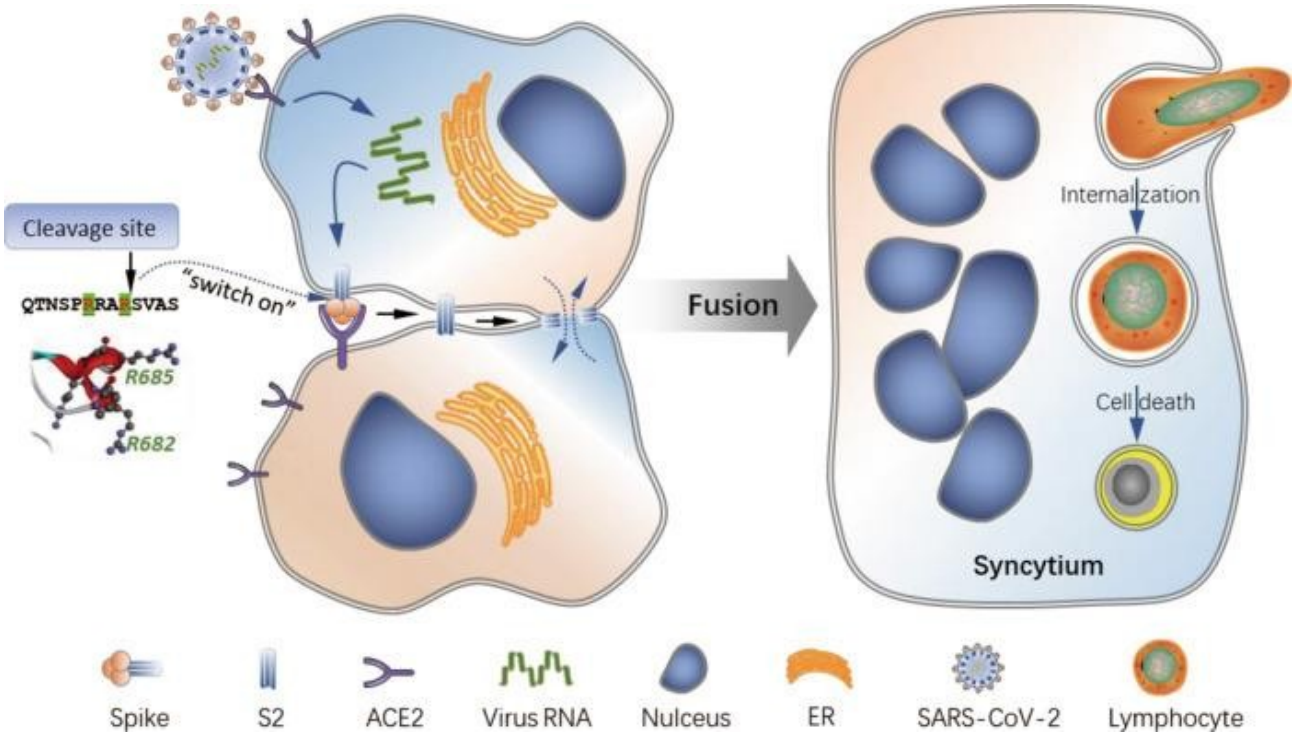
Baudoin NC, Bloomfield M.
Karyotype Aberrations in Action: The Evolution of Cancer Genomes and the Tumor Microenvironment.
Genes (Basel). 2021 Apr 12;12(4):558. doi: 10.3390/genes12040558.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8068843/

[305] Lazebnik Y.
The shock of being united and symphiliosis. Another lesson from plants?
Cell Cycle. 2014;13(15):2323-9. doi: 10.4161/cc.29704.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4128876/

[306] Zhang Z et al
SARS-CoV-2 spike protein dictates syncytium-mediated lymphocyte elimination.
Cell Death Differ. 2021 Sep;28(9):2765-2777. doi: 10.1038/s41418-021-00782-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8056997/

Lin L, Li Q, Wang Y, Shi Y.
Syncytia formation during SARS-CoV-2 lung infection: a disastrous unity to eliminate lymphocytes.
Cell Death Differ. 2021 Jun;28(6):2019-2021. doi: 10.1038/s41418-021-00795-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8114657/

These data thus suggest that blocking surface glycoprotein cleavage, by strategies such as targeting the bi-arginine motif, could serve as a potential strategy to alleviate the pathogenesis caused by SARS-CoV-2 and other highly infectious viruses that contain bi-arginine motifs. Among the drugs tested, hydroxychloroquine was unexpectedly identified as a potent inhibitor of glycoprotein S processing and membrane fusion, as well as syncytium formation, as an additional antiviral mechanism for this substance.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8056997/
A working model for SARS-CoV-2-induced lymphocyte loss through syncytium-mediated cell-to-cell formation.
Infection of ACE2-expressing cells by SARS-CoV-2 virus leads to surface expression of the viral spike glycoprotein, which harbors a bi-arginine motif required for protease-mediated processing and controls membrane fusion. Engagement of the spike protein with its ACE2 receptor triggers membrane fusion, mediated by the S2 domain of the viral spike glycoprotein, between neighboring cells, leading to the production of a multinucleated syncytium. Syncytium are able to target lymphocytes for internalization and cell-to-cell mediated death, plausibly contributing to lymphopenia in patients with COVID-19.

The potential role of syncytia in SARS-Cov-2 infection

It has been proposed, as discussed above, that SARS-CoV-2-mediated syncytium formation contributes to general pathology, however, its overall contribution to viral infection remain to be investigated. [307]
The formation of abnormal multinucleated pneumocytes represents a form of pathological cytopathic effect that differs significantly from physiological syncytial.
During physiological syncytiogenesis, as in placental trophoblast formation, the cell cycle and expression of syncytiotin-2 (SYN2) fusogen are arrested in the G0 phase to allow strict regulation of the physiological cell fusion process.
Transient expression of SYN2 in cells at any other phase (S,G2,M) of the cycle results in the formation of unstable and functionally impaired syncytia.[308]

[307] Rajah MM, Bernier A, Buchrieser J, Schwartz O.
The Mechanism and Consequences of SARS-CoV-2 Spike-Mediated Fusion and Syncytia Formation.
J Mol Biol. 2022 Mar 30;434(6):167280. doi: 10.1016/j.jmb.2021.167280.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8485708/

[308] Lu X, Wang R, Zhu C, Wang H, Lin HY, Gu Y, Cross JC, Wang H.
Fine-Tuned and Cell-Cycle-Restricted Expression of Fusogenic Protein Syncytin-2 Maintains Functional Placental Syncytia.
Cell Rep. 2017 Oct 31;21(5):1150-1159. doi: 10.1016/j.celrep.2017.10.019. Erratum in: Cell Rep. 2018 Jun 26;23(13):3979.

In sharp contrast, unregulated pathogen-induced syncytium formation in cells that do not normally fuse is pathological.
Syncytial cells that form unnaturally are susceptible to a rapid cytopathic effect known as "syncytial apoptosis," due to the ability of the viral envelope protein to trigger a pro-apoptotic signaling pathway leading to karyogamy (nuclear fusion) and DNA damage. [309]
SARS-CoV-2 S protein-mediated syncytia also collapse and die during later stages of the process, and of particular interest is the observation that nuclei within S protein-mediated syncytia cluster together. [310]
Syncytium formation can also have direct virological consequences rather than simply triggering a generalized cytopathic effect or immune response. Indeed, the syncytium may allow viruses to spread directly from cell to cell without having to enter the extracellular environment.
This strategy protects the virus from neutralizing antibodies, physical barriers such as the mucociliary layer, as well as immune system components.
Many respiratory viruses such as measles, influenza, respiratory syncytial virus, parainfluenza virus, and human metapneumonia virus exploit this dissemination mechanism. [311]
Recent cell culture studies suggest that cell-to-cell spread of SARS-CoV-2 is a possible pathway and that in addition to direct cell-to-cell spread, the syncytium may contribute to the overall infectious dose and viral dissemination. In reconstituted primary bronchial epithelia, multiciliated cells and basal cells form syncytia that are then released into the apical lumen.
Vesicular inclusions containing viral particles were detected within the released syncytia, suggesting potential continuous viral replication.[312]
Single, syncytial infected cells that are released can then spread the infection, as is also the case with infection of epithelial cell cultures from macaque respiratory tissue with measles virus.[313]

---

https://doi.org/10.1016/j.celrep.2017.10.019

[309] Nardacci R, Perfettini JL, Grieco L, Thieffry D, Kroemer G, Piacentini M.
Syncytial apoptosis signaling network induced by the HIV-1 envelope glycoprotein complex: an overview.
Cell Death Dis. 2015 Aug 6;6(8):e1846. doi: 10.1038/cddis.2015.204.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4558497/

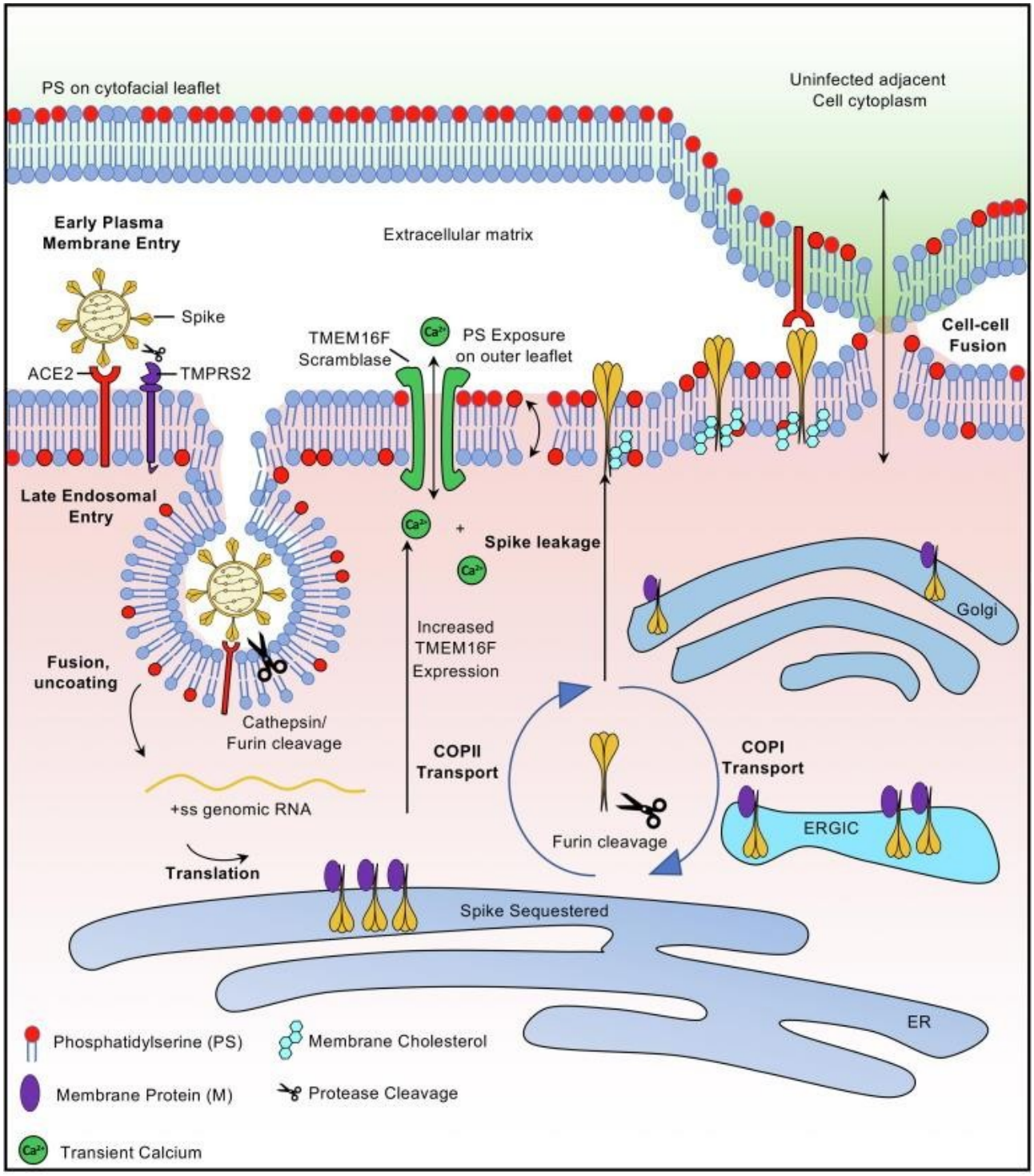
[310] Buchrieser J, et al
Syncytia formation by SARS-CoV-2-infected cells.
EMBO J. 2020 Dec 1;39(23):e106267. doi: 10.15252/embj.2020106267. Epub 2020 Nov 4. Erratum in: EMBO J. 2021 Feb 1;40(3):e107405.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7646020/

Laurelle Jackson, et al COMMIT-KZN Team, Bernadett I. et al.
SARS-CoV-2 cell-to-cell spread occurs rapidly and is insensitive to antibody neutralization
bioRxiv 2021.06.01.446516; doi: https://doi.org/10.1101/2021.06.01.446516
https://www.biorxiv.org/content/10.1101/2021.06.01.446516v1.full.pdf

[311] Cifuentes-Muñoz N, Dutch RE, Cattaneo R.
Direct cell-to-cell transmission of respiratory viruses: The fast lanes.
PLoS Pathog. 2018 Jun 28;14(6):e1007015. doi: 10.1371/journal.ppat.1007015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6023113/

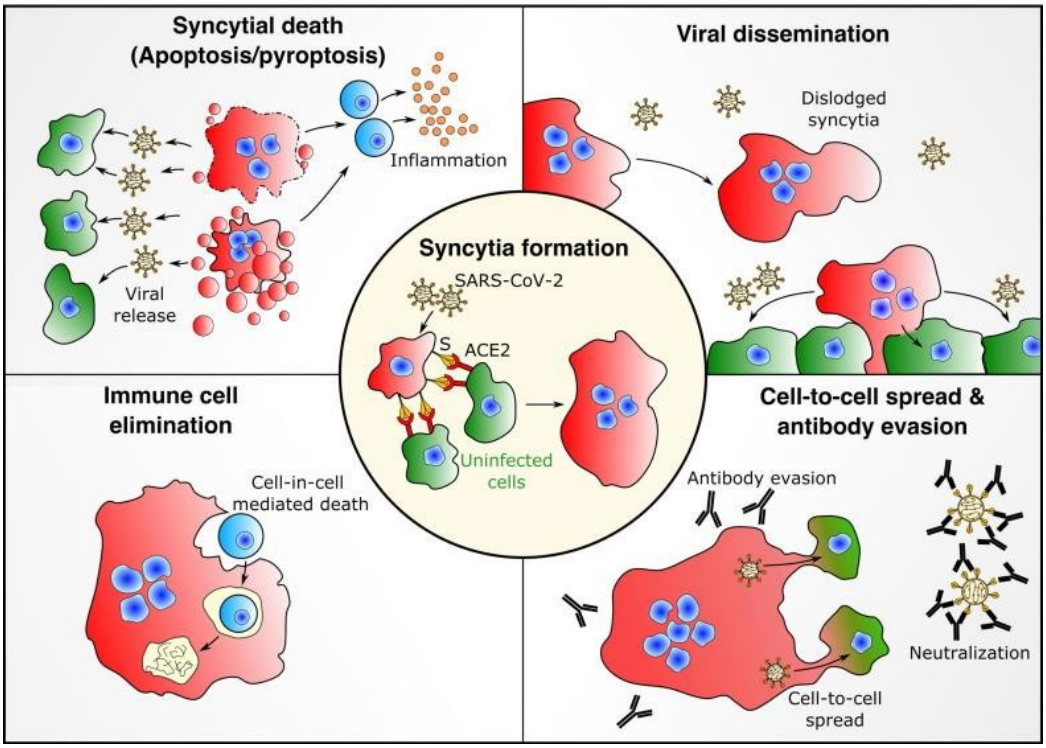
[312] Beucher G, et al
Bronchial epithelia from adults and children: SARS-CoV-2 spread via syncytia formation and type III interferon infectivity restriction.
Proc Natl Acad Sci U S A. 2022 Jul 12;119(28):e2202370119. doi: 10.1073/pnas.2202370119.
https://doi.org/10.1073/pnas.2202370119
https://www.biorxiv.org/content/10.1101/2021.05.28.446159v1.full.pdf

[313] Lin WW, Tsay AJ, Lalime EN, Pekosz A, Griffin DE.
Primary differentiated respiratory epithelial cells respond to apical measles virus infection by shedding multinucleated giant cells.
Proc Natl Acad Sci U S A. 2021 Mar 16;118(11):e2013264118. doi: 10.1073/pnas.2013264118.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7980467/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8485708/

Schematic representation of the different activities of the SARS-CoV-2 spike (S) protein during infection and syncytium formation. Viral infection begins when the S protein on the surface of the virion interacts with the ACE2 receptor. During early entry, the S protein is processed by the protease TMPRSS2 and fusion occurs at the plasma membrane (PM). Protein S can also fuse with endosomal membranes during late entry after being triggered by cathepsins and furin. Single-stranded (+ss) positive-sense viral genomic RNA is deposited in the cytoplasm and translated. Replication and transcription of viral RNA take place on membranes. After being transcribed, the S protein is translocated and inserted into the endoplasmic reticulum (ER) and is generally sequestered within intracellular membranes by the membrane structural protein (M). S protein expression leads to intracellular calcium fluctuations and increased expression of TMEM16F scramblase. TMEM16F exposes phosphatidylserine (PS) from the cytofacial sheet of PM to the exofacial sheet. The transcribed protein S is processed by furin and transported through the ER-Golgi network. During COPI (retrograde) and COPII (anterograde) transport, loss of protein S from vesicles (not shown in the simplified schematic) may occur. The S protein is then translocated into the PM, where it associates with cholesterols and induces syncytial formation by interacting with receptors on nearby uninfected cells. The sequestered S protein is packaged into virions that sprout in the Golgi or ER-Golgi intermediate compartment (ERGIC), and the virions exit the cell via lysosome-dependent deacidified exocytosis (not shown). The ER, ERGIC and Golgi membranes are double layers that are depicted as single lines in this diagram.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8485708/

The potential consequences of syncytium formation on SARS-CoV-2 pathology. Center: Cells infected with SARS-CoV-2 express the surface spike protein and form large multinucleated syncytia. Top left: the process of syncytial death by apoptosis or pyroptosis can release virus to infect neighboring cells and/or trigger an inflammatory response. Top right: infected syncytial cells can take off to contribute to viral spread and overall infectious dose. Bottom left: syncytial cells can target lymphocytes for cell-to-cell mediated death. Bottom right: Syncytia can facilitate the spread of virus from cell to cell and protect the virus from neutralizing antibodies.

Cell fusion and cardiac damage

Viruses can cause myocarditis and cardiomyopathy, but the mechanisms of disease are difficult to characterize experimentally.[314] Cardiac complications frequently observed in COVID-19 patients are also attributed to aberrant host responses to acute respiratory infection, but SARS-CoV-2 replication has occasionally been confirmed in endomyocardial biopsy and autopsy cardiac tissue.

While animal models are being developed to study cardiac infections by SARS-CoV-2, Navaratnarajah et al[315] have characterized the spread of the virus in hiPSC-CM (human induced pluripotent stem cell-derived cardiomyocytes).

Infection of these highly differentiated cells was unexpectedly efficient, with the virus taking control of nearly 90 percent of the cellular transcriptome.

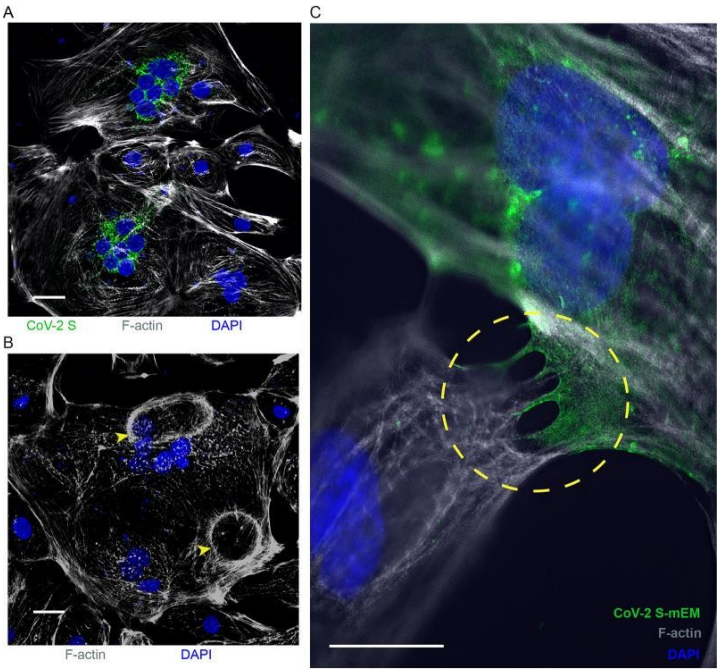
[314] Yajima T, Knowlton KU.
Viral myocarditis: from the perspective of the virus.
Circulation. 2009 May 19;119(19):2615-24. doi: 10.1161/CIRCULATIONAHA.108.766022.
https://pubmed.ncbi.nlm.nih.gov/19451363/

Tschöpe C, et al
Myocarditis and inflammatory cardiomyopathy: current evidence and future directions.
Nat Rev Cardiol. 2021 Mar;18(3):169-193. doi: 10.1038/s41569-020-00435-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7548534/

[315] Navaratnarajah CK,et al Wanek Family Program for HLHS-Stem Cell Pipeline.
Highly Efficient SARS-CoV-2 Infection of Human Cardiomyocytes: Spike Protein-Mediated Cell Fusion and Its Inhibition.
J Virol. 2021 Nov 23;95(24):e0136821. doi: 10.1128/JVI.01368-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8610601/

SARS-CoV-2 infection remodeled subcellular morphologies[316] , secretory vesicles were filled with viral progeny, and spiked virus particles covered the surface of cardiomyocytes. The study also documented a cytopathic effect and syncytium formation in infected hiPSC-CMs, with disassembly/fragmentation of sarcomeres.

In 293T and Vero cells, the multibasic site and the concomitant presence of TMPRSS2 promote this process but are not essential. [317] Because laboratory data indicate that TMPRSS2 and TMPRSS13 are not expressed in cardiomyocytes, it was hypothesized that another protease may trigger fusion in these cells. Cathepsins are candidates based on the transcriptomic data from hiPSC-CMs, as are proteases that confer fusion competence to spike proteins from other coronaviruses. [318]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8610601/
SARS-CoV-2 spike protein induces syncytia in hiPSC-CMs. **(A)** IF confocal microscopy of SARS- CoV-2 spike (CoV-2 S) expressing hiPSC-CMs. Scale bar, 50 µm. Visualized viral and cellular components are indicated with the corresponding color under each panel.
(B) IF confocal microscopy of hiPSC-CM expressing CoV- 2 S with "cadavers" of enucleated actin cytoskeleton (yellow arrows). **(C)** Superresolution confocal microscopy of CoV-2 S-mEM localization on hiPSC-CM filopodia directly contacting the sarcolemma of an adjacent hiPSC-CM (yellow circle). Scale bar, 2 µm.

Cell fusion and COVID-19 vaccines.

If spike expression-induced cell fusion contributes to COVID-19 complications, as suggested by this and previous reports[319] , spike expression by other means, including those used by vaccines, should be expected to have similar effects.

---

[316] Cortese M, et al
Integrative Imaging Reveals SARS-CoV-2-Induced Reshaping of Subcellular Morphologies.
Cell Host Microbe. 2020 Dec 9;28(6):853-866.e5. doi: 10.1016/j.chom.2020.11.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7670925/

Snijder EJ, Limpens RWAL, de Wilde AH, de Jong AWM, Zevenhoven-Dobbe JC, Maier HJ, Faas FFGA, Koster AJ, Bárcena M.
A unifying structural and functional model of the coronavirus replication organelle: Tracking down RNA synthesis.
PLoS Biol. 2020 Jun 8;18(6):e3000715. doi: 10.1371/journal.pbio.3000715.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7302735/

[317] Pope G, Mallery DL, Albecka A, Welch LG, Cattin-Ortolá J, Luptak J, Paul D, McMahon HT, Goodfellow IG, Carter A, Munro S, James LC.
Furin cleavage of SARS-CoV-2 Spike promotes but is not essential for infection and cell-cell fusion.
PLoS Pathog. 2021 Jan 25;17(1):e1009246. doi: 10.1371/journal.ppat.1009246.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7861537/

[318] Heald-Sargent T, Gallagher T.
Ready, set, fuse! The coronavirus spike protein and acquisition of fusion competence.
Viruses. 2012 Apr;4(4):557-80. doi: 10.3390/v4040557.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3347323/

Lamers MM, Mykytyn AZ, Breugem TI, Wang Y, Wu DC, Riesebosch S, van den Doel PB, Schipper D, Bestebroer T, Wu NC, Haagmans BL.
Human airway cells prevent SARS-CoV-2 multibasic cleavage site cell culture adaptation.
Elife. 2021 Apr 9;10:e66815. doi: 10.7554/eLife.66815.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8131099/[31]
[9] Buchrieser J, et al.

The case of the AstraZeneca vaccine is consistent with this possibility, as a characteristic feature of this vaccine is the high incidence of reported complications[320] , including a number of thrombotic complications[321] , attributed to antibodies caused by adenoviruses against platelet factor 4,[322] to alternative spike splicing,[323] and binding of the adenoviral vector or spike to platelets.[324]
If spike-induced cell fusion is pathogenic, the AstraZeneca vaccine is most dangerous for this mechanism because it is the only one of the four vaccines that produces the fully fusogenic native spike,[325] with a vector optimized to express very high levels of the protein.[326]

Syncytia formation by SARS-CoV-2-infected cells.
EMBO J. 2020 Dec 1;39(23):e106267. doi: 10.15252/embj.2020106267. Epub 2020 Nov 4. Erratum in: EMBO J. 2021 Feb 1;40(3):e107405.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7646020/

Bussani R, Schneider E, Zentilin L, Collesi C, Ali H, Braga L, Volpe MC, Colliva A, Zanconati F, Berlot G, Silvestri F, Zacchigna S, Giacca M.
Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology.
EBioMedicine. 2020 Nov;61:103104. doi: 10.1016/j.ebiom.2020.103104.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7677597/

[320] Cari L, Fiore P, Naghavi Alhosseini M, Sava G, Nocentini G.
Blood clots and bleeding events following BNT162b2 and ChAdOx1 nCoV-19 vaccine: An analysis of European data.
J Autoimmun. 2021 Aug;122:102685. doi: 10.1016/j.jaut.2021.102685.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8220408/

Kragholm K, et al
Thrombocytopenia after COVID-19 vaccination.
J Autoimmun. 2021 Sep;123:102712. doi: 10.1016/j.jaut.2021.102712.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8313538/

[321] Østergaard SD, Schmidt M, Horváth-Puhó E, Thomsen RW, Sørensen HT.
Thromboembolism and the Oxford-AstraZeneca COVID-19 vaccine: side-effect or coincidence?
Lancet. 2021 Apr 17;397(10283):1441-1443. doi: 10.1016/S0140-6736(21)00762-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8009607/

Pottegård A, et al
Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study.
BMJ. 2021 May 5;373:n1114. doi: 10.1136/bmj.n1114.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8097496/

[322] Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S.
Thrombotic thrombocytopenia after ChAdOx1 nCov-19 Vaccination.
N Engl J Med. 2021 Jun 3;384(22):2092-2101. doi: 10.1056/NEJMoa2104840.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8095372/

Baker AT, Boyd RJ, Sarkar D, Vant J, Crespo AT, Waraich K, Truong CD, Bates E, Wilson E, Chan CK, Lipka-Lloyd M, Fromme P, Nagalo MB, et al.
The Structure of ChAdOx1/AZD-1222 Reveals Interactions with CAR and PF4 with Implications for Vaccine-induced Immune Thrombotic Thrombocytopenia.
bioRxiv. 2021; 2021.05.19.444882. 10.1101/2021.05.19.444882
https://www.biorxiv.org/content/10.1101/2021.05.19.444882v2.full.pdf

[323] Kowarz E, Krutzke L, Külp M, Streb P, Larghero P, Reis J, Bracharz S, Engler T, Kochanek S, Marschalek
R. Vaccine-induced COVID-19 mimicry syndrome.
Elife. 2022 Jan 27;11:e74974. doi: 10.7554/eLife.74974.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8846585/

[324] Baker AT et al
ChAdOx1 interacts with CAR and PF4 with implications for thrombosis with thrombocytopenia syndrome.
Sci Adv. 2021 Dec 3;7(49):eabl8213. doi: 10.1126/sciadv.abl8213.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8635433/

[325] Watanabe Y, et al
Native-like SARS-CoV-2 Spike Glycoprotein Expressed by ChAdOx1 nCoV-19/AZD1222 Vaccine.
ACS Cent Sci. 2021 Apr 28;7(4):594-602. doi: 10.1021/acscentsci.1c00080.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8043200/

[326] Almuqrin A, Davidson AD, Williamson MK, Lewis PA, Heesom KJ, Morris S, Gilbert SC, Matthews DA.
SARS-CoV-2 vaccine ChAdOx1 nCoV-19 infection of human cell lines reveals low levels of viral backbone gene transcription alongside very high levels of SARS-CoV-2 S glycoprotein gene transcription.
Genome Med. 2021 Mar 15;13(1):43. doi: 10.1186/s13073-021-00859-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7958140/

The spike used in the other three vaccines was made less fusogenic, unintentionally, by stabilizing the structure through the two proline mutations that, as already seen, suppress a conformational change triggered by ACE2 binding. [327]

Because this change is involved in spike activation, its suppression also inhibited cell fusion in a tissue culture assay[328] .

Two additional mutations introduced into the Janssen vaccine further reduced this incidence in the same test[318] by altering the site recognized by furin. It is unclear whether these additional mutations remain as effective in the human body, as other proteases can replace furin and this cleavage may not be necessary[329] .

However, because the abundance of these proteases varies among human tissues, alteration of the furin site could influence on the incidence or location of some complications. In contrast, none of the vaccine developers mutated the S2 site, the cleavage of which exposes the fusion peptide that penetrates the target membrane. [330]

[327] Kirchdoerfer RN, Wang N, Pallesen J, Wrapp D, Turner HL, Cottrell CA, Corbett KS, Graham BS, McLellan JS, Ward AB. Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. Sci Rep. 2018 Oct 24;8(1):15701. doi: 10.1038/s41598-018-34171-7. Erratum in: Sci Rep. 2018 Dec 10;8(1):17823
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6200764/

[328] Bos R, et al
Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses.
NPJ Vaccines. 2020 Sep 28;5:91. doi: 10.1038/s41541-020-00243-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7522255/

[329] Xia S, Lan Q, Su S, Wang X, Xu W, Liu Z, Zhu Y, Wang Q, Lu L, Jiang S.
The role of furin cleavage site in SARS-CoV-2 spike protein-mediated membrane fusion in the presence or absence of trypsin.
Signal Transduct Target Ther. 2020 Jun 12;5(1):92. doi: 10.1038/s41392-020-0184-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7289711/

Hörnich BF, Großkopf AK, Schlagowski S, Tenbusch M, Kleine-Weber H, Neipel F, Stahl-Hennig C, Hahn AS.
SARS-CoV-2 and SARS-CoV Spike-Mediated Cell-Cell Fusion Differ in Their Requirements for Receptor Expression and Proteolytic Activation.
J Virol. 2021 Apr 12;95(9):e00002-21. doi: 10.1128/JVI.00002-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8104116/

[330] Koppisetti RK, Fulcher YG, Van Doren SR.
Fusion Peptide of SARS-CoV-2 Spike Rearranges into a Wedge Inserted in Bilayered Micelles.
J Am Chem Soc. 2021 Aug 25;143(33):13205-13211. doi: 10.1021/jacs.1c05435.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8370118/

CONVALESCENT PLASMA

More than 125 years ago, the first therapeutic serum was developed from animals actively immunized against diphtheria toxin,[331] and Paul Ehrlich later produced a seminal article linking curative antiserum to neutralizing antibodies.[332]
Today, passive immunization involves the infusion of antigen-specific mAbs or polyclonal antibodies derived from nonhuman or human blood products.
Although polyclonal antibodies collected from immunized animals are the primary source of antisera, there is a risk of "serum sickness" (serum sickness[333]), especially after repeated exposures, as the recipient may generate an immune response against antibodies of nonhuman origin.
These risks are mitigated with the use of convalescent plasma from human patients.
With careful screening (e.g., to assess the presence of infectious agents and to establish antibody titer and neutralizing capacity), convalescent plasma therapy (CPT) can be effective with minimal safety risks.[334]

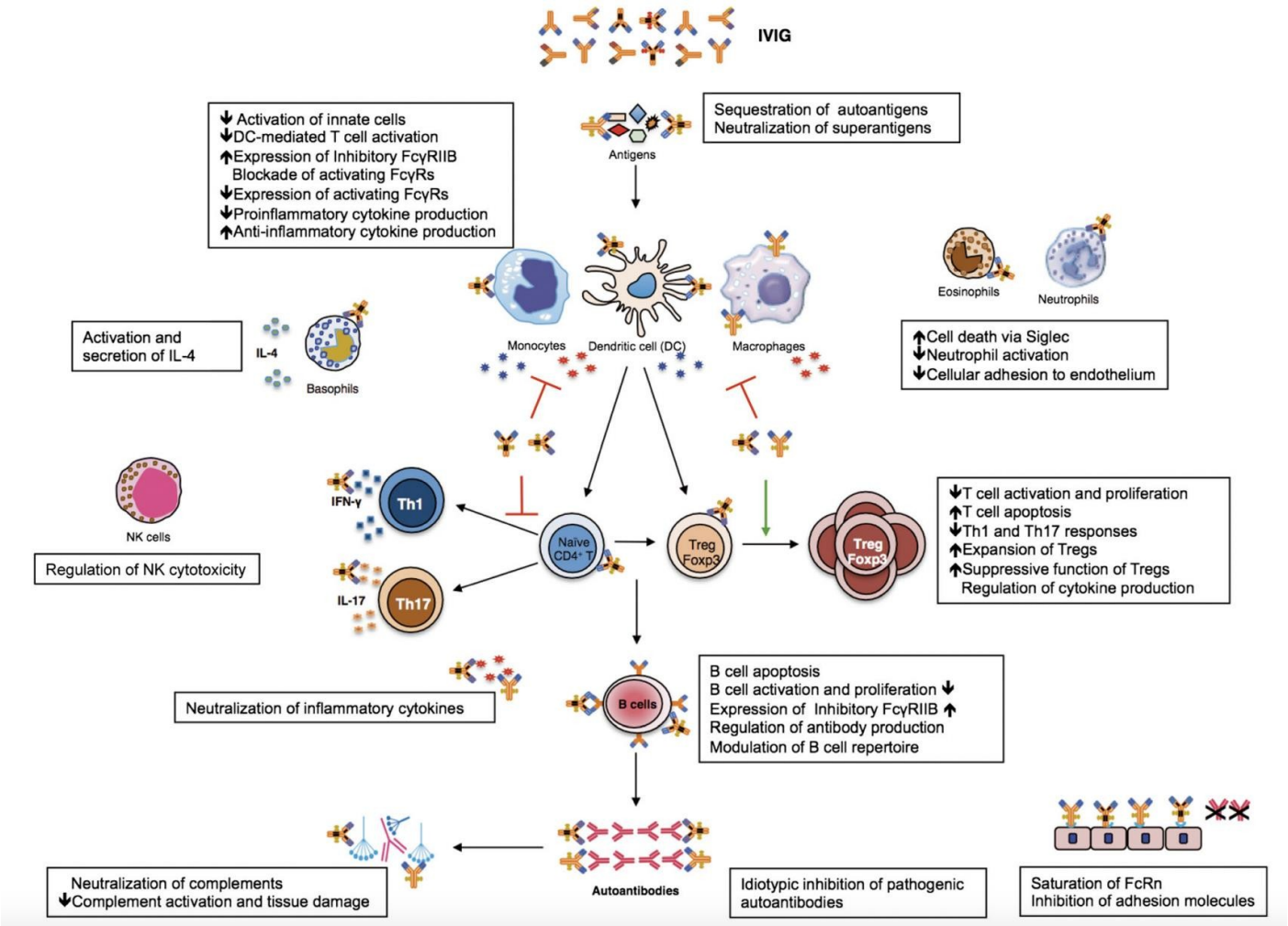
https://pubmed.ncbi.nlm.nih.gov/31161341/
The mechanisms of action of IVIGs on the various branches of autoimmune and inflammatory responses. Autoantigens endocytosed by innate immune cells such as dendritic cells and macrophages are presented to autoreactive T and B cells leading to proliferation of autoreactive cells and production of inflammatory cytokines and autoreactive antibodies. IVIG targets different soluble and cellular compartments of the immune system to exert its therapeutic effects on various autoimmune diseases. IVIG neutralizes autoantigens and superantigens and inhibits the activation of various innate immune cells such as dendritic cells, macrophages, monocytes, granulocytes, and NK cells. Regarding the effector phase of the autoimmune response, IVIG inhibits the activation and proliferation of effector T (Th1, Th17) and B lymphocytes by enhancing the expansion and function of regulatory T lymphocytes (Tregs). IVIG also induces the expression of inhibitory FcγRIIB in a subset of macrophages and B cells. In addition, IVIG saturates neonatal Fc receptors (FcRn), modulates the cytokine network, induces immune cell apoptosis, neutralizes pathogenic autoantibodies by anti-idiotypic interaction, inhibits complement activation, and regulates the B-cell repertoire.
ADCC, antibody-dependent cell-mediated cytotoxicity; FcγR, Fcγ receptors; NK, natural killer cell

[331] Llewelyn MB, Hawkins RE, Russell SJ.
Discovery of antibodies.
BMJ. 1992 Nov 21;305(6864):1269-72. doi: 10.1136/bmj.305.6864.1269.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1883762/

[332] Kaufmann SH.
Immunology's foundation: the 100-year anniversary of the Nobel Prize to Paul Ehrlich and Elie Metchnikoff.
Nat Immunol. 2008 Jul;9(7):705-12. doi: 10.1038/ni070708-705.
https://pubmed.ncbi.nlm.nih.gov/18563076/
[333] Rixe N, Tavarez MM.
Serum Sickness. [Updated 2022 May 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan
https://www.ncbi.nlm.nih.gov/books/NBK538312/

[334] Taylor PC, Adams AC, Hufford MM, de la Torre I, Winthrop K, Gottlieb RL.
Neutralizing monoclonal antibodies for treatment of COVID-19.
Nat Rev Immunol. 2021 Jun;21(6):382-393. doi: 10.1038/s41577-021-00542-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8054133/

Maddur MS, Lacroix-Desmazes S, Dimitrov JD, Kazatchkine MD, Bayry J, Kaveri SV.
Natural Antibodies: from First-Line Defense Against Pathogens to Perpetual Immune Homeostasis.
Clin Rev Allergy Immunol. 2020 Apr;58(2):213-228. doi: 10.1007/s12016-019-08746-9.
https://pubmed.ncbi.nlm.nih.gov/31161341/



Convalescent plasma (CP) treatment involves collecting plasma from patients who are recovering, that is, who have faced an infectious disease and have been successfully cured (known as a convalescent patient), with the intention of administering it to recipient patients who have not yet developed an effective adaptive immune response.[335]

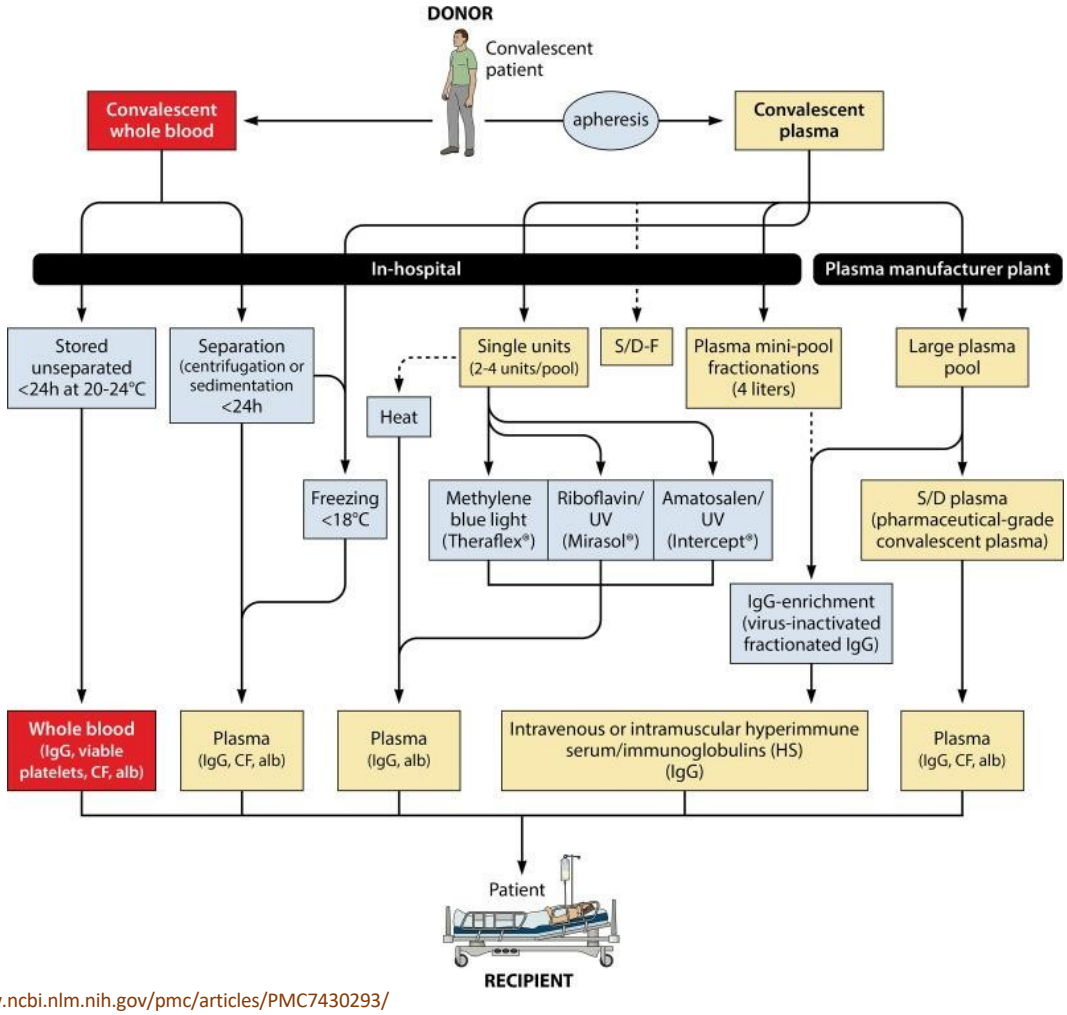
The main goal of this treatment is to reduce the viral load (viremia) in the recipient through the neutralizing action of antibodies produced by the donor, which can occur between 10 and 14 days after infection.
[336]

[335] Brown BL, McCullough J.
Treatment for emerging viruses: Convalescent plasma and COVID-19.
Transfus Apher Sci. 2020 Jun;59(3):102790. doi: 10.1016/j.transci.2020.102790.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194745/

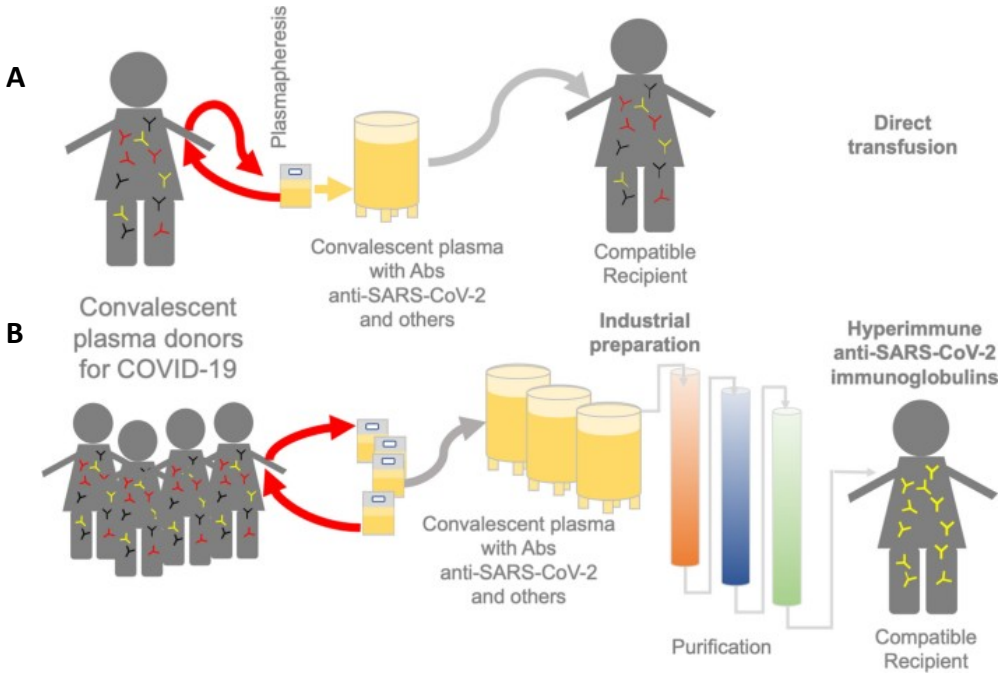
Luke TC, Kilbane EM, Jackson JL, Hoffman SL.
Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment?
Ann Intern Med. 2006 Oct 17;145(8):599-609. doi: 10.7326/0003-4819-145-8-200610170-00139
https://www.acpjournals.org/doi/10.7326/0003-4819-145-8-200610170-00139

[336] Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, Chan P, Wong KC, Leung CB, Cheng G.
Use of convalescent plasma therapy in SARS patients in Hong Kong.
Eur J Clin Microbiol Infect Dis. 2005 Jan;24(1):44-6. doi: 10.1007/s10096-004-1271-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7088355/

Focosi D, Anderson AO, Tang JW, Tuccori M.
Convalescent Plasma Therapy for COVID-19: State of the Art.
Clin Microbiol Rev. 2020 Aug 12;33(4):e00072-20. doi: 10.1128/CMR.00072-20.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7430293/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7430293/
Summary of possible convalescent blood products (CBP). See text of article for explanation.



https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-021-01576-5
Methods of extraction and administration of Convalescent Plasma (CP). **A)** a convalescent donor who has developed antibodies after recovery from the disease could donate plasma (usually through plasmapheresis) that includes antibodies against SARS-CoV-2 for direct transfusion and other antibodies (passive immunity) to patients with severe symptoms of the disease.**B)** plasma from a group of donors could be used to identify

and purify specific antibodies against SARS-CoV-2, eliminating other antibodies and proteins, making this method an alternative for passive immunization.

A crucial factor in the success of CP therapy is donor selection, as one of the main problems that have been identified is the diversity of viral variants found in the population and the neutralizing antibody titer in different plasma samples.[337]

Therefore, it is necessary to ensure that the plasma contains an adequate concentration of neutralizing antibodies, determine the antibody titer, and use an *in vitro* neutralization assay with virus variants. In addition, the potential side effects of CP therapy, particularly serum incompatibility in recipients, must be considered[338] .

As seen above, CP therapy has been used to treat patients infected with SARS-CoV, MERS-CoV, pandemic H1N1 2009 influenza, and suffering from EBOV disease[339] , and has been shown to reduce viral load and mortality rate in the critical stage of infection.[340] Based on these results, the CP of patients with

---

[337] Cunningham AC, Goh HP, Koh D.
Treatment of COVID-19: old tricks for new challenges.
Crit Care. 2020 Mar 16;24(1):91. doi: 10.1186/s13054-020-2818-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7076992/

[338] Li L, et al
Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening covid-19: a randomized clinical trial.
JAMA. 2020 Aug 4;324(5):460-470. doi: 10.1001/jama.2020.10044. Erratum in: JAMA. 2020 Aug 4;324(5):519.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270883/.

Salazar E, et al
Convalescent plasma anti-SARS-CoV-2 spike protein ectodomain and receptor-binding domain IgG correlate with virus neutralization.
J Clin Invest. 2020 Dec 1;130(12):6728-6738. doi: 10.1172/JCI141206.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7685744/

Arabi YM, et al
Feasibility of Using Convalescent Plasma Immunotherapy for MERS-CoV Infection, Saudi Arabia.
Emerg Infect Dis. 2016 Sep;22(9):1554-61. doi: 10.3201/eid2209.151164.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4994343/

Klein SL, et al
Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population.
J Clin Invest. 2020 Nov 2;130(11):6141-6150. doi: 10.1172/JCI142004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7598041/

Franchini M, Marano G, Velati C, Pati I, Pupella S, Maria Liumbruno G.
Operational protocol for donation of anti-COVID-19 convalescent plasma in Italy.
Vox Sang. 2021 Jan;116(1):136-137. doi: 10.1111/vox.12940.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7264735/

[339] Stockman LJ, Bellamy R, Garner P.
SARS: systematic review of treatment effects.
PLoS Med. 2006 Sep;3(9):e343. doi: 10.1371/journal.pmed.0030343.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1564166/

Ko JH, et al
Challenges of convalescent plasma infusion therapy in Middle East respiratory coronavirus infection: a single center experience.
Antivir Ther. 2018;23(7):617-622. doi: 10.3851/IMP3243.
https://pubmed.ncbi.nlm.nih.gov/29923831/

Khalenkov A, He Y, Reed JL, Kreil TR, McVey J, Norton M, Scott J, Scott DE.
Characterization of source plasma from self-identified vaccinated or convalescent donors during the 2009 H1N1 pandemic.
Transfusion. 2018 May;58(5):1108-1116. doi: 10.1111/trf.14530.
https://pubmed.ncbi.nlm.nih.gov/29446442/

Dean CL, Hooper JW, Dye JM, Zak SE, Koepsell SA, Corash L, Benjamin RJ, Kwilas S, Bonds S, Winkler AM, Kraft CS.
Characterization of Ebola convalescent plasma donor immune response and psoralen treated plasma in the United States.
Transfusion. 2020 May;60(5):1024-1031. doi: 10.1111/trf.15739.
https://pubmed.ncbi.nlm.nih.gov/32129478/

[340] Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, Chan P, Wong KC, Leung CB, Cheng G.
Use of convalescent plasma therapy in SARS patients in Hong Kong.

SARS-CoV-2 infection has been administered as an experimental therapy in critically ill patients, along with the use of polyclonal immunoglobulins and plasma derivatives isolated and purified from the blood of COVID- 19 survivors with very promising results.[341]

CP administration in patients leads to an increase in neutralizing IgG and IgM titers,[342] a decrease in short-term mortality in patients with severe respiratory failure[343] , the

---

Eur J Clin Microbiol Infect Dis. 2005 Jan;24(1):44-6. doi: 10.1007/s10096-004-1271-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7088355/

Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, Makki S, Rooney KD, Nguyen-Van-Tam JS, Beck CR;
Convalescent Plasma Study Group. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis.
J Infect Dis. 2015 Jan 1;211(1):80-90. doi: 10.1093/infdis/jiu396.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4264590/

[341] Joyner MJ, et al
Early safety indicators of COVID-19 convalescent plasma in 5000 patients.
J Clin Invest. 2020 Sep 1;130(9):4791-4797. doi: 10.1172/JCI140200
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7456238/

Wu Y, Hong K, Ruan L, Yang X, Zhang J, Xu J, Pan S, Ren L, Chen L, Huang C, Shang Y.
Patients with prolonged positivity of SARS-CoV-2 RNA benefit from convalescent plasma therapy: a retrospective study.
Virol Sin. 2020 Dec;35(6):768-775. doi: 10.1007/s12250-020-00281-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7457444/

Salazar E, et al
Treatment of Coronavirus Disease 2019 patients with convalescent plasma reveals a signal of significantly decreased mortality.
Am J Pathol. 2020 Nov;190(11):2290-2303. doi: 10.1016/j.ajpath.2020.08.001.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7417901/

Joyner MJ, et al
Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: initial three-month experience.
medRxiv [Preprint]. 2020 Aug 12:2020.08.12.20169359. doi: 10.1101/2020.08.12.20169359.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7430623/

Lung T, Kazatchkine MD, Risch L, Risch M, Nydegger EU.
A consideration of convalescent plasma and plasma derivatives in the care of Severely-ill patients with COVID-19.
Transfus Apher Sci. 2020 Oct;59(5):102936. doi: 10.1016/j.transci.2020.102936.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7833822/

Singh N, Pandey A
Blood Plasma from Survivors of COVID-19: A Novel and Next Frontier Approach to Fight against Pandemic Coronavirus.
Int J Immunol Immunother (2020) 7:045. DOI: 10.23937/2378-3672/1410045
https://www.clinmedjournals.org/articles/ijii/international-journal-of-immunology-and-immunotherapy-ijii-7-045.php?jid=ijii

[342] Shen C, et al
Treatment of 5 critically ill patients with COVID-19 with convalescent plasma.
JAMA. 2020 Apr 28;323(16):1582-1589. doi: 10.1001/jama.2020.4783.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7101507/.

Zhang B, Liu S, Tan T, Huang W, Dong Y, Chen L, Chen Q, Zhang L, Zhong Q, Zhang X, Zou Y, Zhang S.
Treatment with convalescent plasma for critically ill patients with Severe Acute Respiratory Syndrome Coronavirus 2 Infection.
Chest. 2020 Jul;158(1):e9-e13. doi: 10.1016/j.chest.2020.03.039.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195335/

Ye M, Fu D, Ren Y, Wang F, Wang D, Zhang F, Xia X, Lv T.
Treatment with convalescent plasma for COVID-19 patients in Wuhan, China.
J Med Virol. 2020 Oct;92(10):1890-1901. doi: 10.1002/jmv.25882.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262027/

Duan K, et al
Effectiveness of convalescent plasma therapy in severe COVID-19 patients.
Proc Natl Acad Sci U S A. 2020 Apr 28;117(17):9490-9496. doi: 10.1073/pnas.2004168117.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196837/

[343] Perotti C, et al
Mortality reduction in 46 severe Covid-19 patients treated with hyperimmune plasma. A proof-of-concept single arm multicenter trial.
Haematologica. 2020 Dec 1;105(12):2834-2840. doi: 10.3324/haematol.2020.261784.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7716363/.

in-hospital mortality and reduced length of hospital stay for critically ill patients[344] . It has been found that CP treatment is most efficient when administered to patients without critical or life-threatening conditions,[345] and is also often accompanied by the integration of different drugs, including antivirals, antibiotics, antifungals, corticosteroids, and anticoagulants,[346] depending on the patient's needs, resulting in greater variability in response, which makes the study of therapy efficacy more complex.

Guidelines for the use of COVID-19 convalescent plasma (CCP) with reference antibody titer table

Convalescent Plasma
https://www.idsociety.org/covid-19-real-time-learning-network/therapeutics-and-interventions/convalescent-plasma/

---

[344] Ibrahim D, Dulipsingh L, Zapatka L, Eadie R, Crowell R, Williams K, Wakefield DB, Cook L, Puff J, Hussain SA.
Factors associated with good patient outcomes following convalescent plasma in COVID-19: A Prospective Phase II Clinical Trial.
Infect Dis Ther. 2020 Dec;9(4):913-926. doi: 10.1007/s40121-020-00341-2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7502154/.

Libster R, et al Fundación INFANT-COVID-19 Group.
Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults.
N Engl J Med. 2021 Feb 18;384(7):610-618. doi: 10.1056/NEJMoa2033700.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7793608/

[345] Zeng QL, Yu ZJ, Gou JJ, Li GM, Ma SH, Zhang GF, Xu JH, Lin WB, Cui GL, Zhang MM, Li C, Wang ZS, Zhang ZH, Liu ZS.
Effect of convalescent plasma therapy on viral shedding and survival in patients with Coronavirus Disease 2019.
J Infect Dis. 2020 Jun 16;222(1):38-43. doi: 10.1093/infdis/jiaa228.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7197534/

Xia X, et al
Improved clinical symptoms and mortality among patients with severe or critical COVID-19 after convalescent plasma transfusion.
Blood. 2020 Aug 6;136(6):755-759. doi: 10.1182/blood.2020007079.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7414593/

Hartman WR, Hess AS, Connor JP.
Hospitalized COVID-19 patients treated with convalescent plasma in a mid-size city in the Midwest.
Transl Med Commun. 2020;5(1):17. doi: 10.1186/s41231-020-00068-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7549340/

[346] Hartman WR, Hess AS, Connor JP.
Hospitalized COVID-19 patients treated with convalescent plasma in a mid-size city in the Midwest.
Transl Med Commun. 2020;5(1):17. doi: 10.1186/s41231-020-00068-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7549340/

Salazar E, et al
Convalescent plasma anti-SARS-CoV-2 spike protein ectodomain and receptor-binding domain IgG correlate with virus neutralization.
J Clin Invest. 2020 Dec 1;130(12):6728-6738. doi: 10.1172/JCI141206.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7685744/

Abolghasemi H, et al
Clinical efficacy of convalescent plasma for treatment of COVID-19 infections: Results of a multicenter clinical study.
Transfus Apher Sci. 2020 Oct;59(5):102875. doi: 10.1016/j.transci.2020.102875.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7362821/

Hegerova L, et al
Use of convalescent plasma in hospitalized patients with COVID-19: case series.
Blood. 2020 Aug 6;136(6):759-762. doi: 10.1182/blood.2020006964.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7414587/

Liu STH, et al
Convalescent plasma treatment of severe COVID-19: a propensity score-matched control study.
Nat Med. 2020 Nov;26(11):1708-1713. doi: 10.1038/s41591-020-1088-9.
https://pubmed.ncbi.nlm.nih.gov/32934372/

Plasma transfusion from convalescents of COVID-19
https://health.ec.europa.eu/blood-tissues-cells-and-organs/covid-19-convalescent-plasma-transfusion_it

An EU program of COVID-19 convalescent plasma collection and transfusion Guidance on collection, testing, processing, storage, distribution and monitored use
https://health.ec.europa.eu/system/files/2021-03/guidance_plasma_covid19_en_0.pdf

COVID-19 Convalescent Plasma
https://www.covid19treatmentguidelines.nih.gov/therapies/anti-sars-cov-2-antibody-products/covid-19-convalescent-plasma/

COVID-19: Convalescent plasma and hyperimmune globulin
https://www.uptodate.com/contents/covid-19-convalescent-plasma-and-hyperimmune-globulin

Recommendations for Investigational COVID-19 Convalescent Plasma
https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-applications-inds-cber-regulated-products/recommendations-investigational-covid-19-convalescent-plasma

Tests Acceptable for Use in the Manufacture of COVID-19 Convalescent Plasma with High Titers of Anti-SARS-CoV-2 Antibodies

Manufacturer (listed alphabetically)	Assay	Qualifying Result	Date of Listing under this EUA
Abbott	AdviseDx SARSCoV-2 IgG II (ARCHITECT and Alinity i)	≥ 1280 AU/mL	December 28, 2021
Diasorin	LIAISON SARS-CoV-2 TrimericS IgG	≥ 87 AU/mL	December 28, 2021
EUROIMMUN	Anti-SARS-CoV-2 S1 Curve ELISA (IgG)	>55 RU/mL	February 9, 2022
GenScript	cPass SARS-CoV-2 Neutralization Antibody Detection Kit	Inhibition ≥ 80%	December 28, 2021
Kantaro	COVID-SeroKlir, Kantaro Semi-Quantitative SARS-CoV-2 IgG Antibody Kit	Spike ELISA > 69 AU/mL	December 28, 2021
Ortho	VITROS Anti-SARS-CoV-2 IgG Quantitative Reagent Pack	>200 BAU/mL	December 28, 2021
Roche	Elecsys Anti-SARS-CoV-2 S	> 210 U/mL	December 28, 2021

https://www.fda.gov/media/141477/download
Table of acceptable tests for use in the production of COVID-19 convalescent plasma with high titers of anti-SARS-CoV-2 antibodies

ADE and plasma from convalescents

The previously mentioned study by Okuya et al[347] , found the presence of neutralizing antibodies in most convalescent phase sera tested collected at 28-73 days after disease onset (median 47; IQR 38.5-50.5), while more than half showed FcγRIIa- or C1q -mediated ADE activity.

[347] Okuya K, Hattori T, Saito T, Takadate Y, Sasaki M, Furuyama W, Marzi A, Ohiro Y, Konno S, Hattori T, Takada A.
Multiple Routes of Antibody-Dependent Enhancement of SARS-CoV-2 Infection.
Microbiol Spectr. 2022 Apr 27;10(2):e0155321. doi: 10.1128/spectrum.01553-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9045191/

Considering the presence of antibodies that potentially aggravate SARS-CoV-2 infection, ADE could raise a potential issue for passive immunization with COVID- 19 convalescent plasma as well as its therapeutic utility against SARS-CoV-2 variants[348] .
Clark et al,[349] to answer this important question, quantified anti-S antibodies of IgG, IgA and IgM isotypes and analyzed neutralization profiles of plasma samples from COVID-19 convalescents (CCP).
A retroviral pseudotype-based infectivity assay was adapted to study the neutralization titer of CCP units in cells expressing ACE2 receptors alone or in combination with FcαR or FcγRIIA.
The results showed that while the neutralizing activity of CCP correlated better with higher titers of anti-S IgG antibodies, the neutralizing titer was not affected when Fc receptors were present on the target cells. These observations support the absence of ADE from the IgG and IgA isotypes found in CCP.

https://www.frontiersin.org/articles/10.3389/fmed.2021.637642/full
Potential mechanisms of monocyte infection and immune potentiation in severe COVID-19. **(a)** Receptor-mediated infection of target cells by SARS-CoV-2 is achieved by binding of the viral receptor binding domain (RBD) with host membrane-bound ACE2, which allows proteasomal cleavage by TMPRSS2 of the viral membrane spike to allow virus-host membrane fusion; **(b)** In antibody-dependent enhancement (ADE), binding of the virus-antibody complex to a gamma Fc receptor stabilizes the viral spike in a manner that mimics the function of the viral receptor, enabling viral membrane cleavage and fusion of virus-host membranes; **(c)** Induction of autoantibodies may result from molecular mimicry by viral proteins having similar sequence to host proteins, anti-idiotype antibodies with cross-reactivity to host receptors, or direct disruption of immunological tolerance, which may be induced by hyperactivation of TLR7 (not shown); **(d)** Increased disease severity may result from maladaptive immune responses to SARS-CoV-2 virus. Viral infection of monocytes/macrophages may contribute to inflammatory pathology by activating downstream cytokine signaling and cell differentiation pathways. Inflammatory responses can also be induced by activation of pattern recognition receptors, including RIG-I- like receptors (RLRs), Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). Receptors expressed on the immune cell membrane mediate adhesion of viral membrane glycoproteins, potentially contributing to infectivity by stabilizing the virus on the host cell membrane. Activation of complement pathway receptors by viral glycoproteins or antibody-bound target proteins can produce tissue damage by inducing complement-dependent cytotoxicity (CDC). Cross-linking of Fc gamma receptors by immune complexes can induce antibody-dependent cellular cytotoxicity (ADCC) and release of neutrophil extracellular traps (not shown). Elevated expression of cytokines and chemokines promotes cell recruitment, increased vascular permeability, and inflammatory damage to infected tissues

[348] Volz E, et al
Transmission of SARS-CoV-2 Lineage B.1.1.7 in England: Insights from linking epidemiological and genetic data
Nature. 2021 May;593(7858):266-269. doi: 10.1038/s41586-021-03470-x.
https://eprints.whiterose.ac.uk/175263/1/2020.12.30.20249034v2.full.pdf

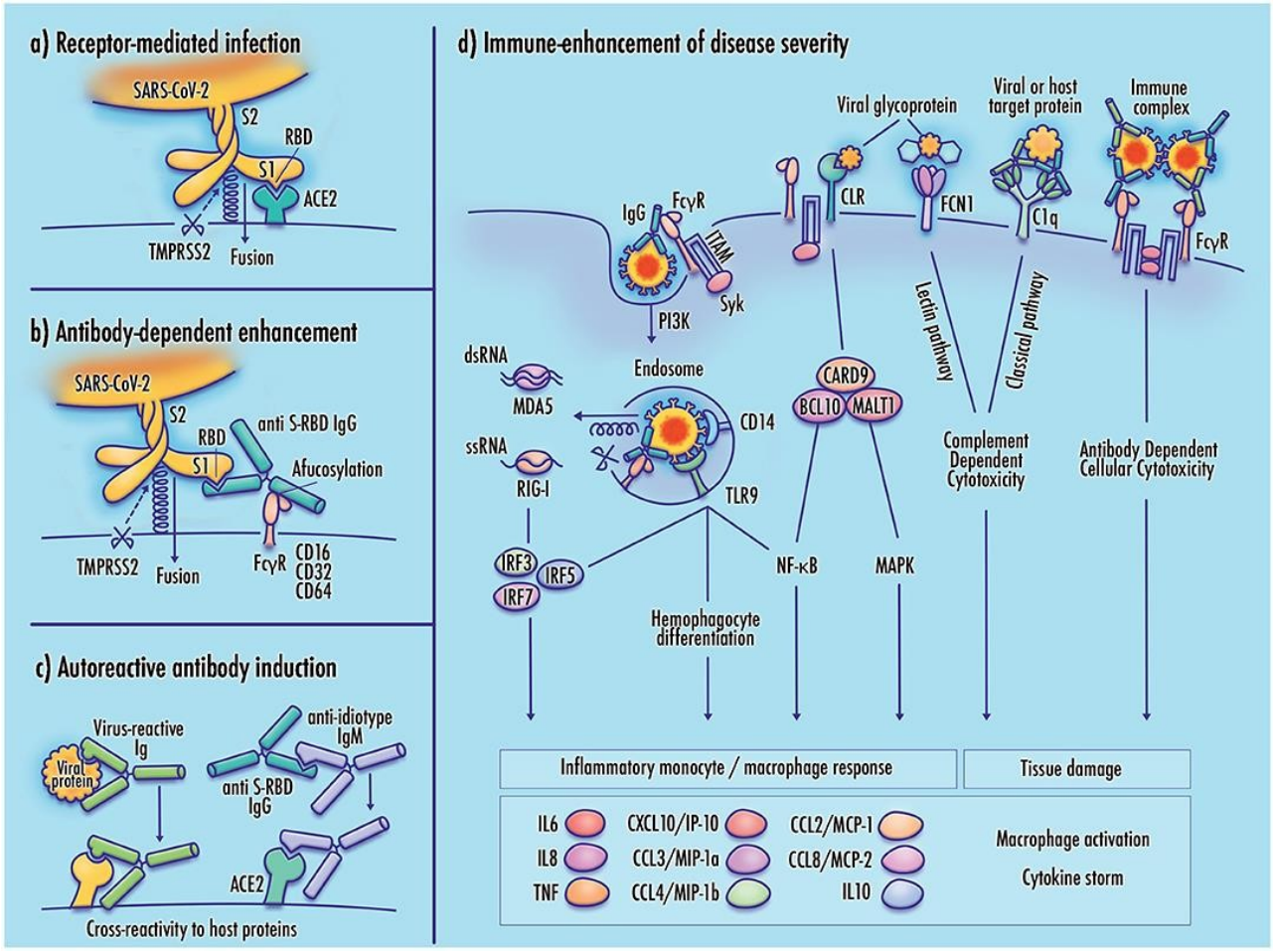
Tegally H, et al
Detection of a SARS-CoV-2 variant of concern in South Africa.
Nature. 2021 Apr;592(7854):438-443. doi: 10.1038/s41586-021-03402-9.
https://pubmed.ncbi.nlm.nih.gov/33690265/

Lauring AS, Hodcroft EB.
Genetic Variants of SARS-CoV-2-What Do They Mean?
JAMA. 2021 Feb 9;325(6):529-531. doi: 10.1001/jama.2020.27124.
https://pubmed.ncbi.nlm.nih.gov/33404586/

Faria NR, et al
Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil.
Science. 2021 May 21;372(6544):815-821. doi: 10.1126/science.abh2644.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8139423/

Lee WS, et al
Decay of Fc-dependent antibody functions after mild to moderate COVID-19.
Cell Rep Med. 2021 Jun 15;2(6):100296. doi: 10.1016/j.xcrm.2021.100296.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8106889/

[349] Clark NM, Janaka SK, Hartman W, Stramer S, Goodhue E, Weiss J, Evans DT, Connor JP.
Anti-SARS-CoV-2 IgG and IgA antibodies in COVID-19 convalescent plasma do not enhance viral infection.
PLoS One. 2022 Mar 8;17(3):e0257930. doi: 10.1371/journal.pone.0257930.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8903276/



Italian clinical trials of efficacy of plasma from convalescents

The retrospective cohort study published in March 2022 by Franchini, Gligani and De Donno et al "*Convalescent Plasma for Hospitalized COVID-19 Patients: A Single-Center Experience*" [350], reports the experience of Mantua City Hospital on the compassionate use of CCP in patients hospitalized for severe COVID-19.

Between April 2020 and April 2021, 405 consecutive COVID-19 patients received 657 units of CCP[351] with a median anti-SARS-CoV-2 neutralizing antibody (nAb) titer[352] of 160 (interquartile range (IQR), 80- 320).

[350] Franchini M, et al
On behalf of convalescent plasma study group. convalescent plasma for hospitalized COVID-19 Patients: a single-center experience.
Life (Basel). 2022 Mar 14;12(3):420. doi: 10.3390/life12030420.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8950373/

[351] Franchini M., Marano G., Velati C., Pati I., Pupella S., Maria Liumbruno G.
Operational protocol for donation of anti-COVID-19 convalescent plasma in Italy.
Vox Sang. 2021;116:136-137. doi: 10.1111/vox.12940.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7264735/

Accorsi P., Berti P., de Angelis V., De Silvestro G., Mascaretti L., Ostuni A.
Position paper on the preparation of immune plasma to be used in the treatment of patients with COVID-19.
Transfus. Apher. Sci. 2020;59:102817. doi: 10.1016/j.transci.2020.102817.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7239775/

[352] Perotti C., Del Fante C., Baldanti F., Franchini M., Percivalle E., Nepita E.V., Seminari E., De Silvestri A., Bruno R., Klersy C.
Plasma from donors recovered from the new Coronavirus 2019 as therapy for critical patients with COVID-19 (COVID-19 plasma study): A multicenter study protocol.
Intern. Emerg. Med. 2020;15:819-824. doi: 10.1007/s11739-020-02384-2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8849045/

The SARS-CoV-2 neutralizing antibody titration test was performed at the Molecular Virology Unit of the University Hospital of Pavia and was based on the determination of the cytopathic effect.

Reference articles for the method:

- *Mortality reduction in 46 patients with severe COVID-19 treated with hyperimmune plasma. A proof-of-concept, single-arm, multicenter trial.*
- *Plasma from donors recovered from the new Coronavirus 2019 as therapy for critical patients with COVID-19 (COVID-19 plasma study): A multicenter study protocol.*

Their mean age was 68 years (IQR, 56-78 years) and 62% were male. At enrollment, 55% of patients had an increased body mass index (BMI) and 25.6% had at least three comorbidities. The crude mortality rate at 28 days was 12.6% (51/405). Young age (<68 years), mild disease (admission to low-intensity wards) and early treatment (<7 days from symptom onset) with high nAb titer (≥320) were independently associated with favorable response to CCP treatment .
No safety issues were reported, with a CCP-related adverse reaction rate (all mild) of 1.3%. Other studies on the use of hyperimmune plasma for the treatment of Covid-19 by Dr. De Donno's group have confirmed the validity of this approach, even in pregnant women. [353]

Perotti C., Baldanti F., Bruno R., Del Fante C., Seminari E., Casari S., Percivale E., Glingani C., Musella V., Belliato V., et al.
Mortality reduction in 46 patients with severe COVID-19 treated with hyperimmune plasma. A proof-of-concept, single-arm, multicenter trial.
Haematologica. 2020;105:2834. doi: 10.3324/haematol.2020.261784.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8950373/

Franchini M., Mengoli C., Caruso B., Petilino R., Ballotari A., Glingani C.
Measuring accuracy of the neutralizing activity of COVID-19 convalescent plasma.
Clin. Chem. Lab. Med. 2021;60:e4–e6. doi: 10.1515/cclm-2021-0810.
https://www.degruyter.com/document/doi/10.1515/cclm-2021-0810/html

Luther A. et al
Outcomes of convalescent plasma with defined high- versus lower-neutralizing antibody titers against SARS-CoV-2 among hospitalized patients: CoronaVirus Inactivating Plasma (CoVIP), double-blind phase 2 study
medRxiv 2022.04.29.22274387; doi: https://doi.org/10.1101/2022.04.29.22274387
https://www.medrxiv.org/content/10.1101/2022.04.29.22274387v1.full.pdf

Focosi D, Franchini M.
Clinical predictors of SARS-CoV-2 neutralizing antibody titers in COVID-19 convalescents: Implications for convalescent plasma donor recruitment.
Eur J Haematol. 2021 Jul;107(1):24-28. doi: 10.1111/ejh.13630.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8250676/

Gilchuk P, et al
Standardized two-step testing of antibody activity in COVID-19 convalescent plasma.
iScience. 2022 Jan 21;25(1):103602. doi: 10.1016/j.isci.2021.103602.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8653399/

[353] Franchini M, Glingani C, Bellani A, Cicorella N, Amadini A, De Donno G, Casari S.
Early and persistent viral clearance in COVID-19 patients treated with convalescent plasma.
Transfus Clin Biol. 2021 Aug;28(3):309-310. doi: 10.1016/j.tracli.2021.04.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8055517/

Franchini M, et al
Safety and Efficacy of Convalescent Plasma in Elderly COVID-19 Patients: The RESCUE Trial.
Mayo Clin Proc Innov Qual Outcomes. 2021 Apr;5(2):403-412. doi: 10.1016/j.mayocpiqo.2021.01.010
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7869678/

Perotti C, et al
Covid-Plasma Task Force. Mortality reduction in 46 severe Covid-19 patients treated with hyperimmune plasma. A proof-of-concept single arm multicenter trial.
Haematologica. 2020 Dec 1;105(12):2834-2840. doi: 10.3324/haematol.2020.261784.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7716363/

Equally, the randomized controlled trial by De Silvestro et al "*Outcome of SARS CoV-2 in patients treated with convalescent plasma: One-year of data from the Veneto region (Italy) Registry*" [354] reports that of the 1,517 patients treated with CCP, 209 died at the 30-day follow-up (14%).
Death was significantly associated with older age, longer hospitalization time before PCC infusion, more inclusion criteria) and associated comorbidities.
Conditions significantly associated with an increased rate of death were $_{PaO2/FiO2}$ ≤ 200 and tachypnea with RR>30 at admission, concomitant hypertension, cardiovascular disease, chronic kidney disease, dyslipidemia, and cancer.
In addition, factors that led to a poor prognosis were a life-threatening illness, admission to an intensive care unit, high-flow oxygen therapy or mechanical ventilation, and an area of consolidation evidenced by a chest radiograph.
From the analysis of the reports of hospitalized patients, a comparison of mortality by age group was made with respect to the series of patients treated with CCP.
Mortality was lower overall in CCP-treated patients (14% vs 25%), especially in the elderly patient group (23% vs 40%,), with strong significance (p<0.001).
Regarding the safety of CCP administration, 16 adverse events were recorded out of a total of 3,937 TF (therapeutic fraction) transfused (0.4%).

IN-DEPTH STUDY

THE NATURAL AND VACCINE ANTIBODY RESPONSE

Reducing viral infectivity by binding antibodies to the surface of viral particles (virions) is called neutralization, and it works by blocking the phase of the viral replication cycle that precedes virus-encoded transcription or synthesis.[355]
Classically, the term was applied only to antibodies and antibody fragments, Fab and F (ab')2, but in later was extended to recombinant single-domain antigen-binding fragments and natural nanocarriers[356] .

An optimal immune response against viruses depends on several functions of antibodies:

(1) effector functions aimed at eliminating infected cells,

(2) Enhancement of the host's endogenous antiviral immunity response and

---

Grisolia G, et al
Convalescent plasma for coronavirus disease 2019 in pregnancy: a case report and review.
Am J Obstet Gynecol MFM. 2020 Aug;2(3):100174. doi: 10.1016/j.ajogmf.2020.100174.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7332432/

[354] De Silvestro G, et al Veneto Hospitals.
Outcome of SARS CoV-2 inpatients treated with convalescent plasma: One-year of data from the Veneto region (Italy) Registry.
Eur J Intern Med. 2022 Mar;97:42-49. doi: 10.1016/j.ejim.2021.12.023.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8710400/

[355] Klasse PJ, Sattentau QJ.
Occupancy and mechanism in antibody-mediated neutralization of animal viruses.
J Gen Virol. 2002 Sep;83(Pt 9):2091-2108. doi: 10.1099/0022-1317-83-9-2091.
https://pubmed.ncbi.nlm.nih.gov/12185262/

[356] https://didattica-2000.archived.uniroma2.it/Immunotecnologia/deposito/LeScienze.pdf

https://www.proteogenix.science/it/produzione-di-anticorpi/phage-display-antibody-library-screening-services/produzione-anticorpi-vhh-dominio-single/

https://en.wikipedia.org/wiki/Single-domain_antibody

(3) neutralization of the virus, preventing initial infection and viral spread.[357]

There are a number of antibody effector mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent antibody-mediated cytotoxicity (CDC), each directed at the removal of infected cells. [358]

The following figure shows the mechanisms of neutralization (Figure 2B) referring to blocking the initial phase of the viral replication cycle (Fig. 2A).[359]

Viruses with envelopes enter the host cell by binding to the receptor on the cell surface, while viruses without envelopes enter through lysis of the cell membrane or by creating pores in the membrane.

Fusion of the virus (without envelope/with envelope) with the host cell membrane requires special conformational changes in the viral protein induced by low pH in endosomes.[360]

https://www.mdpi.com/2076-393X/9/12/1376/htm

Interaction of SARS-CoV-2 with its receptors and mechanisms of neutralization. **(A)** S1 contains the receptor binding domain (RBD) and binds directly to ACE2 to gain entry into host cells. **(B)** Neutralizing mechanisms: *(1)* NAbs bind to the receptor-binding protein (S) and block its interaction with ACE2; *(2)* the virion makes contact between its binding protein and the receptor on the cell surface, and the NAbs block subsequent steps, such as binding to a coreceptor; *(3)* the virion is about to fuse with the cell membrane, but the NAbs are bound to proteins that are not essential for cell receptor binding but exert conformational changes that do not allow internalization of the

---

[357] Dörner T, Radbruch A.
Antibodies and B cell memory in viral immunity.
Immunity. 2007 Sep;27(3):384-92. doi: 10.1016/j.immuni.2007.09.002.
https://doi.org/10.1016/j.immuni.2007.09.002

Hua CK, Ackerman ME.
Increasing the Clinical Potential and Applications of Anti-HIV Antibodies.
Front Immunol. 2017 Nov 28;8:1655. doi: 10.3389/fimmu.2017.01655.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5712301/

Murin CD, Wilson IA, Ward AB.
Antibody responses to viral infections: a structural perspective across three different enveloped viruses.
Nat Microbiol. 2019 May;4(5):734-747. doi: 10.1038/s41564-019-0392-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6818971/

[358] van Erp EA, Luytjes W, Ferwerda G, van Kasteren PB.
Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease.
Front Immunol. 2019 Mar 22;10:548. doi: 10.3389/fimmu.2019.00548.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6438959/

Lu LL, Suscovich TJ, Fortune SM, Alter G.
Beyond binding: antibody effector functions in infectious diseases.
Nat Rev Immunol. 2018 Jan;18(1):46-61. doi: 10.1038/nri.2017.106.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6369690/

[359] Klasse PJ.
Neutralization of Virus Infectivity by Antibodies: Old Problems in New Perspectives.
Adv Biol. 2014;2014:157895. doi: 10.1155/2014/157895.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4835181/.

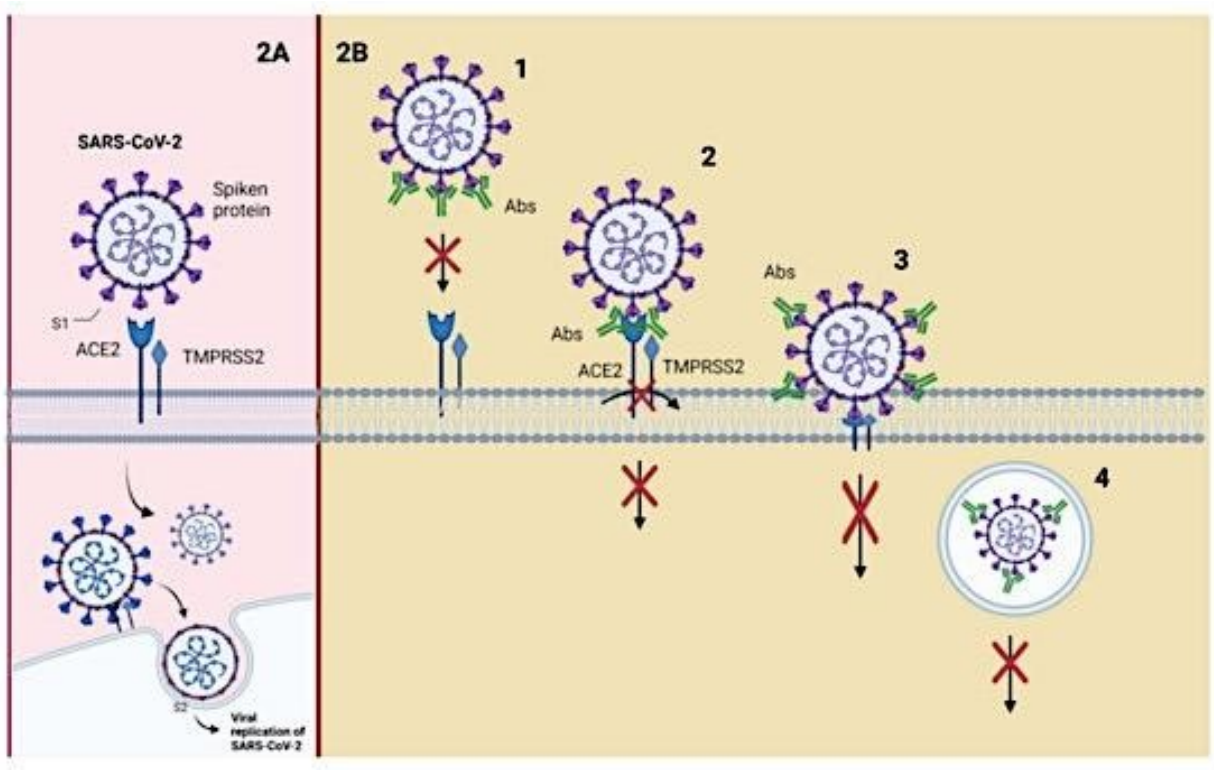
Cheedarla, N.; Hanna, L.E.
Chapter 7-Functional and Protective Role of Neutralizing Antibodies (NAbs) Against Viral Infections.
In Recent Developments in Applied Microbiology and Biochemistry; Buddolla, V., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 83-93.
https://doi.org/10.1016/B978-0-12-816328-3.00007-6

[360] Ali MG, Zhang Z, Gao Q, Pan M, Rowan EG, Zhang J.
Recent advances in therapeutic applications of neutralizing antibodies for virus infections: an overview.
Immunol Res. 2020 Dec;68(6):325-339. doi: 10.1007/s12026-020-09159-z.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7648849/

Dimitrov DS.
Virus entry: molecular mechanisms and biomedical applications.
Nat Rev Microbiol. 2004 Feb;2(2):109-22. doi: 10.1038/nrmicro817.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7097642/

Thorley JA, McKeating JA, Rappoport JZ.
Mechanisms of viral entry: sneaking in the front door.
Protoplasm. 2010 Aug;244(1-4):15-24. doi: 10.1007/s00709-010-0152-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3038234/

virus with the cell membrane; *(4)* NAbs prevent the virion from fusing its envelope with the vesicular membrane (endosome) and initiate viral replication, and binding of the antibody to the virus inhibits the conformational changes necessary for membrane fusion. Abbreviations: ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane serine protease 2.



Neutralization can occur through multiple mechanisms:

(1) NAbs bind to viral surface proteins and block their interaction with the host cell receptor and consequently infection; [361]

(2) NAbs bind to viral protein epitopes that interact with host cell co-receptors critical for viral infection; [362]

---

[361] Zhou G, Zhao Q.
Perspectives on therapeutic neutralizing antibodies against the Novel Coronavirus SARS-CoV-2.
Int J Biol Sci. 2020 Mar 15;16(10):1718-1723. doi: 10.7150/ijbs.45123.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7098029/

Zheng Q, et al
Viral neutralization by antibody-imposed physical disruption.
Proc Natl Acad Sci U S A. 2019 Dec 9;116(52):26933-40. doi: 10.1073/pnas.1916028116.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6936700/

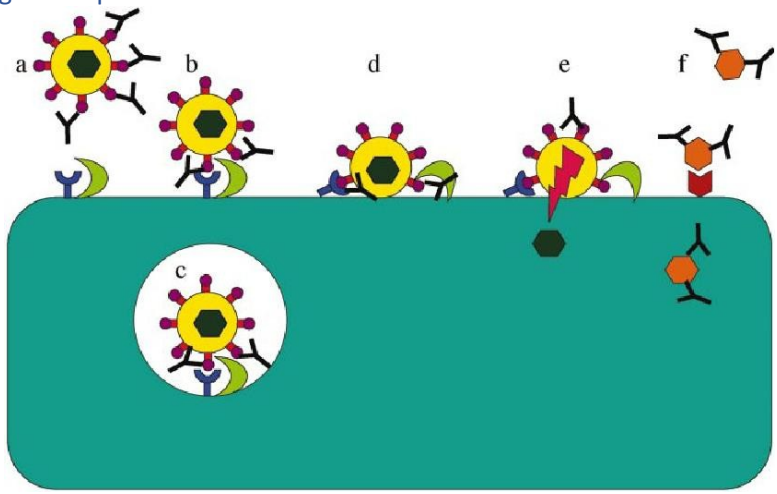
[362] Klasse PJ.
Neutralization of Virus Infectivity by Antibodies: Old Problems in New Perspectives.
Adv Biol. 2014;2014:157895. doi: 10.1155/2014/157895.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4835181/

Klasse PJ, Bron R, Marsh M.
Mechanisms of enveloped virus entry into animal cells.
Adv Drug Deliv Rev. 1998 Oct 5;34(1):65-91. doi: 10.1016/s0169-409x(98)00002-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7125647/

Reading SA, Dimmock NJ.
Neutralization of animal virus infectivity by antibody.
Arch Virol. 2007;152(6):1047-59. doi: 10.1007/s00705-006-0923-8.
https://pubmed.ncbi.nlm.nih.gov/17516034/

(3) NAbs bind to viral epitopes that are not essential for binding to the host cell receptor but are required for the conformational changes induced for membrane fusion.[363]

These mechanisms can be classified as inhibition of virus entry into host cells; other mechanisms can induce post-binding inhibition. In fact, some viruses require internalization and a decrease in pH to trigger conformational changes and perform viral membrane fusion.[364]



https://www.researchgate.net/figure/Mechanisms-of-virus-neutralization-by-Ab-a-Ab-binding-to-a-proportion-of-the_fig1_11202732
Mechanisms of virus neutralization by Ab. **(a)** Binding of Ab to part of the receptor-interacting structures on the virion can block virus attachment to the target cell surface. In the schematic example shown, Abs are bound to protein spikes on an enveloped virus, which is then prevented from making contact with either of the two cell surface receptors it uses for attachment and entry.
(b) Inhibition by Ab of interactions between the viral envelope protein and cell surface receptors has been shown to occur after the virion has attached by binding through receptors. For example, a receptor can act as an attachment point and trigger conformational changes that allow interactions with a coreceptor, which in turn mediates subsequent events such as membrane fusion. Interference by Ab with any of these necessary links in a chain of events leading to entry would constitute a neutralizing mechanism. **(c)** To infect, some viruses require internalization by endocytosis and pH lowering in the endosome as a trigger for conformational changes in viral proteins. Antibodies that did not block virus attachment but allowed internalization are shown. Blocking the necessary interactions between viral and cell membrane proteins would delay or prevent penetration of the viral core into the cytoplasm of the target cell. The virion can then ultimately be destroyed through lysosomal degradation. Such Ab effects would constitute a mechanism of virus neutralization. Furthermore, Ab-mediated hijacking of viruses that preferentially enter directly through the cell surface into a less permissive endosomal compartment may abrogate infectivity. **(d)** Ab intercalation at the fusion interface between the cell membrane and the envelope of a virus may block fusion at the cell surface, as illustrated, or in an endosome. **(e) It** has been hypothesized that even very low antibody occupancy on the virion can cause global or internal changes by transmitting a signal through the viral envelope or outer layer. These hypothesized changes

---

[363] Nelson CD, Palermo LM, Hafenstein SL, Parrish CR.
Different mechanisms of antibody-mediated neutralization of parvoviruses revealed using the Fab fragments of monoclonal antibodies.
Virology. 2007 May 10;361(2):283-93. doi: 10.1016/j.virol.2006.11.032.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1991280/.

Xiao Y, Dong X, Chen YH.
Neutralizing antibodies mechanism of neutralization and protective activity against HIV-1.
Immunol Res. 2002;25(3):193-200. doi: 10.1385/IR:25:3:193.
https://pubmed.ncbi.nlm.nih.gov/12018459/
Klasse PJ, Sattentau QJ.

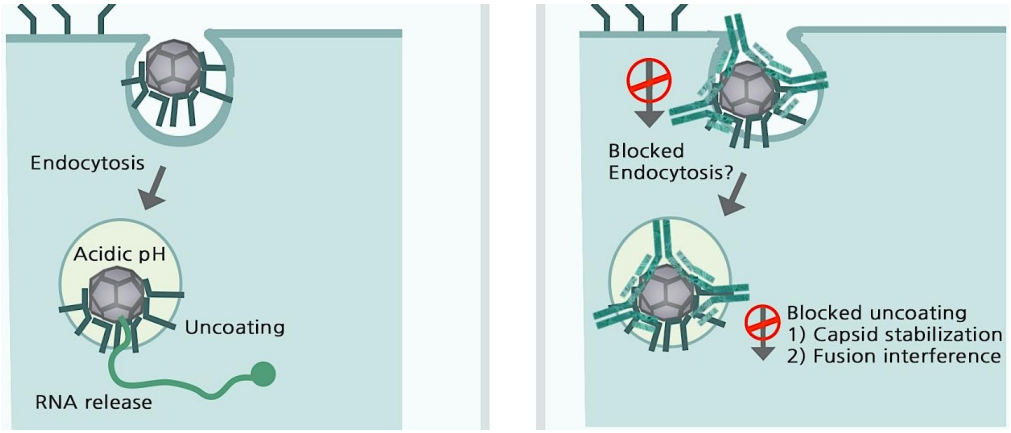
Occupancy and mechanism in antibody-mediated neutralization of animal viruses.
J Gen Virol. 2002 Sep;83(Pt 9):2091-2108. doi: 10.1099/0022-1317-83-9-2091.
https://www.researchgate.net/publication/11202732_Occupancy_and_mechanism_in_antibody-mediated_neutralization_of_animal_viruses

[364] Ali MG, Zhang Z, Gao Q, Pan M, Rowan EG, Zhang J.
Recent advances in therapeutic applications of neutralizing antibodies for virus infections: an overview.
Immunol Res. 2020 Dec;68(6):325-339. doi: 10.1007/s12026-020-09159-z.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7648849/

Marsh M, Helenius A.
Virus entry: open sesame.
Cell. 2006 Feb 24;124(4):729-40. doi: 10.1016/j.cell.2006.02.007.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7112260/

would allow the viral core to enter the cytoplasm but compromise further replicative steps. **(f)** Neutralization of naked viruses could potentially differ from that of enveloped viruses. Although naked viruses have been shown in several cases to be neutralized by blocking viral attachment to target cells, as in (a), conformational effects on the whole virion via Ab binding have also been recorded as pI shifts [islet pH shift: pH value at which a molecule has zero net electrical charge]. The possibility of the Ab-virion complex entering the cytoplasm and Ab blocking further replicative events is shown.

A fourth mechanism of neutralization may occur once the virus is inside endosomes, and the association of antibodies to viral surface proteins inhibits the changes necessary for viral membrane fusion, causing neutralization.
The latter mechanism could target enveloped and naked viral particles, and is known as post-internalization neutralization.[365]



https://bpsbioscience.com/sars-cov-2-antibody-detection

To date, it is unclear whether all the neutralization mechanisms described occur in all viruses, but most likely not; in fact, the mechanism activated will depend on the target of the viral protein, whether it is a virus with or without an envelope.

Generation and characteristics of a neutralizing antibody

A feature of humoral immunity is the production of antibodies, whose affinity for antigen develops during the immune response, a process known as affinity maturation.[366]
Affinity maturation is based on somatic mutation of immunoglobulin germline genes, a process called somatic hypermutation (SHM), which consists of point mutations performed by the activation-induced cytosine deaminase (AID) protein that can induce increased antibody affinity or, conversely, decreased affinity.[367]

---

[365] Jiang S, Du L.
Effect of Low-Pathogenic Human Coronavirus-Specific Antibodies on SARS-CoV-2.
Trends Immunol. 2020 Oct;41(10):853-854. doi: 10.1016/j.en.2020.08.003
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7418642/

Padilla-Quirarte HO, Lopez-Guerrero DV, Gutierrez-Xicotencatl L, Esquivel-Guadarrama F.
Protective Antibodies Against Influenza Proteins.
Front Immunol. 2019 Jul 18;10:1677. doi: 10.3389/fimmu.2019.01677.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6657620/

[366] Tas JM, Mesin L, Pasqual G, Targ S, Jacobsen JT, Mano YM, Chen CS, Weill JC, Reynaud CA, Browne EP, Meyer-Hermann M, Victora GD.
Visualizing antibody affinity maturation in germinal centers.
Science. 2016 Mar 4;351(6277):1048-54. doi: 10.1126/science.aad3439.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4938154/

[367] Pilzecker B, Jacobs H.
Mutating for Good: DNA Damage Responses During Somatic Hypermutation.
Front Immunol. 2019 Mar 12;10:438. doi: 10.3389/fimmu.2019.00438.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6423074/

Teng G, Papavasiliou FN.
Immunoglobulin somatic hypermutation.

This process is carried out in germinal centers (GCs), specialized microstructures formed in secondary lymphoid tissues following infection or immunization, which produce long-lived plasma cells and memory B cells and protect against reinfection.[368]
GCs are organized into two regions, the dark zone (DZ) and the light zone (LZ).[369] In the DZ are B cells called centroblasts with a high proliferation rate and SHM.[370]
Centroblasts enter the LZ becoming centrocytes, where they capture and process antigens present on follicular dendritic cells (FDCs)[371] and subsequently present antigenic peptides to follicular helper T cells (Tfh) to receive critical survival signals and undergo selection.[372]
FDCs have the ability to retain native antigen to carry out the previous process, and also produce cytokines that support the survival of selected B cells.[373]
Class-switching recombination (CSR) also occurs in the LZ, in which the constant region of the antibody heavy chain is changed, allowing B lymphocytes to produce IgG, IgA or IgE antibodies.[374]

Annu Rev Genet. 2007;41:107-20. doi: 10.1146/annurev.genet.41.110306.130340.
https://pubmed.ncbi.nlm.nih.gov/17576170/

[368] Stebegg M, Kumar SD, Silva-Cayetano A, Fonseca VR, Linterman MA, Graca L.
Regulation of the Germinal Center Response.
Front Immunol. 2018 Oct 25;9:2469. doi: 10.3389/fimmu.2018.02469.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6209676/

[369] Oropallo MA, Cerutti A.
Germinal center reaction: antigen affinity and presentation explain it all.
Trends Immunol. 2014 Jul;35(7):287-9. doi: 10.1016/j.en.2014.06.001.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4174395/

[370] Pilzecker B, Jacobs H.
Mutating for Good: DNA Damage Responses During Somatic Hypermutation.
Front Immunol. 2019 Mar 12;10:438. doi: 10.3389/fimmu.2019.00438.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6423074/

Nouri N, Kleinstein SH.
Somatic hypermutation analysis for improved identification of B cell clonal families from next-generation sequencing data.
PLoS Comput Biol. 2020 Jun 23;16(6):e1007977. doi: 10.1371/journal.pcbi.1007977.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7347241/

[371] Mesin L, Ersching J, Victora GD.
Germinal Center B Cell Dynamics.
Immunity. 2016 Sep 20;45(3):471-482. doi: 10.1016/j.immuni.2016.09.001.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5123673/

Stavnezer J, Guikema JE, Schrader CE.
Mechanism and regulation of class switch recombination.
Annu Rev Immunol. 2008;26:261-92. doi: 10.1146/annurev.immunol.26.021607.090248.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2707252/

[372] Liechti T.
CD40 signaling-germinal center b cells go at their own pace.
Cytometry A. 2019 Apr;95(4):419-421. doi: 10.1002/cyto.a.23639.
https://doi.org/10.1002/cyto.a.23639

Zhang M, Srivastava G, Lu L.
The pre-B cell receptor and its function during B cell development.
Cell Mol Immunol. 2004 Apr;1(2):89-94. PMID: 16212894.
http://www.cmi.ustc.edu.cn/1/2/89.pdf

[373] Pope I, Vinuesa CG.
Synaptic Interactions in Germinal Centers.
Front Immunol. 2018 Aug 13;9:1858. doi: 10.3389/fimmu.2018.01858.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6099157/

[374] Chen Z, Wang JH.
Signaling control of antibody isotype switching.
Adv Immunol. 2019;141:105-164. doi: 10.1016/bs.ai.2019.01.001.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7875453/.

In the final stage of the process in the germinal center, centrocytes emerge from the GC as memory B cells or plasma cells that secrete high-affinity antibodies. [375]
Specifically, plasma cells reside in the bone marrow and constitutively secrete antibodies, do not possess BCR receptors, are not reactivated by antigenic reexposure, and are responsible for serum antibody production for years after infection or vaccination. [376]
On the other hand, memory B lymphocytes express BCR but do not constitutively secrete antibodies. When they encounter the antigen again, they can reactivate and form GC to produce antibodies with higher affinity; in addition, these cells can give rise to plasma cells and reside in the circulation or peripheral lymphoid tissue. [377]

Insight

Humoral immunity

Jhamnani RD, Nunes-Santos CJ, Bergerson J, Rosenzweig SD.
Class-Switch Recombination (CSR)/Hyper-IgM (HIGM) Syndromes and Phosphoinositide 3-Kinase (PI3K) Defects.
Front Immunol. 2018 Sep 26;9:2172. doi: 10.3389/fimmu.2018.02172.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6168630/

[375] Ruth H Florese and Marjorie Robert-Guroff
Non-neutralizing antibodies and vaccine-induced protection against HIV
Future HIV Therapy 2007 1:3, 259-271
https://doi.org/10.2217/17469600.1.3.259

[376] Brynjolfsson SF, Persson Berg L, Olsen Ekerhult T, Rimkute I, Wick M-J, Mårtensson I-L and Grimsholm O
Long-Lived Plasma Cells in Mice and Men.
Front. Immunol. (2018) 9:2673. doi: 10.3389/fimmu.2018.02673
https://www.frontiersin.org/articles/10.3389/fimmu.2018.02673/full

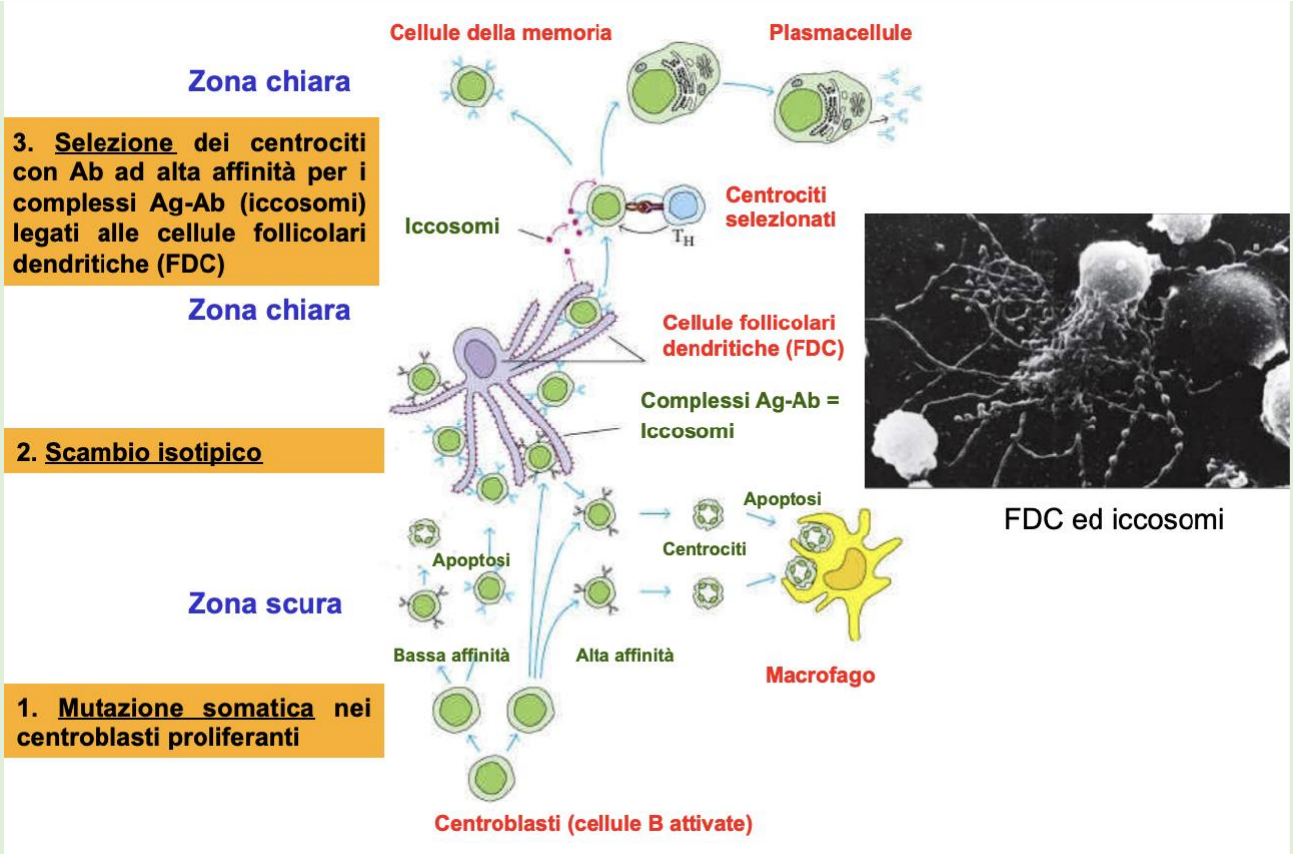
Lightman SM, Utley A, Lee KP.
Survival of Long-Lived Plasma Cells (LLPCs): Piecing Together the Puzzle.
Front Immunol. 2019 May 3;10:965. doi: 10.3389/fimmu.2019.00965.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6510054/

Slifka MK, Antia R, Whitmire JK, Ahmed R.
Humoral immunity due to long-lived plasma cells.
Immunity. 1998 Mar;8(3):363-72. doi: 10.1016/s1074-7613(00)80541-5
https://doi.org/10.1016/S1074-7613(00)80541-5

[377] Akkaya M, Kwak K, Pierce SK.
B cell memory: building two walls of protection against pathogens.
Nat Rev Immunol. 2020 Apr;20(4):229-238. doi: 10.1038/s41577-019-0244-2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7223087/

Weisel F, Shlomchik M.
Memory B Cells of Mice and Humans.
Annu Rev Immunol. 2017 Apr 26;35:255-284. doi: 10.1146/annurev-immunol-041015-055531.
https://pubmed.ncbi.nlm.nih.gov/28142324/

Wong R, Bhattacharya D.
Basics of memory B-cell responses: lessons from and for the real world.
Immunology. 2019 Feb;156(2):120-129. doi: 10.1111/imm.13019.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6328991/



Characteristics of immunoglobulin subclasses.

The subclasses IgG1, IgG2, IgG3 and IgG4 differ in the size of the hinge region (the position of disulfide bonds between chains and molecular weight), in particular IgG3 has a molecular weight of 170 kDal, while the other subtypes have a molecular weight of 146 kDal.[378]

IgG1 and IgG3 are usually produced in response to proteins; IgG2 and IgG4 are produced in response to carbohydrate antigens; in addition, IgG4 undergoes a process called Fab-arm exchange (FAE), in which bi-specific and functionally monovalent antibodies are created.

This contributes to the anti-inflammatory properties of IgG4 and limits its ability to form immune complexes and activate complement.[379]

The subclasses also differ in their ability to activate complement or bind to and react with Fc receptors in phagocytic cells.[380]

Complement activation by IgG1 and IgG3 is 40 times higher than that of IgG2,[381] while the IgG4 subclass is unable to activate the classical complement pathway. Based on this, IgG1 and IgG3 are.

---

[378] Vukovic N, van Elsas A, Verbeek JS, Zaiss DMW.
Isotype selection for antibody-based cancer therapy.
Clin Exp Immunol. 2021 Mar;203(3):351-365. doi: 10.1111/cei.13545.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7874837/

[379] Davies AM, Sutton BJ.
Human IgG4: a structural perspective.
Immunol Rev. 2015 Nov;268(1):139-59. doi: 10.1111/imr.12349.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4670484/

[380] Demonbreun AR, Sancilio A, Velez MP, Ryan DT, Saber R, Vaught LA, Reiser NL, Hsieh RR, D'Aquila RT, Mustanski B, McNally EM, McDade TW.
Comparison of IgG and neutralizing antibody responses after one or two doses of COVID-19 mRNA vaccine in previously infected and uninfected individuals.
EClinicalMedicine. 2021 Aug;38:101018. doi: 10.1016/j.eclinm.2021.101018.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8276631/

[381] Palm AE, Henry C.
Remembrance of Things Past: Long-Term B Cell Memory After Infection and Vaccination.

subclasses of IgG most related to NAb activity against enveloped viruses, as they primarily target peptides derived from viral proteins.
Regarding SARS-CoV-2, Kallolimath et al. showed that IgG3 manifested up to 50 times more neutralizing potency than other IgG subclasses.[382]

IgA in serum is mainly monomeric and comprises about 90% IgA1 and 10% IgA2.
In IgA2, there are generally no light to heavy chain disulfide bonds; rather, there is a disulfide bridge between the light chains when dimers are formed.[383]
A key mediator of IgA effector function is FcαRI, which can trigger various elimination processes by neutrophils, monocytes, eosinophils and some macrophages and dendritic cells. [384]
IgA does not activate the classical complement pathway, so one of the main differences between IgG and IgA in terms of function is the ability to activate complement.
In the case of SARS-CoV-2, Sterlin et al. showed that IgA1 dominates the NAbs response, reaching its maximum values three weeks after infection; this subclass predominance may be explained because the lungs (and other tissues damaged by the virus) consist mainly of mucous tissue. It has been proposed that the greater flexibility and longer zipper in IgA1 than in IgG would be more favorable for interactions between immunoglobulins and the SARS-CoV-2 trimer. [385]
Regarding IgM levels, it is essential to take into account that it is the first immunoglobulin to appear, but its actions are more directed to effector functions mediated by its pentameric structure capable of activating complement through the classical pathway, via C1q binding to the Fc regions of these immunoglobulins.[386]
However, IgM, through its FcμR, as will be discussed in more detail later, plays a role in B-cell development, maturation and activation, humoral immune responses, host defense and immunological tolerance.[387]

Front Immunol. 2019 Jul 31;10:1787. doi: 10.3389/fimmu.2019.01787.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6685390/

[382] Kallolimath S, Sun L, Palt R, Stiasny K, Mayrhofer P, Gruber C, Kogelmann B, Chen Q, Steinkellner H.
Highly active engineered IgG3 antibodies against SARS-CoV-2.
Proc Natl Acad Sci U S A. 2021 Oct 1;118(42):e2107249118. doi: 10.1073/pnas.2107249118.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8545452/

[383] Corthésy B.
Multi-faceted functions of secretory IgA at mucosal surfaces.
Front Immunol. 2013 Jul 12;4:185. doi: 10.3389/fimmu.2013.00185.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3709412/

[384] Woof JM, Russell MW.
Structure and function relationships in IgA.
Mucosal Immunol. 2011 Nov;4(6):590-7. doi: 10.1038/mi.2011.39.
https://pubmed.ncbi.nlm.nih.gov/21937984/

[385] Sterlin D, et al
IgA dominates the early neutralizing antibody response to SARS-CoV-2.
Sci Transl Med. 2021 Jan 20;13(577):eabd2223. doi: 10.1126/scitranslmed.abd2223.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857408/

[386] Sharp TH, Boyle AL, Diebolder CA, Kros A, Koster AJ, Gros P.
Insights into IgM-mediated complement activation based on in situ structures of IgM-C1-C4b.
Proc Natl Acad Sci U S A. 2019 Jun 11;116(24):11900-11905. doi: 10.1073/pnas.1901841116.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6575175/.

Daha NA, Banda NK, Roos A, Beurskens FJ, Bakker JM, Daha MR, Trouw LA.
Complement activation by (auto-) antibodies.
Mol Immunol. 2011 Aug;48(14):1656-65. doi: 10.1016/j.molimm.2011.04.024.
https://pubmed.ncbi.nlm.nih.gov/21757235/

[387] Liu J, Wang Y, Xiong E, Hong R, Lu Q, Ohno H, Wang JY.
Role of the IgM Fc Receptor in Immunity and Tolerance.
Front Immunol. 2019 Mar 22;10:529. doi: 10.3389/fimmu.2019.00529.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6438924/

Neutralizing antibodies and SARS-Cov-2

NAbs induced by vaccines or natural infections play crucial roles in the control of viral infections. [388] In SARS-CoV-2 infection, epitopes that bind to NAbs are predominantly located in the receptor binding domain (RBD) of the viral "S" protein.[389]

The development of SARS-CoV-2 vaccines moved at an unprecedented speed, yet vaccine developers did not have a homogeneous system for measuring immune responses after vaccination, which made immunogenicity comparisons difficult.

Initiatives such as CEPI Global Centralized Laboratory Network have been launched for the harmonization of immune response assessment among COVID-19 vaccine candidates.

A key tool for this harmonization is the global use of an international standard to calibrate all assays to an arbitrary unit; therefore, it has been proposed that immunogenicity results should be reported as the international standard unit (IU/mL) for neutralizing antibodies.[390] .

The international standard is based on human plasma from convalescent patients, lyophilized in vials, with an assigned unit of 250 IU/ vial for neutralizing activity.[391]

NAbs induced by natural infections and their protective role

In natural infection, most patients infected with SARS-CoV-2 develop variable titers of NAbs between days 14 and 20 after infection.[392]

Studies have shown that most patients had detectable SARS-CoV-2 antibody responses up to 13 months after infection, giving hope that it could last even longer than expected.[393]

Flacco et al in a retrospective cohort study of 1,293,941 patients through February 2022 found that the incidence of reinfection 18-22 months after primary infection was 6.7 ‰ thus suggesting that the protection conferred by natural immunity may last beyond 12 months.[394]

---

[388] Jiang S, Hillyer C, Du L.
Neutralizing Antibodies against SARS-CoV-2 and Other Human Coronaviruses.
Trends Immunol. 2020 May;41(5):355-359. doi: 10.1016/j.en.2020.03.007. Epub 2020 Apr 2. Erratum in: Trends Immunol. 2020 Apr 24
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7129017/

[389] Putcharoen O, et al
Early detection of neutralizing antibodies against SARS-CoV-2 in COVID-19 patients in Thailand.
PLoS One. 2021 Feb 12;16(2):e0246864. doi: 10.1371/journal.pone.0246864.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7880427/

[390] Krammer F.
A correlate of protection for SARS-CoV-2 vaccines is urgently needed.
Nat Med. 2021 Jul;27(7):1147-1148. doi: 10.1038/s41591-021-01432-4.
https://pubmed.ncbi.nlm.nih.gov/34239135/

[391] Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, Plotkin S, Knezevic I.
WHO International Standard for anti-SARS-CoV-2 immunoglobulin.
Lancet. 2021 Apr 10;397(10282):1347-1348. doi: 10.1016/S0140-6736(21)00527-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7987302/

[392] Lau EHY, Tsang OTY, Hui DSC, Kwan MYW, Chan WH, Chiu SS, Ko RLW, Chan KH, Cheng SMS, Perera RAPM, Cowling BJ, Poon LLM, Peiris M.
Neutralizing antibody titers in SARS-CoV-2 infections.
Nat Commun. 2021 Jan 4;12(1):63. doi: 10.1038/s41467-020-20247-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7782739/

[393] Altawalah H.
Antibody Responses to Natural SARS-CoV-2 Infection or after COVID-19 Vaccination.
Vaccines (Basel). 2021 Aug 16;9(8):910. doi: 10.3390/vaccines9080910.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8402626/

[394] Flacco ME, Soldato G, Acuti Martellucci C, Di Martino G, Carota R, Caponetti A, Manzoli L.
Risk of SARS-CoV-2 Reinfection 18 Months After Primary Infection: Population-Level Observational Study.
Front Public Health. 2022 May 2;10:884121. doi: 10.3389/fpubh.2022.884121.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9108359/

Chemaitelly et al in the recent article "*Duration of immune protection of SARS-CoV-2 natural infection against reinfection in Qatar*"[395] report an efficacy of primary infection against pre-Omicron reinfection of 85.5% (95% CI: 84.8-86.2%).
Efficacy peaked at 90.5% (95% CI: 88.4-92.3%) in the 7th month after primary infection, and declined to about 70% by the 16th month. Extrapolation of this declining trend using a Gompertz curve suggested efficacy of 50% at month 22 and <10% at month 32.
The efficacy of pre-Omicron primary infection against Omicron reinfection was 38.1% (95% CI: 36.3-39.8%) and decreased with time since primary infection. A Gompertz curve suggested <10% efficacy by month 15.
The efficacy of primary infection against severe, critical, or fatal COVID-19 reinfection was 97.3% (95% CI: 94.9-98.6%), regardless of the variant of primary infection or reinfection and with no evidence of decline. Similar results were found in subgroup analyses for those aged ≥50 years.
Seow et al. showed that the kinetics of the neutralizing antibody response is typical of acute viral infection, with declining neutralizing antibody titers observed after an initial peak and that the magnitude of this peak depends on the severity of the disease.[396]
In addition, Beltran et al. showed that higher neutralization titers are good predictors of survival in patients with severe COVID-19 and that patients who have recovered from severe disease have higher NAbs than patients with mild or asymptomatic infections.[397] .
This may be due to prolonged stimulation of the B lymphocyte receptor[398] or high production of type I interferon (IFN-I) in the course of severe disease.
IFN-I plays a key role in the early stages of the viral immune response and is part of the innate response. It also induces dendritic cell activation and enables these cells to present antigens to $CD4^+$ and CD8 T $cells^+$ naïve.[399]
Regarding outpatients and asymptomatic patients, Röltgen et al. observed that SARS-CoV-2 antibodies progressively decreased five months after infection.[400]

---

[395] Hiam Chemaitelly, et al.
Duration of immune protection of SARS-CoV-2 natural infection against reinfection in Qatar
medRxiv 2022.07.06.22277306; doi:https://doi.org/10.1101/2022.07.06.22277306
https://www.medrxiv.org/content/10.1101/2022.07.06.22277306v1.full

[396] Seow J, et al
Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans.
Nat Microbiol. 2020 Dec;5(12):1598-1607. doi: 10.1038/s41564-020-00813-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7610833/

[397] Garcia-Beltran WF, et al
COVID-19-neutralizing antibodies predict disease severity and survival.
Cell. 2021 Jan 21;184(2):476-488.e11. doi: 10.1016/j.cell.2020.12.015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7837114/

Klein S, Cortese M, Winter SL, Wachsmuth-Melm M, Neufeldt CJ, Cerikan B, Stanifer ML, Boulant S, Bartenschlager R, Chlanda P.
SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography.
Nat Commun. 2020 Nov 18;11(1):5885. doi: 10.1038/s41467-020-19619-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7676268/

[398] Liu L, To KK, Chan KH, Wong YC, Zhou R, Kwan KY, Fong CH, Chen LL, Choi CY, Lu L, Tsang OT, Leung WS, To WK, Hung IF, Yuen KY, Chen Z.
High neutralizing antibody titer in intensive care unit patients with COVID-19.
Emerg Microbes Infect. 2020 Dec;9(1):1664-1670. doi: 10.1080/22221751.2020.1791738.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7473321/

[399] Schreiber G.
The Role of Type I Interferons in the Pathogenesis and Treatment of COVID-19.
Front Immunol. 2020 Sep 30;11:595739. doi: 10.3389/fimmu.2020.595739.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7561359/

[400] Röltgen K, et al
Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome.
Sci Immunol. 2020 Dec 7;5(54):eabe0240. doi: 10.1126/sciimmunol.abe0240.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857392/

Dugan et al. in particular showed that several months after infection there was a change in the persistence of NAbs against the S protein of SARS-CoV-2, and the rate of these NAbs began to decline, while there was a transition in the production of antibodies to nonneutralizing viral targets, such as NP and ORF8.[401] However, other authors have detected memory B cells producing IgG against S-glycoprotein and RBD in the blood of COVID-19 patients, and that therefore there are memory responses after natural infection that have the potential to be activated and rapidly produce neutralizing antibodies upon re-exposure to SARS-CoV-2.[402]
The "SIREN" study in The Lancet investigated the relationships between seropositivity in people with previous COVID-19 infection and the subsequent risk of severe acute respiratory syndrome due to SARS-CoV-2 infection over the next 7-12 months,[403] demonstrating that previous infection reduced the risk of symptomatic reinfection by 93%.
A large cohort study published in JAMA Internal Medicine examined 3.2 million U.S. patients and showed that the risk of infection was significantly lower (0.3%) in HIV-positive patients than in HIV-negative patients (3%)[404] .
Even more important to the question of the durability of immunity are recent studies showing the presence of long-lived memory immune cells in those who have recovered from COVID-19.[405]

[401] Dugan HL, et al
Profiling B cell immunodominance after SARS-CoV-2 infection reveals antibody evolution to non-neutralizing viral targets.
Immunity. 2021 Jun 8;54(6):1290-1303.e7. doi: 10.1016/j.immuni.2021.05.001.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8101792/

[402] Rodda LB, et al
Functional SARS-CoV-2-Specific Immune Memory Persists after Mild COVID-19.
Cell. 2021 Jan 7;184(1):169-183.e17. doi: 10.1016/j.cell.2020.11.029.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7682481/

[403] Hall VJ, et al Hopkins S; SIREN Study Group.
SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicenter, prospective cohort study (SIREN).
Lancet. 2021 Apr 17;397(10283):1459-1469. doi: 10.1016/S0140-6736(21)00675-9. Epub 2021 Apr 9. Erratum in: Lancet. 2021 May 8;397(10286):1710.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8040523/

Block J.
Vaccinating people who have had covid-19: why doesn't natural immunity count in the US?
BMJ. 2021 Sep 13;374:n2101. doi: 10.1136/bmj.n2101. Erratum in: BMJ. 2021 Sep 15;374:n2272.
https://www.bmj.com/content/374/bmj.n2101
https://www.bmj.com/content/374/bmj.n2101/rr-0

[404] Harvey RA, et al
Association of SARS-CoV-2 Seropositive Antibody Test With Risk of Future Infection.
JAMA Intern Med. 2021 May 1;181(5):672-679. doi: 10.1001/jamainternmed.2021.0366.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7905701/

[405] Clara Schnizer, et al the CoNAN study group.
Persistent immunity after mild SARS CoV-2 infection - THE CoNAN-long term study -
medRxiv 2022.07.05.22277237; doi: https://doi.org/10.1101/2022.07.05.22277237
https://www.medrxiv.org/content/10.1101/2022.07.05.22277237v1

Turner JS, Kim W, Kalaidina E, Goss CW, Rauseo AM, Schmitz AJ, Hansen L, Haile A, Klebert MK, Pusic I, O'Halloran JA, Presti RM, Ellebedy AH.
SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans.
Nature. 2021 Jul;595(7867):421-425. doi: 10.1038/s41586-021-03647-4.
https://pubmed.ncbi.nlm.nih.gov/34030176/

Wang Z, Yang X, Zhong J, Zhou Y, Tang Z, Zhou H, He J, Mei X, Tang Y, Lin B, Chen Z, McCluskey J, Yang J, Corbett AJ, Ran P.
Exposure to SARS-CoV-2 generates T-cell memory in the absence of a detectable viral infection.
Nat Commun. 2021 Mar 19;12(1):1724. doi: 10.1038/s41467-021-22036-z.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7979809/

NAbs induced by vaccination

Although NAbs have been determined for all approved vaccines, the specific tests have varied and thus are not directly comparable. Most studies have reported a good humoral response a few days after vaccination, but NAbs tend to decline over time.[406]

Song et al. measured the ability of pre-existing antibodies due to previous HCoV infection (SARS-CoV-1, MERS-CoV, HCoV-HKU1, HCoV-OC43, HCoV-NL63 and HCoV-229E) to neutralize SARS-CoV-2.

The study showed that pre-existing antibodies do not cross-react with SARS-CoV-2 to neutralize them, but pre-existing memory B cells can cross-react and generate antibodies against SARS-CoV-2 more rapidly.[407]

Comparison of natural and vaccine immunity

The following is an excerpt from the article "*Health-care workers recovered from natural SARS-CoV-2 infection should be exempt from mandatory vaccination edicts*" [408], in which some considerations are made about the difference between natural and vaccine immunity. This topic will be explored in detail in subsequent papers.

"According to etymology, the word immune comes from the Latin *immunis*, meaning exempt from public service, not taxed, relieved.

By extension, the term "immunity" means exempt from a particular infectious disease, but the term is now in danger of being equated with exemption from work because of vaccination mandates that have been implemented or proposed in some countries. Many vaccination mandates include those who are naturally immune, which constitutes a large percentage of health care workers in view of exposure to SARS-CoV-2 in the workplace.

However, there are compelling arguments against such unilateral mandates that deserve to be taken up from the perspective of accumulated knowledge on respiratory tract viral infections and immunity.

First, it is well known that for single-stranded RNA viruses such as influenza, natural immunity after recovery from infection provides better protection than vaccination, which must be repeated every year because of the decline in vaccine immunity.[409]

The same was demonstrated for SARS-CoV -2; in one study, individuals exposed to natural infection were 10 times less likely to be reinfected than vaccinated individuals without natural infection (adjusted hazard ratio 0.02, 95% CI 0.01-0.04 for previous infection vs 0.26 , 0-24-0-28 for vaccination).

---

[406] Dolgin E.
COVID vaccine immunity is waning - how much does that matter?
Nature. 2021 Sep;597(7878):606-607. doi: 10.1038/d41586-021-02532-4.
https://pubmed.ncbi.nlm.nih.gov/34548661/

Francesco Menegale, et al
Waning of SARS-CoV-2 vaccine-induced immunity: A systematic review and secondary data analysis
medRxiv 2022.07.04.22277225; doi: https://doi.org/10.1101/2022.07.04.22277225
https://www.medrxiv.org/content/10.1101/2022.07.04.22277225v1.full

[407] Song G, et al
Cross-reactive serum and memory B-cell responses to spike protein in SARS-CoV-2 and endemic coronavirus infection.
Nat Commun. 2021 May 19;12(1):2938. doi: 10.1038/s41467-021-23074-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8134462/

[408] McGonagle DG.
Health-care workers recovered from natural SARS-CoV-2 infection should be exempt from mandatory vaccination edicts.
Lancet Rheumatol. 2022 Mar;4(3):e170. doi: 10.1016/S2665-9913(22)00038-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8820743/

[409] Krammer F.
The human antibody response to influenza A virus infection and vaccination.
Nat Rev Immunol. 2019 Jun;19(6):383-397. doi: 10.1038/s41577-019-0143-6.
https://pubmed.ncbi.nlm.nih.gov/30837674/

Individuals exposed to natural infections were also less likely to be hospitalized with COVID-19.[410] [Studies done in relation to infection with the Delta variant have confirmed these findings, which will be discussed further in the paper on vaccine variants. [411]]
Second, prior to the COVID-19 pandemic, it was a well-established principle that although systemic vaccination against respiratory tract viral pathogens protects vaccinated individuals from severe infections*, these individuals can still transmit the virus to unvaccinated individuals due to lack of mucosal immunity. [412]

> * this author's claim is disputed by the study "*Influenza vaccine effectiveness in an Italian elderly population during the 2016-2017 season.*" As can be seen, the effectiveness of vaccination was virtually nil, with even a statistically significant increase in hospitalizations and deaths among the vaccinated group, compared to the unvaccinated, for those who received the tetravalent vaccine. In fact, tetravalent vaccination has the highest risk, with an average 47% increase (from 0% to as much as 215%) in hospitalizations for complications (influenza and pneumonia) and 12% increase in deaths (from 3% to 54%). [413]

Therefore, individuals with immunity derived from natural infection are probably less likely to transmit the infection to vulnerable patients (who should be vaccinated themselves) than those who are vaccinated but not naturally immune.
Long-term immunity in the upper airway cannot be measured directly, and serum antibody levels are not a surrogate for mucosal immunity.[414]

Third, numerous studies have shown that vaccination in individuals with previous natural SARS-CoV-2 infection induces so-called superimmunity (or hybrid immunity), that is, a greater antibody and T-cell response than vaccination alone.[415]

---

[410] Shrestha NK, Burke PC, Nowacki AS, Terpeluk P, Gordon SM.
Necessity of COVID-19 Vaccination in Persons Who Have Already Had COVID-19.
Clin Infect Dis. 2022 Jan 13:ciac022. doi: 10.1093/cid/ciac022.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8807217/

[411] Gazit S, Shlezinger R, Perez G, Lotan R, Peretz A, Ben-Tov A, Herzel E, Alapi H, Cohen D, Muhsen K, Chodick G, Patalon T.
SARS-CoV-2 Naturally Acquired Immunity vs. Vaccine-induced Immunity, Reinfections versus Breakthrough Infections: a Retrospective Cohort Study.
Clin Infect Dis. 2022 Apr 5:ciac262. doi: 10.1093/cid/ciac262.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9047157/

Sivan Gazit, Roei Shlezinger, Galit Perez, Roni Lotan, Asaf Peretz, Amir Ben-Tov, Dani Cohen, Khitam Muhsen, Gabriel Chodick, Tal Patalon
Comparing SARS-CoV-2 natural immunity to vaccine-induced immunity: reinfections versus breakthrough infections
medRxiv 2021.08.24.21262415; doi: https://doi.org/10.1101/2021.08.24.21262415
https://www.medrxiv.org/content/10.1101/2021.08.24.21262415v1

[412] Connell AR, Connell J, Leahy TR, Hassan J.
Mumps Outbreaks in Vaccinated Populations-Is It Time to Re-assess the Clinical Efficacy of Vaccines?
Front Immunol. 2020 Sep 18;11:2089. doi: 10.3389/fimmu.2020.02089.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7531022/

[413] https://www.onb.it/2018/11/02/vaccini-antinfluenzali-risposte-ed-efficacia-per-fasce-di-eta/

Valent F, Gallo T.
Influenza vaccine effectiveness in an Italian elderly population during the 2016-2017 season.
Ann Ist Super Sanita. 2018 Jan-Mar;54(1):67-71. doi: 10.4415/ANN_18_01_13.
https://www.iss.it/documents/20126/45616/ANN_18_01_13.pdf

[414] Pérez-Alós L, et al
waning immunity after SARS-CoV-2 vaccination and influencing factors.
Nat Commun. 2022 Mar 28;13(1):1614. doi: 10.1038/s41467-022-29225-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8960902/

[415] Crotty S.
Hybrid Immunity
Science 25 Jun 2021 372, 6549, pp 1392-3 DOI: 10.1126/science.abj2258
https://www.science.org/doi/full/10.1126/science.abj2258

This concept is often evoked in favor of vaccination, but this superimmune state has no proven long-term clinical correlates, and a growing number of studies show marginal, if any, additional benefits of vaccination in individuals with natural immunity.
Attributing higher serum antibody responses in vaccinated individuals as superior to natural infection is erroneous, as much time may have elapsed since natural infection with the expected decline in antibody levels.
Furthermore, natural infection, with the induction of strong interferon-dependent immunity in the upper airway, could lead to interferon-related flu-like symptoms, but with an innate cytokine response that prevents sufficient mucosal barrier aggression for clinically significant antibody generation.
Intramuscular vaccination readily generates an antibody response, which is measurable as serum antibodies, albeit transiently, but this phenomenon cannot be used to claim that vaccines are better than natural infections." [416]

Factors influencing the production of NAb

In a Japanese cohort, immunosuppressive medications, age, glucocorticoids, and alcohol consumption were identified as predictors of lower antibody titers after vaccination, while previous SARS-CoV-2 infection, female sex, time between two vaccine doses, and allergy medications were identified as predictors of higher serum antibody titers.[417] These same associations for age and previous infection have been reported in another study.[418]

Yair Goldberg, Micha Mandel, Yinon M. Bar-On, Omri Bodenheimer, Laurence Freedman, Nachman Ash, Sharon Alroy-Preis, Amit Huppert, Ron Milo
Protection and waning of natural and hybrid COVID-19 immunity
medRxiv 2021.12.04.21267114; doi: https://doi.org/10.1101/2021.12.04.21267114
https://www.medrxiv.org/content/10.1101/2021.12.04.21267114v1.full.pdf

Pilz S, Theiler-Schwetz V, Trummer C, Krause R, Ioannidis JPA.
SARS-CoV-2 reinfections: Overview of efficacy and duration of natural and hybrid immunity.
Environ Res. 2022 Jun;209:112911. doi: 10.1016/j.envres.2022.112911.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8824301/

Seven A, Crotty S.
Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines.
Immunol Rev. 2022 Jun 22:10.1111/imr.13089. doi: 10.1111/imr.13089.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9349657/

Bates TA, McBride SK, Leier HC, Guzman G, Lyski ZL, Schoen D, Winders B, Lee JY, Lee DX, Messer WB, Curlin ME, Tafesse FG.
Vaccination before or after SARS-CoV-2 infection leads to robust humoral response and antibodies that effectively neutralize variants.
Sci Immunol. 2022 Feb 18;7(68):eabn8014. doi: 10.1126/sciimmunol.abn8014.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8939472/

Castro Dopico X, Ols S, Loré K, Karlsson Hedestam GB.
Immunity to SARS-CoV-2 induced by infection or vaccination.
J Intern Med. 2022 Jan;291(1):32-50. doi: 10.1111/joim.13372.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8447342/

[416] Milne G, Hames T, Scotton C, Gent N, Johnsen A, Anderson RM, Ward T.
Does infection with or vaccination against SARS-CoV-2 lead to lasting immunity?
Lancet Respir Med. 2021 Dec;9(12):1450-1466. doi: 10.1016/S2213-2600(21)00407-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8530467/

Misra A, Theel ES.
Immunity to SARS-CoV-2: What Do We Know and Should We Be Testing for It?
J Clin Microbiol. 2022 Jun 15;60(6):e0048221. doi: 10.1128/jcm.00482-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9199399/

[417] Kageyama T, et al
Antibody responses to BNT162b2 mRNA COVID-19 vaccine and their predictors among healthcare workers in a tertiary referral hospital in Japan.
Clin Microbiol Infect. 2021 Dec;27(12):1861.e1-1861.e5. doi: 10.1016/j.cmi.2021.07.042.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8349446/

[418] Ebinger JE, Fert-Bober J, Printsev I, Wu M, Sun N, Prostko JC, Frias EC, Stewart JL, Van Eyk JE, Braun JG, Cheng S, Sobhani K.
Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2.

Levin et al. reported that six months after receiving the second dose, neutralizing antibody titers were lower among those aged 65 years and older than those aged 18 to less than 45 years (ratio of means, 0.58; 95% confidence interval (CI), 0.48 to 0.70), substantially lower among men than women (ratio of means, 0.64; 95% (CI), 0.55 to 0.75), and lower among participants with immunosuppression than those without immunosuppression (ratio of means, 0.30; 95% CI, 0.20 to 0.46).[419]

Comorbidities, such as diabetes, obesity, hypertension, dermatitis, and overweight, have not been associated with seronegativity or low production of neutralizing antibodies[420] , unlike kidney and liver disease. It is possible that this poor response is related to immune system alterations in renal disease, since uremia is associated with a state of immune dysfunction characterized by immunodepression.[421]

Regarding natural infection, Gozalbo-Rovira et al. reported weak correlations between antibody levels and inflammatory biomarkers (ferritin, D-dimer, CRP, lactate dehydrogenase (LDH) and interleukin-6).[422]

These correlations could be explained by the relationship between the magnitude of antibody response with hyperactivation of the immune system in patients with severe COVID-19.

Cytokine storm is believed to play a key role in disease progression and thus in the prognosis of COVID-19.[423] Therefore, NAb levels in patients cured of COVID-19 are positively related to disease severity.[424]

Nat Med. 2021 Jun;27(6):981-984. doi: 10.1038/s41591-021-01325-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8205849/

[419] Levin EG, et al
Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months.
N Engl J Med. 2021 Dec 9;385(24):e84. doi: 10.1056/NEJMoa2114583.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8522797/

[420] Morales-Núñez JJ, et al.
Neutralizing Antibodies Titers and Side Effects in Response to BNT162b2 Vaccine in Healthcare Workers with and without Prior SARS-CoV-2 Infection.
Vaccines (Basel). 2021 Jul 5;9(7):742. doi: 10.3390/vaccines9070742
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8310237/

[421] Zimmermann P, Curtis N.
Factors That Influence the Immune Response to Vaccination.
Clin Microbiol Rev. 2019 Mar 13;32(2):e00084-18. doi: 10.1128/CMR.00084-18.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6431125/

[422] Pang NY, Pang AS, Chow VT, Wang DY.
Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice.
Mil Med Res. 2021 Aug 31;8(1):47. doi: 10.1186/s40779-021-00342-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8405719/

Gozalbo-Rovira R, et al.
SARS-CoV-2 antibodies, serum inflammatory biomarkers and clinical severity of hospitalized COVID-19 patients.
J Clin Virol. 2020 Oct;131:104611. doi: 10.1016/j.jcv.2020.104611.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7459327/
[423] Eroshenko N, Gill T, Keaveney MK, Church GM, Trevejo JM, Rajaniemi H.
Implications of antibody-dependent enhancement of infection for SARS-CoV-2 countermeasures.
Nat Biotechnol. 2020 Jul;38(7):789-791. doi: 10.1038/s41587-020-0577-1.
https://pubmed.ncbi.nlm.nih.gov/32504046/

[424] Chen W, Zhang J, Qin X, Wang W, Xu M, Wang LF, Xu C, Tang S, Liu P, Zhang L, Liu X, Zhang Y, Yi C, Hu Z, Yi Y.
SARS-CoV-2 neutralizing antibody levels are correlated with severity of COVID-19 pneumonia.
Biomed Pharmacother. 2020 Oct;130:110629. doi: 10.1016/j.biopha.2020.110629.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7425713/

NATURAL IgM

Antibodies are a vital part of the adaptive immune system's resources for recognizing and responding to external threats.
However, in health there are certain types of antibodies that instead recognize autoantigens and these contribute to the enhancement of primitive innate functions.
Removal of dying cells is one of the most essential responsibilities of the immune system, necessary to prevent uncontrolled inflammation and autoimmunity, and natural IgM antibodies that recognize apoptotic cells have been shown to enhance phagocytic clearance (elimination) of dead and dying cells and suppress innate immune signaling pathways.[425]

[425] Avrameas S, Alexopoulos H, Moutsopoulos HM.
Natural Autoantibodies: An Undersugn Hero of the Immune System and Autoimmune Disorders-A Point of View.
Front Immunol. 2018 Jun 12;9:1320. doi: 10.3389/fimmu.2018.01320.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6005843/

Fereidan-Esfahani M, Nayfeh T, Warrington A, Howe CL, Rodriguez M.
IgM Natural Autoantibodies in Physiology and the Treatment of Disease.
Methods Mol Biol. 2019;1904:53-81. doi: 10.1007/978-1-4939-8958-4_3.
https://pubmed.ncbi.nlm.nih.gov/30539466/

Ehrenstein MR, Notley CA.
The importance of natural IgM: scavenger, protector and regulator.
Nat Rev Immunol. 2010 Nov;10(11):778-86. doi: 10.1038/nri2849.
https://pubmed.ncbi.nlm.nih.gov/20948548/

Grönwall C, Vas J, Silverman GJ.
Protective Roles of Natural IgM Antibodies.
Front Immunol. 2012 Apr 4;3:66. doi: 10.3389/fimmu.2012.00066.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3341951/

Wang H, Coligan JE, Morse HC 3rd.
Emerging Functions of Natural IgM and Its Fc Receptor FCMR in Immune Homeostasis.
Front Immunol. 2016 Mar 15;7:99. doi: 10.3389/fimmu.2016.00099.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4791374/

Kaveri SV, Silverman GJ, Bayry J.
Natural IgM in immune equilibrium and harnessing their therapeutic potential.
J Immunol. 2012 Feb 1;188(3):939-45. doi: 10.4049/jimmunol.1102107.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3266110/

Panda S, Ding JL.
Natural antibodies bridge innate and adaptive immunity.
J Immunol. 2015 Jan 1;194(1):13-20. doi: 10.4049/jimmunol.1400844.
https://www.jimmunol.org/content/194/1/13.long

Blandino R, Baumgarth N.
Secreted IgM: New tricks for an old molecule.
J Leukoc Biol. 2019 Nov;106(5):1021-1034. doi: 10.1002/JLB.3RI0519-161R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6803036/

Vas J, Grönwall C, Silverman GJ.
Fundamental roles of the innate-like repertoire of natural antibodies in immune homeostasis.
Front Immunol. 2013 Feb 5;4:4. doi: 10.3389/fimmu.2013.00004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3564042/

New JS, King RG, Kearney JF.
Glycan Reactive Natural Antibodies and Viral Immunity.
Viral Immunol. 2020 May;33(4):266-276. doi: 10.1089/vim.2019.0136.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7247037/

Binder CJ, Silverman GJ.
Natural antibodies and the autoimmunity of atherosclerosis.
Springer Semin Immunopathol. 2005 Mar;26(4):385-404. doi: 10.1007/s00281-004-0185-z.

IgM exists in two forms: membrane-bound (mIgM) and secreted (sIgM), with sIgM further divided into natural and antigen-induced IgM[426] .
"Natural IgM" antibodies represent the majority of secreted IgM antibodies present in normal serum and are also found in the pleural and peritoneal compartments[427] . This class of IgM antibodies is evolutionarily conserved in all jawed vertebrates[428] , is produced spontaneously by a subset of B cells, and often binds to specific antigens in the absence of immunization[429] . Natural IgM is encoded by unmutated germline variable gene segments, with polyreactive binding specificity to epitopes of self and non-self antigens, and is found at higher frequencies in infants than in adults, both in humans and mice[430] .

---

https://link.springer.com/article/10.1007/s00281-004-0185-z

Holodick NE, Rodríguez-Zhurbenko N, Hernández AM.
Defining Natural Antibodies.
Front Immunol. 2017 Jul 26;8:872. doi: 10.3389/fimmu.2017.00872.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5526850/

New JS, King RG, Kearney JF.
Manipulation of the glycan-specific natural antibody repertoire for immunotherapy.
Immunol Rev. 2016 Mar;270(1):32-50. doi: 10.1111/imr.12397.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4755354/

New JS, King RG, Kearney JF.
Glycan Reactive Natural Antibodies and Viral Immunity.
Viral Immunol. 2020 May;33(4):266-276. doi: 10.1089/vim.2019.0136.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7247037/

Khasbiullina NR, Bovin NV.
Hypotheses of the origin of natural antibodies: a glycobiologist's opinion.
Biochemistry (Mosc). 2015 Jul;80(7):820-35. doi: 10.1134/S0006297915070032.
http://protein.bio.msu.ru/biokhimiya/contents/v80/full/80070980.html

[426] Keyt BA, Baliga R, Sinclair AM, Carroll SF, Peterson MS.
Structure, Function, and Therapeutic Use of IgM Antibodies.
Antibodies (Basel). 2020 Oct 13;9(4):53. doi: 10.3390/antib9040053.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7709107/

Ehrenstein MR, Notley CA.
The importance of natural IgM: scavenger, protector and regulator.
Nat Rev Immunol. 2010 Nov;10(11):778-86. doi: 10.1038/nri2849.
https://pubmed.ncbi.nlm.nih.gov/20948548/

Michaud E, Mastrandrea C, Rochereau N, Paul S.
Human Secretory IgM: An Elusive Player in Mucosal Immunity.
Trends Immunol. 2020 Feb;41(2):141-156. doi: 10.1016/j.en.2019.12.005.
https://pubmed.ncbi.nlm.nih.gov/31928913/

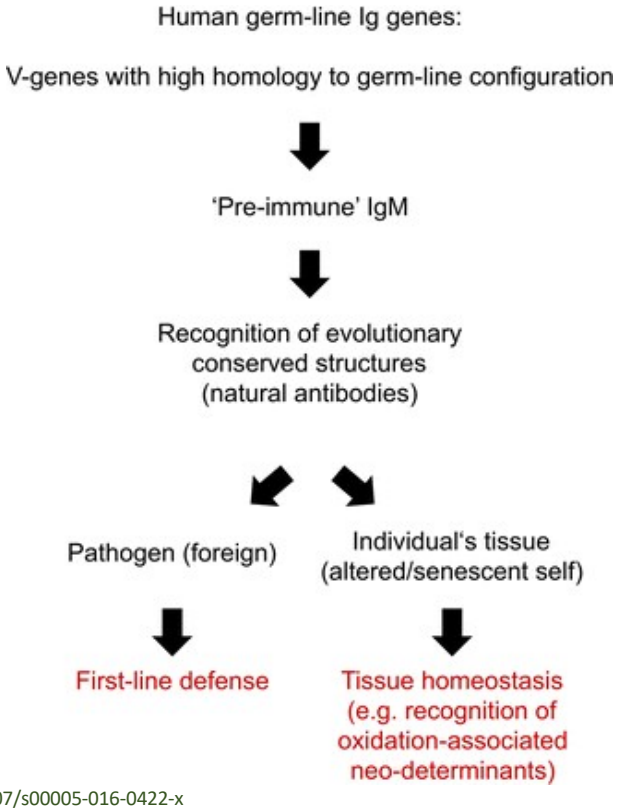
[427] Boes M.
Role of natural and immune IgM antibodies in immune responses.
Mol Immunol. 2000 Dec;37(18):1141-9. doi: 10.1016/s0161-5890(01)00025-6.
https://pubmed.ncbi.nlm.nih.gov/11451419/

Holodick NE, Tumang JR, Rothstein TL.
Immunoglobulin secretion by B1 cells: differential intensity and IRF4-dependence of spontaneous IgM secretion by peritoneal and splenic B1 cells.
Eur J Immunol. 2010 Nov;40(11):3007-16. doi: 10.1002/eji.201040545.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3139744/

[428] Flajnik MF.
Comparative analyses of immunoglobulin genes: surprises and portents.
Nat Rev Immunol. 2002 Sep;2(9):688-98. doi: 10.1038/nri889.
https://pubmed.ncbi.nlm.nih.gov/12209137/

[429] Jayasekera JP, Moseman EA, Carroll MC.
Natural antibody and complement mediate neutralization of influenza virus in the absence of prior immunity.
J Virol. 2007 Apr;81(7):3487-94. doi: 10.1128/JVI.02128-06.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1866020/

[430] Chen ZJ, Wheeler CJ, Shi W, Wu AJ, Yarboro CH, Gallagher M, Notkins AL.



https://link.springer.com/article/10.1007/s00005-016-0422-x
Concurrent recognition by the same natural IgM of evolutionarily conserved epitopes shared by microorganisms and autoantigens mediates first-line defense and tissue homeostasis. Tissue homeostasis by "natural" IgM antibodies is mediated by recognition of phosphorylcholine epitopes that are often neo-determinants associated with oxidation. These are common inflammatory danger signals of cellular damage, oxidative stress, and bacterial capsules

Key differences in effector B-cell subsets. [431]
Classical memory takes up to 4 days to develop and can be slow compared to the rapid invasion of encapsulated bacteria and viruses. It is during this period that innate-type B lymphocytes, which produce rapid cross-reactive natural IgM or long-lasting antigen-specific IgM responses, can interfere with the initial infection. The subsets of IgM-producing cells are listed below:

Short-lived plasma cells (SLPCs): IgM-producing antibody SLPCs are typically found in the spleen at the periphery of B-cell follicles, showing little or no SHM (somatic hypermutation).
SLPCs release the early wave of antibodies after antigen exposure and provide an initial protective response before the emergence of high-affinity antibodies.

Long-lived plasma cells (LLPCs): LLPCs continuously secrete antibodies at a constant titer, appear to be more stringently selected than SLPCs, and appear in the last germinal centers (GCs). LLPCs reside in lymphoid tissues associated with bone marrow, spleen, and intestine (GALT).

IgM memory B lymphocytes (MBCs-M): MBCs secrete antibodies in response to "cognate antigen challenge"[432] . MBCs maintain greater diversity and appear much earlier in the

Polyreactive antigen-binding B cells are the predominant cell type in the newborn B cell repertoire.
Eur J Immunol. 1998 Mar;28(3):989-94. doi: 10.1002/(SICI)1521-4141(199803)28:03<989::AID-IMMU989>3.0.CO;2-1.
https://onlinelibrary.wiley.com/doi/10.1002/(SICI)1521-4141(199803)28:03%3C989::AID-IMMU989%3E3.0.CO;2-1

[431] Jones K, Savulescu AF, Brombacher F, Hadebe S.
Immunoglobulin M in Health and Diseases: How Far Have We Come and What Next,
Front Immunol. 2020 Oct 30;11:595535. doi: 10.3389/fimmu.2020.595535.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7662119/
[432] Benson MJ, Elgueta R, Schpero W, Molloy M, Zhang W, Usherwood E, Noelle RJ.

GC than other B cells and may be tissue resident or found recirculating in secondary lymphoid organs. [433] MBC-Ms show lower mutation load than their CSR (class switch recombination) counterparts and broad cross-reactivity, particularly against conserved N-glycans of bacteria and retroviruses.
In humans, there are unswitched IgM memory B cells that are more abundant in local tissues such as GALT, lung, and SLO than in mice[434] . MBC-Ms have also been found in the blood circulation (identified as IgM^+ IgD^+ $CD27^+$) and show clonal correlation with gut-specific MBC-Ms, IgM and IgA only from plasma cells, providing significantly faster CSR and providing protection to blood-borne infections, probably through cross-reactivity. [435]

https://www.frontiersin.org/articles/10.3389/fimmu.2020.595535/full
Developmental pathways of immunoglobulin M (IgM) through B1 and B2 B cells from fetal liver (FL) and bone marrow (BM). B1 cells develop FL where they pass through pro-B cells, pre-B cells, immature B cells, and naïve B cells expressing IgM and CD5 differentiating B1a and B1b cells, both capable of secreting natural IgM (A). B2 cells develop from the common BM lymphoid progenitor to become immature B cells that migrate to splenic B cells that secrete IgM. Expression of IgD differentiates marginal zones from follicular B (B) lymphocytes. Follicular B cells after antigen stimulation may undergo germinal center maturation creating long-lived plasma cells, memory B cells, class switching, or remain short-lived unswitched plasma cells (C).

Distinction of the memory B cell response to cognate antigen versus bystander inflammatory signals.
J Exp Med. 2009 Aug 31;206(9):2013-25. doi: 10.1084/jem.20090667.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2737154/

Blink EJ, Light A, Kallies A, Nutt SL, Hodgkin PD, Tarlinton DM.
Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. J Exp Med. 2005 Feb 21;201(4):545-54. doi: 10.1084/jem.20042060.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2213050/

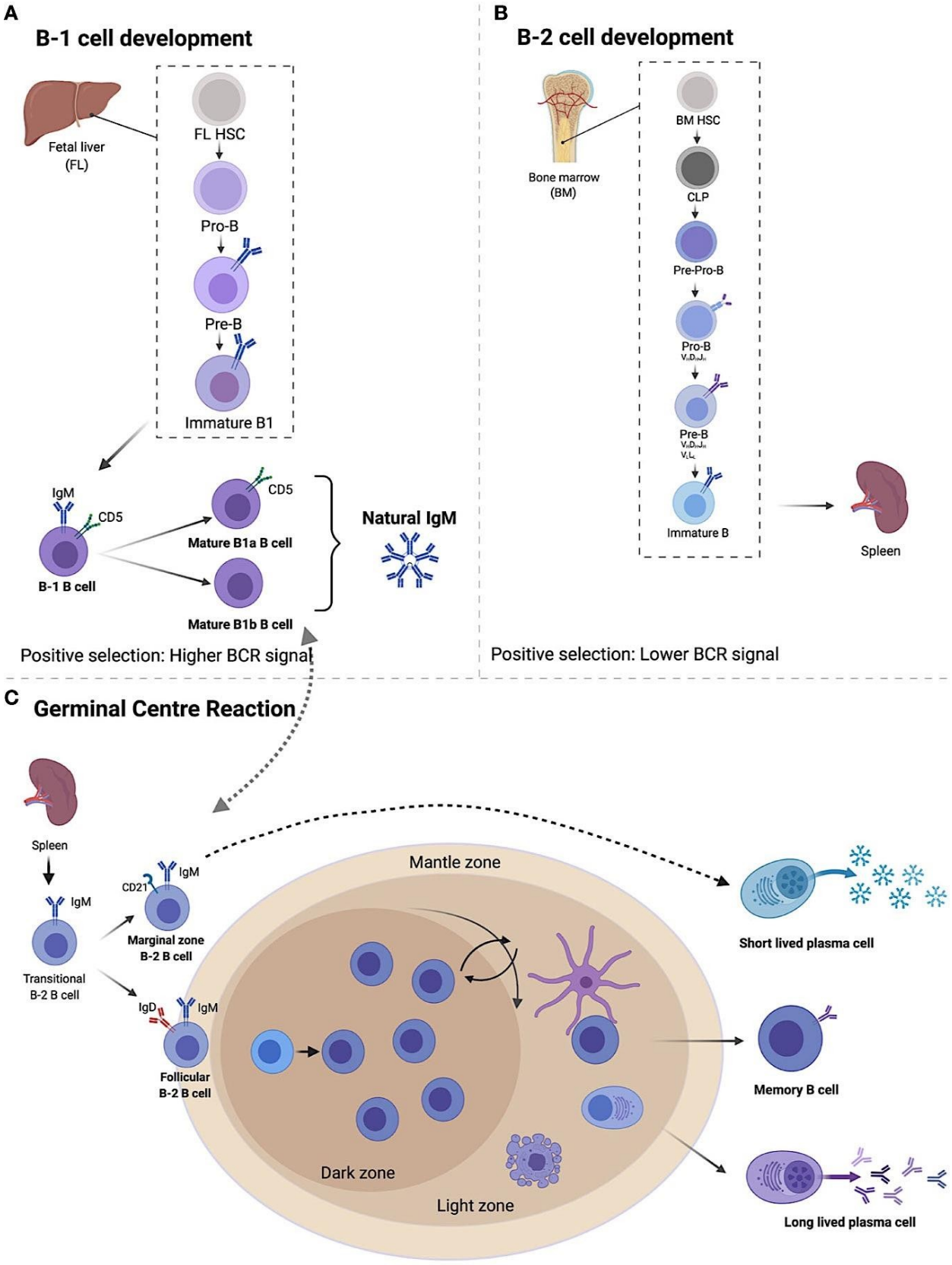
[433] Burton BR, Tennant RK, Love J, Titball RW, Wraith DC, White HN.
Variant proteins stimulate more IgM+ GC B-cells revealing a mechanism of cross-reactive recognition by antibody memory.
Elife. 2018 May 1;7:e26832. doi: 10.7554/eLife.26832.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5959717/

Weisel F, Shlomchik M.
Memory B Cells of Mice and Humans.
Annu Rev Immunol. 2017 Apr 26;35:255-284. doi: 10.1146/annurev-immunol-041015-055531.
https://pubmed.ncbi.nlm.nih.gov/28142324/

Tarlinton D, Good-Jacobson K.
Diversity among memory B cells: origin, consequences, and utility.
Science. 2013 Sep 13;341(6151):1205-11. doi: 10.1126/science.1241146.
https://pubmed.ncbi.nlm.nih.gov/24031013/
[434] Magri G, et al
Human Secretory IgM Emerges from Plasma Cells Clonally Related to Gut Memory B Cells and Targets Highly Diverse Commensals.
Immunity. 2017 Jul 18;47(1):118-134.e8. doi: 10.1016/j.immuni.2017.06.013.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5519504/

[435] Seifert M, Przekopowitz M, Taudien S, Lollies A, Ronge V, Drees B, Lindemann M, Hillen U, Engler H, Singer BB, Küppers R.
Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions.
Proc Natl Acad Sci U S A. 2015 Feb 10;112(6):E546-55. doi: 10.1073/pnas.1416276112.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4330750/

Klein U, Rajewsky K, Küppers R.
Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells.
J Exp Med. 1998 Nov 2;188(9):1679-89. doi: 10.1084/jem.188.9.1679.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2212515/


A
B-1 cell development
Fetal liver
(FL)
FL HSC
Pro-B
Pre-B
Immature B1
IgM
CD5
B-1 B cell
CD5
Mature B1a B cell
Mature B1b B cell
Natural IgM
Positive selection: Higher BCR signal
B
B-2 cell development
Bone marrow
(BM)
BM HSC
CLP
Pre-Pro-B
Pro-B
Pre-B
Immature B
Spleen
Positive selection: Lower BCR signal
C
Germinal Centre Reaction
Spleen
IgM
Transitional
B-2 B cell
CD21
IgM
Marginal zone
B-2 B cell
IgD
IgM
Follicular
B-2 B cell
Mantle zone
Dark zone
Light zone
Short lived plasma cell
Memory B cell
Long lived plasma cell

B cells and microbiota

The microbiota colonizes mucosal sites soon after birth in humans and plays a key role in homeostasis[436] . The dominant antibodies found at mucosal sites are secretory IgAs, which bind to and shape the microbiota[437] .

Most IgA from plasma cells is generated by memory B cells residing in the lamina propria (LP) of the intestine[438] . In addition to IgA, new evidence positions secreted IgM as a key player in maintaining local homeostasis at mucosal sites, such as the gut and lung, and helping to shape the local microbiota[439] .

Several studies have found that IgM, together with human IgA secreted into the human intestinal mucosa coat the human microbiota[440] , enhance the binding repertoire of IgA, and in some cases are even more potent in neutralizing enteric bacteria alone. In particular, IgM has been found to promote bacterial species beneficial to healthy intestinal homeostasis, such as *Firmicutes* (e.g., *Bacillus cereus, Lachnospiraceae spp.* and *Ruthenibacterium spp.*) and *Bacteroidetes* (*Bacteroides vulgatus*)[441] .

---

[436] Belkaid Y, Hand TW.
Role of the microbiota in immunity and inflammation.
Cell. 2014 Mar 27;157(1):121-41. doi: 10.1016/j.cell.2014.03.011.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4056765/

[437] Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P.
The immune geography of IgA induction and function.
Mucosal Immunol. 2008 Jan;1(1):11-22. doi: 10.1038/mi.2007.6.
https://pubmed.ncbi.nlm.nih.gov/19079156/

Nakajima A, et al
IgA regulates the composition and metabolic function of gut microbiota by promoting symbiosis between bacteria.
J Exp Med. 2018 Aug 6;215(8):2019-2034. doi: 10.1084/jem.20180427.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6080902/

[438] Kubinak JL, Round JL.
Do antibodies select a healthy microbiota?
Nat Rev Immunol. 2016 Dec;16(12):767-774. doi: 10.1038/nri.2016.114.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9004535/

[439] Magri G, et al
Human Secretory IgM Emerges from Plasma Cells Clonally Related to Gut Memory B Cells and Targets Highly Diverse Commensals.
Immunity. 2017 Jul 18;47(1):118-134.e8. doi: 10.1016/j.immuni.2017.06.013.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5519504/

Wesemann DR, Portuguese AJ, Meyers RM, Gallagher MP, Cluff-Jones K, Magee JM, Panchakshari RA, Rodig SJ, Kepler TB, Alt FW.
Microbial colonization influences early B-lineage development in the gut lamina propria.
Nature. 2013 Sep 5;501(7465):112-5. doi: 10.1038/nature12496.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3807868/

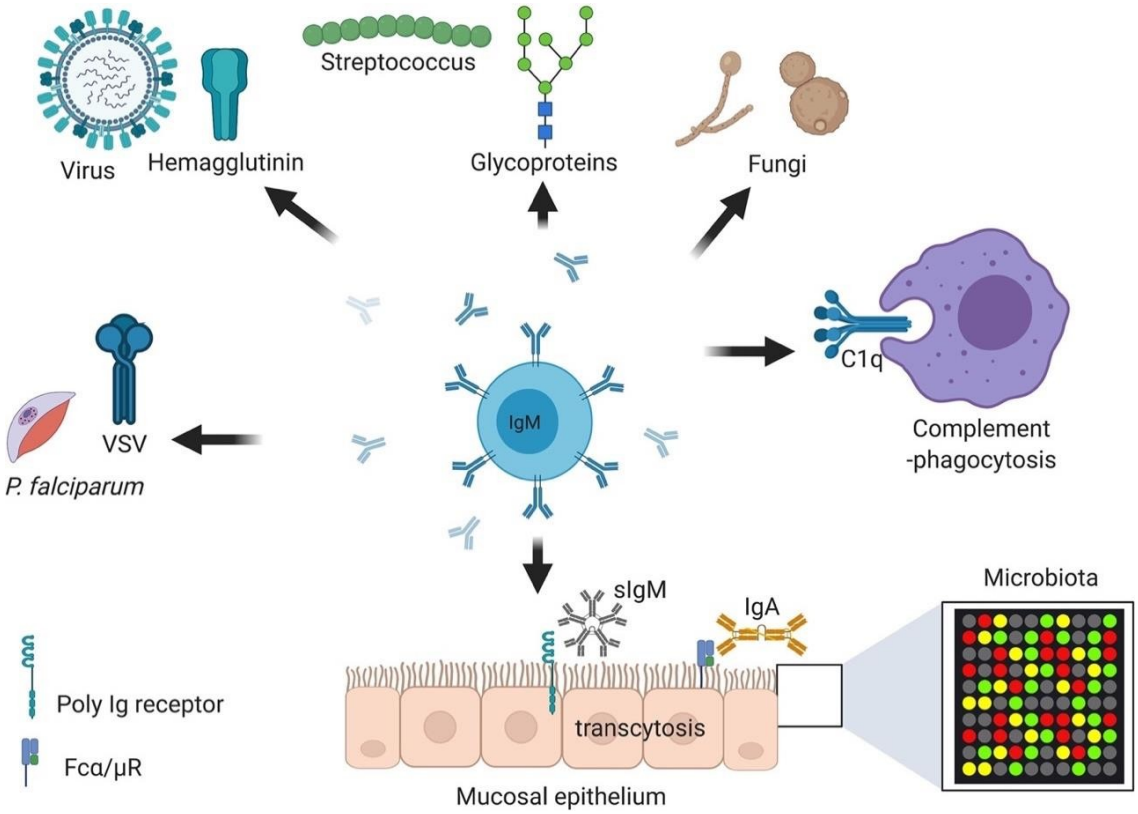
[440] Longet S, Vonarburg C, Lötscher M, Miescher S, Zuercher A, Corthésy B.
Reconstituted human polyclonal plasma-derived secretory-like IgM and IgA maintain the barrier function of epithelial cells infected with an enteropathogen.
J Biol Chem. 2014 Aug 1;289(31):21617-26. doi: 10.1074/jbc.M114.549139.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4118121/

Bioley G, Monnerat J, Lötscher M, Vonarburg C, Zuercher A, Corthésy B.
Plasma-Derived Polyreactive Secretory-Like IgA and IgM Opsonizing Salmonella enterica Typhimurium Reduces Invasion and Gut Tissue Inflammation through Agglutination.
Front Immunol. 2017 Aug 29;8:1043. doi: 10.3389/fimmu.2017.01043.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5581814/

[441] Belkaid Y, Hand TW.
Role of the microbiota in immunity and inflammation.
Cell. 2014 Mar 27;157(1):121-41. doi: 10.1016/j.cell.2014.03.011. ì
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4056765/

Shen X, Miao J, Wan Q, Wang S, Li M, Pu F, Wang G, Qian W, Yu Q, Marotta F, He F.
Possible correlation between gut microbiota and immunity among healthy middle-aged and elderly people in southwest China.
Gut Pathog. 2018 Feb 9;10:4. doi: 10.1186/s13099-018-0231-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5806246/

It is known that age negatively correlates with the presence of these bacteria, resulting in dysbiosis in the adult population, and therefore secreted IgM/MCB-M is believed to have evolved to help IgA preserve the homeostasis of the microbiota by interacting directly with the bacteria to promote the biodiversity of the healthy microbiota and possibly eliminate pathogenic bacteria.



https://www.frontiersin.org/articles/10.3389/fimmu.2020.595535/full
Immunoglobulin M (IgM) is critical at the stationary stage and against infections and noncommunicable diseases. Secretory IgM is important on mucosal surfaces in maintaining a healthy microbiota along with secreted IgA. Secretory IgM together with the IgM B cell receptor are important in the initiation of protective immunity against various respiratory pathogens including fungal species, viruses, and bacteria. Secreted IgM are essential in parasitic infections, including those that cause malaria and sleeping sickness. Secreted IgM play an important role in the diagnosis of cancer and autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Secreted IgM have a high affinity for C1q, a component of complement that enables the degradation of antibody-coated pathogens and apoptotic debris.

It has also been shown that natural IgM antibodies play a role in controlling the development, selection, and induction of central B-cell tolerance to prevent autoimmunity.
The rare condition of selective IgM deficiency in humans, associated with recurrent infections, is characterized by an increased risk of developing autoimmune diseases such as arthritis and systemic lupus erythematosus[442] .
In a study conducted by Nguyen et al. [443], it was found that mice with deficiency of the μ secretory chain (μs-/-) mimic the selective IgM deficiency phenotype observed in humans.
Although the phenotype may be due to reduced clearance of autoantigen, these knockout mice showed a blockage in differentiation at the pre/pro-B cell stage of development and a

[442] Louis AG, Gupta S.
Primary selective IgM deficiency: an ignored immunodeficiency.
Clin Rev Allergy Immunol. 2014 Apr;46(2):104-11. doi: 10.1007/s12016-013-8375-x.
https://pubmed.ncbi.nlm.nih.gov/23760686/

[443] Nguyen TT, Elsner RA, Baumgarth N.
Natural IgM prevents autoimmunity by enforcing B cell central tolerance induction.
J Immunol. 2015 Feb 15;194(4):1489-502. doi: 10.4049/jimmunol.1401880.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4324358/

escape from the induction of central tolerance with subsequent accumulation of autoantibody-secreting cells, a phenotype reversible by administration of polyclonal IgM.
Thus, these data support that naturally secreted IgM antibodies facilitate normal B-cell development, which imposes negative selection of self-reactive B cells, although the precise mechanism is unclear.

Glycosylation of IgM

Antibodies are glycoproteins with N-linked glycosylation, as will be detailed in the dedicated discussion. In the case of IgG, there is N-linked (nitrogen-bound) glycosylation in Asn 297, which influences binding to Fc-gamma receptors and thus plays a role in modulating antibody-dependent cellular cytotoxicity (ADCC).
Significantly, IgM has more glycosylation sites than IgG: while IgG heavy chains have a single glycosylation site, IgM heavy chains of human and nonhuman primates show five N-linked glycosylation sites[444] . These glycans are believed to facilitate polymerization and assembly of the IgM oligomeric structure[445] , as well as providing IgM with increased solubility and a longer *in vivo* half-life[446] .
Recently Colucci et al. also demonstrated a role for sialylation on IgM in mediating internalization in T lymphocytes and IgM-mediated immune suppression[447] .
The additional glycosylation sites increase the complexity of IgM antibodies, and it is interesting to note that the three sites of Asn 171, Asn 332 and Asn 395 (in domains 1, 2 and 3) show complex carbohydrate portions with sialylated endings.
In addition, the carboxyl terminal sites in Asn 402 and Asn 563 (in domains 3 and 4) contain high mannose structures[436] , and this pattern of glycosylation is consistent with the fact that the terminal amine regions of IgM are more accessible to glycosylation enzymes in the Golgi intracellular apparatus, while the carboxy-terminal regions (the "core" structure) are not fully processed, perhaps due to the steric hindrance and lack of accessibility of these glycans in the oligomerized form of IgM.
The fourth glycosylation site on IgM (Asn 402) is homologous to the single site on IgG, known to have limited accessibility and does not show fully developed complex carbohydrates in either IgM or IgG.

Natural IgM and innate immunity

Natural IgM antibodies, along with natural killer (NK) cells, mast cells and dendritic and macrophages are part of the innate immune system, the first line of defense against invading microorganisms and aberrant human cells[448] .

---

[444] Moh ES, Lin CH, Thaysen-Andersen M, Packer NH.
Site-Specific N-Glycosylation of Recombinant Pentameric and Hexameric Human IgM.
J Am Soc Mass Spectrom. 2016 Jul;27(7):1143-55. doi: 10.1007/s13361-016-1378-0.
https://pubmed.ncbi.nlm.nih.gov/27038031/

[445] Muraoka S, Shulman MJ.
Structural requirements for IgM assembly and cytolytic activity. Effects of mutations in the oligosaccharide acceptor site at Asn402.
J Immunol. 1989 Jan 15;142(2):695-701.
https://pubmed.ncbi.nlm.nih.gov/2911015/

[446] Maiorella BL, Winkelhake J, Young J, Moyer B, Bauer R, Hora M, Andya J, Thomson J, Patel T, Parekh R.
Effect of culture conditions on IgM antibody structure, pharmacokinetics and activity.
Biotechnology (N Y). 1993 Mar;11(3):387-92. doi: 10.1038/nbt0393-387. ì
https://pubmed.ncbi.nlm.nih.gov/7763441/.

[447] Colucci M, Stöckmann H, Butera A, Masotti A, Baldassarre A, Giorda E, Petrini S, Rudd PM, Sitia R, Emma F, Vivarelli M.
Sialylation of N-linked glycans influences the immunomodulatory effects of IgM on T cells.
J Immunol. 2015 Jan 1;194(1):151-7. doi: 10.4049/jimmunol.1402025.
https://www.jimmunol.org/content/194/1/151.long

[448] Vollmers HP, Brändlein S.
Natural IgM antibodies: the orphaned molecules in immune surveillance.
Adv Drug Deliv Rev. 2006 Aug 7;58(5-6):755-65. doi: 10.1016/j.addr.2005.08.007.
https://pubmed.ncbi.nlm.nih.gov/16820243/

This response involves binding to specific antigenic motifs, such as specific carbohydrates on glycoproteins or glycolipids and repetitive structures such as lipopolysaccharides, recognized by IgM antibodies encoded by germline (i.e., nonmutated) genes. Thus, these natural IgM antibodies play an important role in primary defense mechanisms by recognizing foreign bacteria and viruses or mutated human cells such as cancer cells.

Typically, these natural IgM antibodies use low-affinity binding to a range of similar foreign antigens, and their ability to eliminate these foreign antigens is thus amplified by the high avidity offered by having 10 (in the pentamer) or 12 (in the hexamer) binding sites.

The powerful ability of IgM antibodies to fix complement and opsonize particles makes them particularly effective against bacteria and viruses[449] .

IgM antibodies also differ from IgG isotypes regarding effector mechanisms. IgG can cause antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) through the action of Natural Killer cells.

In contrast, IgMs do not bind to Fcγ receptors, and therefore do not show ADCC, but have very potent CDC activity.

Their hexameric or pentameric structure allows very avid binding to the complement component C1q, and thus IgM are able to bind complement better than IgG[450] .

Recent work by Sharp et al.,[451] using phase-plate cryoelectron microscopy, provided a detailed model of how complement fixation results in a large conformational change in antigen- IgM binding.

The planar or discoidal structure of free IgM changes to a "squatting" or "flake-like" structure when Fab regions bind antigen on a cell surface. The antigen-binding Fab regions move out of the plane of the ring formed by Cμ3, Cμ4 and chorder because of the flexibility of the Cμ2 regions, the equivalent of the hinge regions of IgG.

This allows many or all Fab arms to make contact with antigens on a surface by exploiting the avidity of IgM. Other effector mechanisms, such as antibody-dependent cell phagocytosis, have also been implicated in IgM action[452] .

Keyt BA, Baliga R, Sinclair AM, Carroll SF, Peterson MS.
Structure, Function, and Therapeutic Use of IgM Antibodies.
Antibodies (Basel). 2020 Oct 13;9(4):53. doi: 10.3390/antib9040053.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7709107/

[449] Wibroe PP, Helvig SY, Moein Moghimi S.
The Role of Complement in Antibody Therapy for Infectious Diseases.
Microbiol Spectr. 2014 Apr;2(2). doi: 10.1128/microbiolspec.AID-0015-2014.
https://doi.org/10.1128/microbiolspec.AID-0015-2014

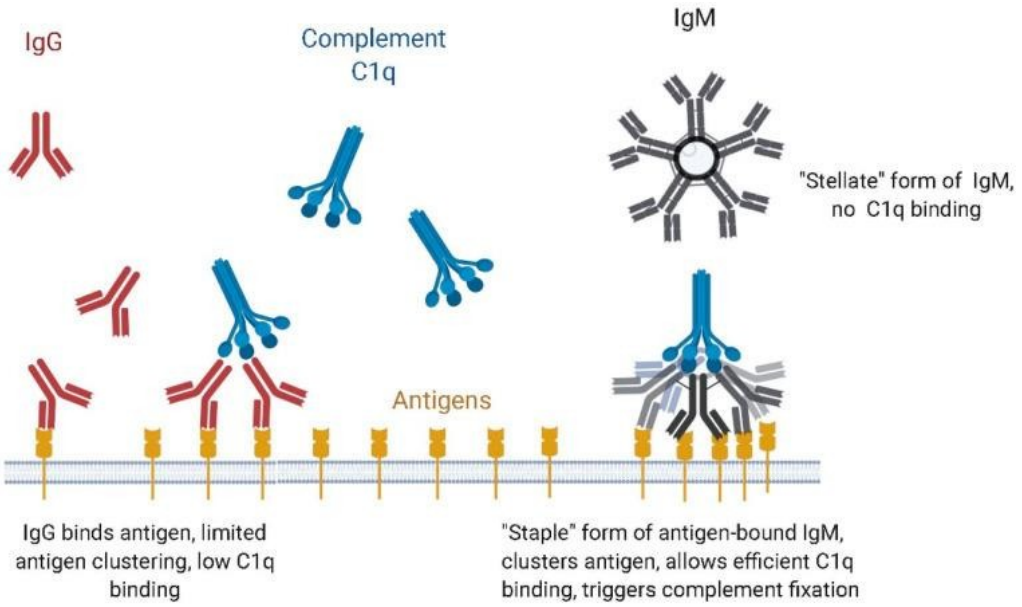
Strohl W.R., Strohl L.M.
Therapeutic Antibody Engineering. Woodhead Publishing; Sawston, UK: 2012. pp. 197-223
Parren PWHI, Lugovskoy AA.
Therapeutic antibody engineering: Current and future advances driving the strongest growth area in the pharmaceutical industry.
MAbs. 2013 Mar 1;5(2):175-7. doi: 10.4161/mabs.23654.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3893228/

[450] Klimovich VB.
IgM and its receptors: structural and functional aspects.
Biochemistry (Mosc). 2011 May;76(5):534-49. doi: 10.1134/S0006297911050038.
https://pubmed.ncbi.nlm.nih.gov/21639833/

[451] Sharp TH, Boyle AL, Diebolder CA, Kros A, Koster AJ, Gros P.
Insights into IgM-mediated complement activation based on in situ structures of IgM-C1-C4b.
Proc Natl Acad Sci U S A. 2019 Jun 11;116(24):11900-11905. doi: 10.1073/pnas.1901841116.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6575175/

[452] Shibuya A, et al
Fc alpha/mu receptor mediates endocytosis of IgM-coated microbes.
Nat Immunol. 2000 Nov;1(5):441-6. doi: 10.1038/80886.
https://pubmed.ncbi.nlm.nih.gov/11062505/

Weinstein JR, Quan Y, Hanson JF, Colonna L, Iorga M, Honda S, Shibuya K, Shibuya A, Elkon KB, Möller T.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7709107/

According to more recent data, IgM is an asymmetric pentamer that also forms a pocket for an effector protein, called "macrophage apoptosis inhibitor" (AIM/CD5L), whose serum half-life is greatly enhanced by binding to IgM.[453]
In addition to direct IgM effector functions and its AIM transport function, studies conducted for more than 30 years have demonstrated direct immunoregulatory functions for sIgM (secreted IgM).
Of particular interest are the immune enhancing effects of sIgM to induce maximal IgG responses, and the immune protective effects against the development of antibody-mediated autoimmune diseases. [454]

IgM-Dependent Phagocytosis in Microglia Is Mediated by Complement Receptor 3, Not Fcα/μ Receptor. J Immunol. 2015 Dec 1;195(11):5309-17. doi: 10.4049/jimmunol.1401195.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4655136/

[453] Blandino R, Baumgarth N.
Secreted IgM: New tricks for an old molecule.
J Leukoc Biol. 2019 Nov;106(5):1021-1034. doi: 10.1002/JLB.3RI0519-161R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6803036/

Hiramoto E, Tsutsumi A, Suzuki R, Matsuoka S, Arai S, Kikkawa M, Miyazaki T.
The IgM pentamer is an asymmetric pentagon with an open groove that binds the AIM protein.
Sci Adv. 2018 Oct 10;4(10):eaau1199. doi: 10.1126/sciadv.aau1199.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6179379/

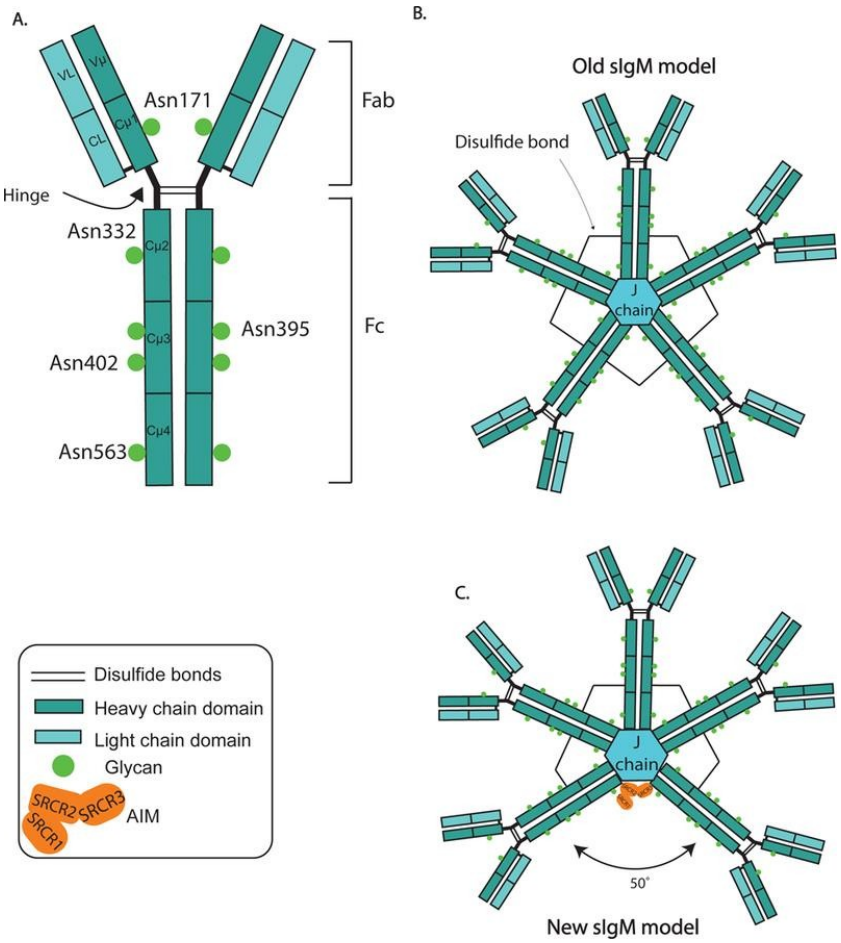
[454] Baumgarth N, Herman OC, Jager GC, Brown LE, Herzenberg LA, Chen J.
B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection.
J Exp Med. 2000 Jul 17;192(2):271-80. doi: 10.1084/jem.192.2.271.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2193249/

Boes M, Esau C, Fischer MB, Schmidt T, Carroll M, Chen J.
Enhanced B-1 cell development but impaired IgG antibody responses in mice deficient in secreted IgM.
J Immunol. 1998 May 15;160(10):4776-87.
https://www.jimmunol.org/content/160/10/4776.long

Heyman B, Pilström L, Shulman MJ.
Complement activation is required for IgM-mediated enhancement of the antibody response.
J Exp Med. 1988 Jun 1;167(6):1999-2004. doi: 10.1084/jem.167.6.1999.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2189680/

Nguyen TTT, Graf BA, Randall TD, Baumgarth N.
sIgM-FcμR Interactions Regulate Early B Cell Activation and Plasma Cell Development after Influenza Virus Infection.
J Immunol. 2017 Sep 1;199(5):1635-1646. doi: 10.4049/jimmunol.1700560.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5568459/

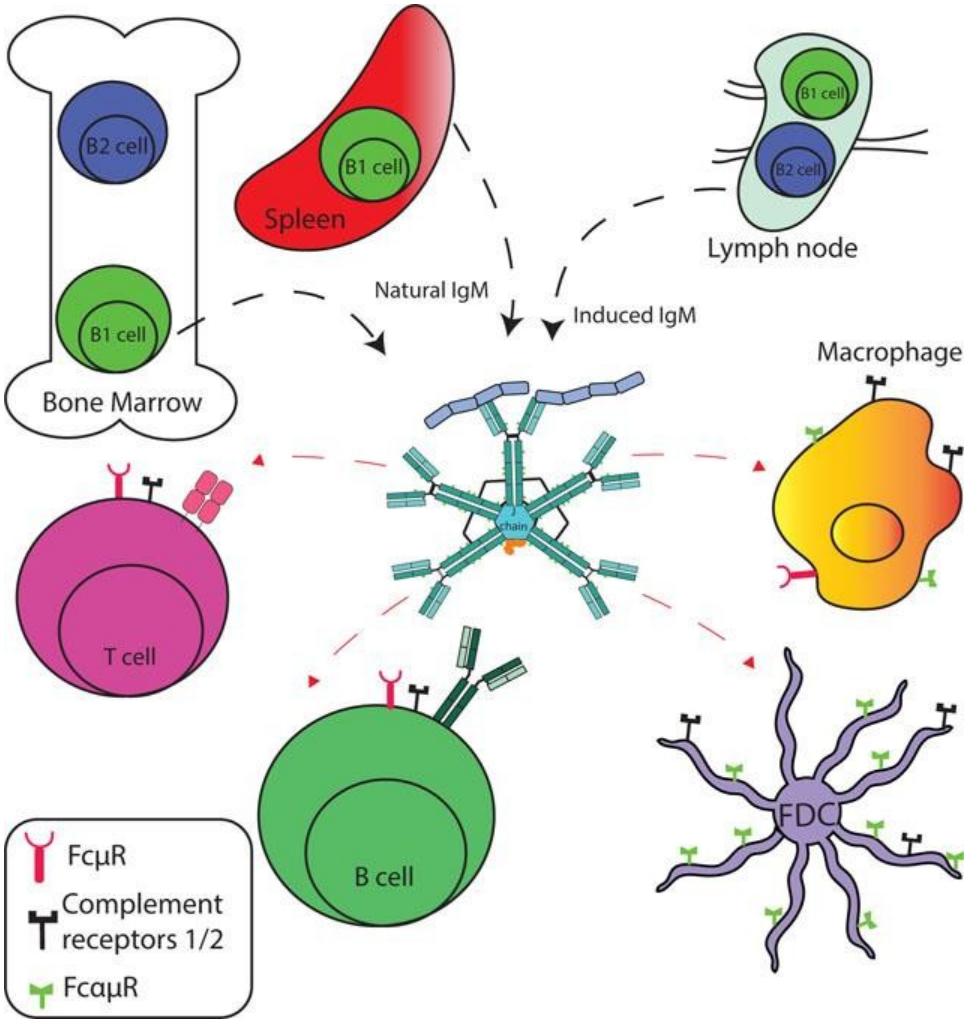
The mechanisms by which sIgMs regulate these processes remain poorly understood, but it is known that FcμR facilitates the direct interaction of sIgMs with cells of the immune system, particularly B and T cells. Mice lacking FcμR also show reduced IgG responses after immunization and infection and develop increased circulating autoantibody titers.[455]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6803036/
A new structure for sigM.
(A) Schematic diagram of an IgM monomer possessing two immunoglobulin heavy and light chains each. The Fab region (antibody binding fragment) encodes antigen binding sites, and the Fc region (constant fragment) regulates its function. The heavy chain contains five glycosylation sites, while there are no glycosylation sites encoded on the light chains. **(B)** Secreted IgM was thought to form a symmetric pentamer in which five monomers are joined together by a J chain and disulfide bonds. **(C)** New data now show that pentameric IgM is asymmetrical with a 50-degree groove that allows an AIM (macrophage apoptosis inhibitor/CD5L) molecule to bind, stabilizing their serum half-life.

[455] Choi SC, Wang H, Tian L, Murakami Y, Shin DM, Borrego F, Morse HC 3rd, Coligan JE.
Mouse IgM Fc receptor, FCMR, promotes B cell development and modulates antigen-driven immune responses.
J Immunol. 2013 Feb 1;190(3):987-96. doi: 10.4049/jimmunol.1202227
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3552009/

Nguyen TT, Kläsener K, Zürn C, Castillo PA, Brust-Mascher I, Imai DM, Bevins CL, Reardon C, Reth M, Baumgarth N.
The IgM receptor FcμR limits tonic BCR signaling by regulating expression of the IgM BCR.
Nat Immunol. 2017 Mar;18(3):321-333. doi: 10.1038/ni.3677.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5310993/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6803036/

IgM receptors

In healthy individuals, circulating polyclonal IgM is generally present at a concentration of 1-2 mg/ml blood, with a half-life of about 5 days.[456]

The following receptors have been recognized as IgM binding sites:

(a) Complement receptors (CRs), widely expressed by different cell types.

For example, B lymphocytes express cell surface complement receptor type 1 (CR1/CD35) and complement receptor type 2 (CR2/CD21), which can bind to antigen-IgM complexes with aggregated activated complement molecules.[457]

(b) Fcα/μ receptors (Fcα/μR), type I transmembrane proteins that bind both IgA and IgM isotypes.[458]

[456] Kaveri SV, Silverman GJ, Bayry J.
Natural IgM in immune equilibrium and harnessing their therapeutic potential.
J Immunol. 2012 Feb 1;188(3):939-45. doi: 10.4049/jimmunol.1102107.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3266110/

[457] Carroll MC.
The role of complement and complement receptors in induction and regulation of immunity.
Annu Rev Immunol. 1998;16:545-68. doi: 10.1146/annurev.immunol.16.1.545.
https://pubmed.ncbi.nlm.nih.gov/9597141/

[458] Sakamoto N, Shibuya K, Shimizu Y, Yotsumoto K, Miyabayashi T, Sakano S, Tsuji T, Nakayama E, Nakauchi H, Shibuya A.
A novel Fc receptor for IgA and IgM is expressed on both hematopoietic and non-hematopoietic tissues.
Eur J Immunol. 2001 May;31(5):1310-6. doi: 10.1002/1521-4141(200105)31:5<1310::AID-IMMU1310>3.0.CO;2-N.

These receptors are constitutively expressed on marginal zone B lymphocytes, the follicular dendritic cells, and binding of IgM to these cells can suppress germinal center formation, affinity maturation, and the generation of memory B cells in response to T-cell-independent antigen challenge.[459]

(c) Polymeric Ig receptors (poly-IgRs), expressed on epithelial cells that bind polymeric IgA and IgM through the J-chain.

These receptors mediate the transport of polymeric Ig containing J chains to mucosal sites.[460]

(d) FAIM3/TOSO receptors, initially identified as "Fas apoptosis inhibitory molecule 3" (FAIM3). These FcμR receptors have recently been rediscovered as an IgM-specific Fc receptor, the only one that binds exclusively to the Fc portion of pentameric IgM with high affinity[461] and is present on a variety of cell types such as macrophages, dendritic cells, and T cells, with the highest expression observed in B cells.[462]

Binding of IgM, particularly pentameric IgM, to this receptor enhances B- and T-cell cooperation and potentiates antibody-dependent cell-mediated cytotoxicity and complement activation.[463]

---

https://doi.org/10.1002/1521-4141(200105)31:5<1310::AID-IMMU1310>3.0.CO;2-N

[459] Shibuya A, et al
Fc alpha/mu receptor mediates endocytosis of IgM-coated microbes.
Nat Immunol. 2000 Nov;1(5):441-6. doi: 10.1038/80886.
https://pubmed.ncbi.nlm.nih.gov/11062505/

[460] Kaetzel CS.
The polymeric immunoglobulin receptor: bridging innate and adaptive immune responses at mucosal surfaces.
Immunol Rev. 2005 Aug;206:83-99. doi: 10.1111/j.0105-2896.2005.00278.x.
https://pubmed.ncbi.nlm.nih.gov/16048543/

Johansen FE, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C, Brandtzaeg P.
Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice.
J Exp Med. 1999 Oct 4;190(7):915-22. doi: 10.1084/jem.190.7.915.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2195652/

[461] Kubagawa H, et al
Nomenclature of Toso, Fas apoptosis inhibitory molecule 3, and IgM FcR.
J Immunol. 2015 May 1;194(9):4055-7. doi: 10.4049/jimmunol.1500222.
https://doi.org/10.4049/jimmunol.1500222

[462] Kubagawa H, Kubagawa Y, Jones D, Nasti TH, Walter MR, Honjo K.
The old but new IgM Fc receptor (FcμR).
Curr Top Microbiol Immunol. 2014;382:3-28. doi: 10.1007/978-3-319-07911-0_1.
https://pubmed.ncbi.nlm.nih.gov/25116093/

Kubagawa H, Oka S, Kubagawa Y, Torii I, Takayama E, Kang DW, Gartland GL, Bertoli LF, Mori H, Takatsu H, Kitamura T, Ohno H, Wang JY.
Identity of the elusive IgM Fc receptor (FcmuR) in humans.
J Exp Med. 2009 Nov 23;206(12):2779-93. doi: 10.1084/jem.20091107.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2806608/

463 Shima H, Takatsu H, Fukuda S, Ohmae M, Hase K, Kubagawa H, Wang JY, Ohno H.
Identification of TOSO/FAIM3 as an Fc receptor for IgM.
Int Immunol. 2010 Mar;22(3):149-56. doi: 10.1093/intimm/dxp121.
https://pubmed.ncbi.nlm.nih.gov/20042454/

Berland R, Wortis HH.
Origins and functions of B-1 cells with notes on the role of CD5.
Annu Rev Immunol. 2002;20:253-300. doi: 10.1146/annurev.immunol.20.100301.064833.
https://pubmed.ncbi.nlm.nih.gov/11861604/

(e) Lectins similar to sialic acid-binding immunoglobulins (Siglec) such as SiglecG and CD22, expressed on B-cell membranes and binding sialic acid residues on IgM, resulting in inhibition of downstream B-cell receptor (BCR) signaling.[464]

CD22, an inhibitory co-receptor on B lymphocytes, also plays a role as a receptor for glycoconjugates on soluble IgM through its sialoprotein binding domain. The absence of Siglec- G or CD22 alone does not lead to the development of autoimmune disease, but the lack of both receptors causes spontaneous lupus-like disease in mice,[465] as B cells become hypereactive due to increased BCR signaling. [466]

Characteristic	NAbs	Conventional Abs
Sequence	Germline or near germline with few somatic mutations, no affinity maturation	Somatically mutated, affinity maturated
Immunoglobulin subtype	Mainly IgM; also IgA and IgG	Mainly IgG; also IgM and IgA
Antigen	Bind to structurally diverse and unrelated antigens	Bind to a single antigen
Affinity, kDa	Low (10^{-4} to 10^{-7} M)	High (10^{-7} to 10^{-11} M)
No. of potentially allowed conformations of antigen-binding pocket	>1 (Conformational selection or induced fit hypothesis)	1 (Lock-and-key fit mechanism)
Half-life, approximate time, h	IgM, 8; IgG, 10; IgA, 8	IgM, 35 h; IgG, 280 h; IgA, 26 h

https://www.researchgate.net/publication/281976288_Naturally_Occurring_Monoclonal_Antibodies_and_Their_Therapeutic_Potential_for_Neurologic_Diseases

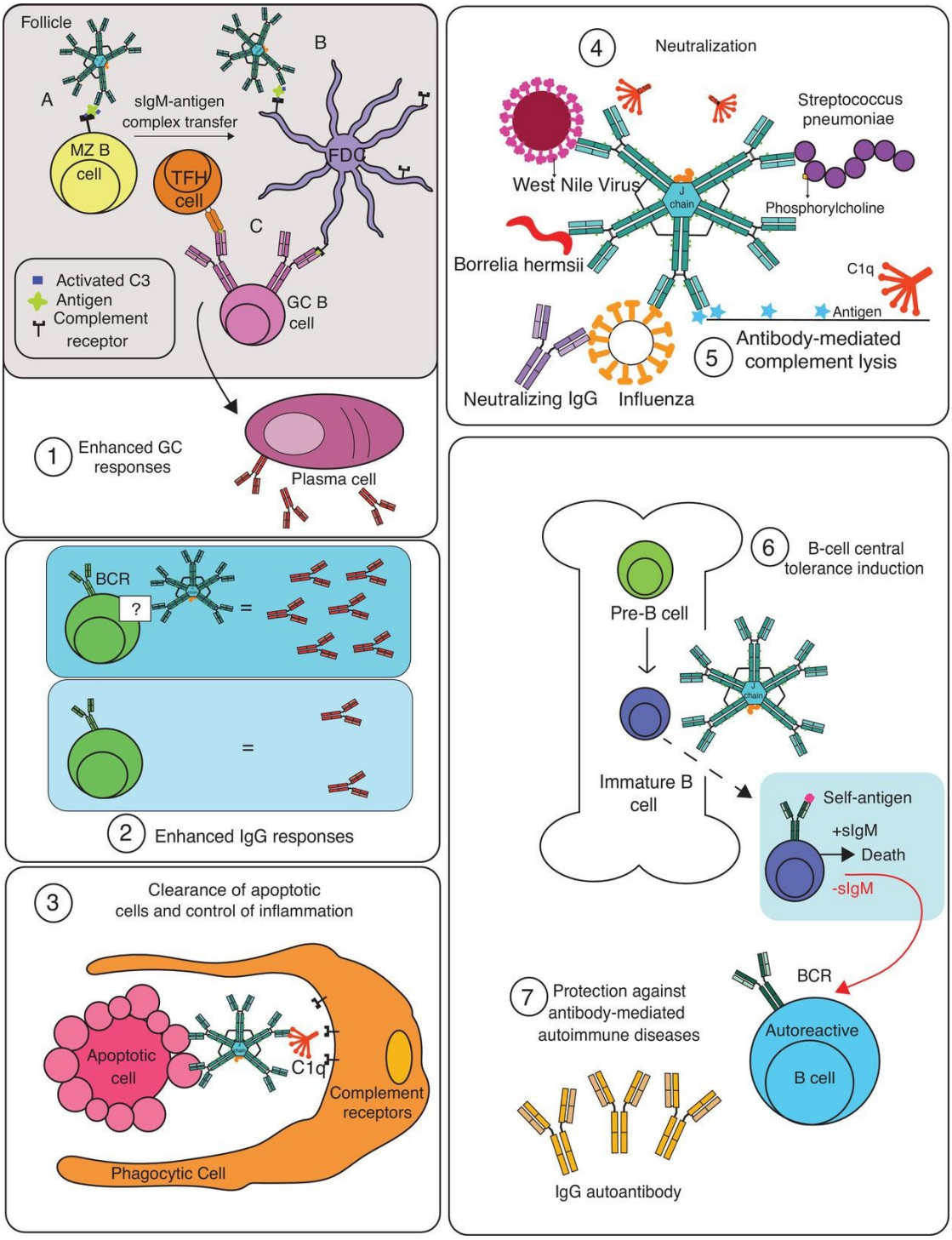
https://jlb.onlinelibrary.wiley.com/doi/full/10.1002/JLB.3RI0519-161R
Functions of secreted IgM. **(1A)**. Splenic B cells in the marginal zone capture sIgM-antigen/complement C3 complexes and **(B)** migrates into the B cell follicle where it transfers the complexes onto CR1/2 expressed by FDCs, where **(C)** the FDC presents antigen to germinal B cells in support of T-dependent antibody production. **(2)** sIgM strongly supports the development of IgG response by additional but poorly understood processes. **(3)** Opsonization of antigen by sIgM via C1q-mediated uptake of complement by macrophages. This process may also be mediated by mannose-binding lectin (not shown). **(4)** Neutralization of pathogens to block pathogen entry or induce pathogen aggregation. Natural IgM are polyreactive and recognize conserved antigens, such as phosphorylcholine present in Streptococcus pneumoniae cell walls, as well as on dead or dying mammalian host cells. **(5)** sIgM can recruit complement components to initiate the classical complement activation pathway, eventually leading to the formation of the membrane attack complex that can result in pathogen lysis. **(6)** Although the mechanisms are incompletely resolved, sIgM prevents autoimmune antibody formation through multiple mechanisms, including the effect of central tolerance induction in the bone marrow and through the removal of DAMPS, such as dead and dying cellular debris. **(7)** Absence of sIgM causes changes in the use of the V gene that encodes for increased self-reactivity

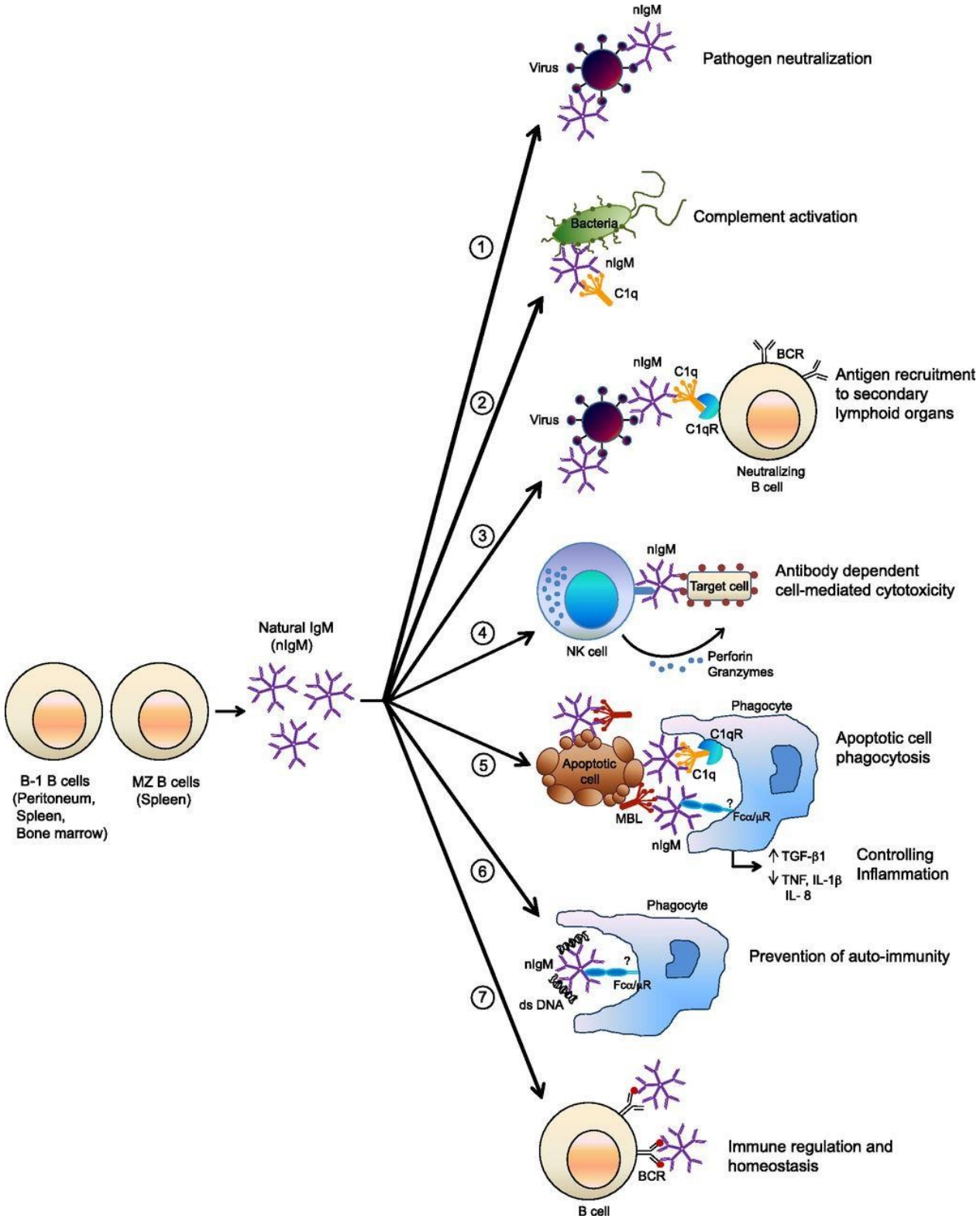
[464] Peaker CJ, Neuberger MS.
Association of CD22 with the B cell antigen receptor.
Eur J Immunol. 1993 Jun;23(6):1358-63. doi: 10.1002/eji.1830230626.
https://pubmed.ncbi.nlm.nih.gov/7684686/

[465] Müller J, Nitschke L.
The role of CD22 and Siglec-G in B-cell tolerance and autoimmune disease.
Nat Rev Rheumatol. 2014 Jul;10(7):422-8. doi: 10.1038/nrrheum.2014.54.
https://pubmed.ncbi.nlm.nih.gov/24763061/

[466] O'Keefe TL, Williams GT, Davies SL, Neuberger MS.
Hyperresponsive B cells in CD22-deficient mice.
Science. 1996 Nov 1;274(5288):798-801. doi: 10.1126/science.274.5288.798.
https://pubmed.ncbi.nlm.nih.gov/8864124/

Jellusova J, Nitschke L.
Regulation of B cell functions by the sialic acid-binding receptors siglec-G and CD22.
Front Immunol. 2012 Jan 11;2:96. doi: 10.3389/fimmu.2011.00096.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342095/


Follicle
A
B
C
sIgM-antigen complex transfer
MZ B cell
TFH cell
FDC
GC B cell
Activated C3
Antigen
Complement receptor
1 Enhanced GC responses
Plasma cell
BCR
?
=
=
2 Enhanced IgG responses
3 Clearance of apoptotic cells and control of inflammation
Apoptotic cell
C1q
Complement receptors
Phagocytic Cell
4 Neutralization
Streptococcus pneumoniae
West Nile Virus
J chain
Phosphorylcholine
Borrelia hermsii
C1q
Antigen
5 Antibody-mediated complement lysis
Neutralizing IgG
Influenza
6 B-cell central tolerance induction
Pre-B cell
Immature B cell
Self-antigen
+sIgM
Death
-sIgM
7 Protection against antibody-mediated autoimmune diseases
BCR
Autoreactive
B cell
IgG autoantibody



https://www.jimmunol.org/content/194/1/13

Different roles of natural IgM (nIgM) in immunity. nIgM, produced by B-1 cells in the peritoneum, bone marrow, and splenic B MZ cells, plays a crucial role in many immune processes: **(1)** direct neutralization of the pathogen; **(2)** activation of classical complement in cooperation with C1q; **(3)** Ag recruitment to secondary lymphoid organs and triggering of subsequent adaptive TI immunity; **(4)** Ab-dependent cell-mediated cytotoxicity: nIgM eliminates target cells through NK-cell-mediated release of perforin and granzymes; **(5)** Apoptotic cellular phagocytosis in cooperation with C1q and MBL through C1qR and Fcα/μR, which reduces inflammation and restores homeostasis; **(6)** Prevention of autoimmunity by clearance of DAMPs, such as dsDNA; and (**7**) Immune regulation and homeostasis of B lymphocytes.

Roles of IgM

It has been shown that IgM-NAA (Natural IgM Autoantibodies) play several roles in the immune system, including immediate protection from infection, regulation of B lymphocyte responses[467] , control of B lymphocyte development[468] , selection of the B lymphocyte repertoire[469] , suppression of allergic responses[470] and protection against cancer[471] by the following mechanisms:

(a) They provide the first line of defense against pathogens:
while the adaptive arm of the immune system generates specific, long-term immunity to pathogens, IgM-NAA serve as a parallel, immediate, innate response to invading microbes through neutralization of pathogens, activation of the classical complement pathway, opsonization of pathogens, enhancement of phagocytosis, and transport of antigens to lymphoid tissue.[472]

---

[467] Lobo PI.
Role of Natural IgM Autoantibodies (IgM-NAA) and IgM Anti-Leukocyte Antibodies (IgM-ALA) in Regulating Inflammation.
Curr Top Microbiol Immunol. 2017;408:89-117. doi: 10.1007/82_2017_37.
https://pubmed.ncbi.nlm.nih.gov/28698955/

Boes M, Esau C, Fischer MB, Schmidt T, Carroll M, Chen J.
Enhanced B-1 cell development but impaired IgG antibody responses in mice deficient in secreted IgM.
J Immunol. 1998 May 15;160(10):4776-87.
https://www.jimmunol.org/content/160/10/4776.long

[468] Nguyen TT, Elsner RA, Baumgarth N.
Natural IgM prevents autoimmunity by enforcing B cell central tolerance induction.
J Immunol. 2015 Feb 15;194(4):1489-502. doi: 10.4049/jimmunol.1401880.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4324358/

[469] Freitas AA, Viale AC, Sundblad A, Heusser C, Coutinho A.
Normal serum immunoglobulins participate in the selection of peripheral B-cell repertoires.
Proc Natl Acad Sci U S A. 1991 Jul 1;88(13):5640-4. doi: 10.1073/pnas.88.13.5640.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC51933/

[470] Kearney JF, Patel P, Stefanov EK, King RG.
Natural antibody repertoires: development and functional role in inhibiting allergic airway disease.
Annu Rev Immunol. 2015;33:475-504. doi: 10.1146/annurev-immunol-032713-120140.
https://pubmed.ncbi.nlm.nih.gov/25622195/

Patel PS, Kearney JF.
Neonatal exposure to pneumococcal phosphorylcholine modulates the development of house dust mite allergy during adult life.
J Immunol. 2015 Jun 15;194(12):5838-50. doi: 10.4049/jimmunol.1500251.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4456637/

[471] Vollmers HP, Brändlein S.
Natural antibodies and cancer.
N Biotechnol. 2009 Jun;25(5):294-8. doi: 10.1016/j.nbt.2009.03.016.
https://pubmed.ncbi.nlm.nih.gov/19442595/

Madi A, Bransburg-Zabary S, Maayan-Metzger A, Dar G, Ben-Jacob E, Cohen IR.
Tumor-associated and disease-associated autoantibody repertoires in healthy colostrum and maternal and newborn cord sera.
J Immunol. 2015 Jun 1;194(11):5272-81. doi: 10.4049/jimmunol.1402771.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4432729/

[472] Heyman B.
Regulation of antibody responses via antibodies, complement, and Fc receptors.
Annu Rev Immunol. 2000;18:709-37. doi: 10.1146/annurev.immunol.18.1.709.
https://pubmed.ncbi.nlm.nih.gov/10837073/

Heyman B, Pilström L, Shulman MJ.
Complement activation is required for IgM-mediated enhancement of the antibody response.
J Exp Med. 1988 Jun 1;167(6):1999-2004. doi: 10.1084/jem.167.6.1999.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2189680/

Ochsenbein AF, Fehr T, Lutz C, Suter M, Brombacher F, Hengartner H, Zinkernagel RM.
Control of early viral and bacterial distribution and disease by natural antibodies.
Science. 1999 Dec 10;286(5447):2156-9. doi: 10.1126/science.286.5447.2156.
https://pubmed.ncbi.nlm.nih.gov/10591647/

(b) They shape the subsequent immune response to the antigen:
pentameric structure and polyreactivity of IgM provide the ability to interact directly with pathogens. IgM-NAA can bind simultaneously to several conserved structures, such as nucleic acids, phospholipids and carbohydrates on the same pathogen, promote recognition and presentation by APCs, ultimately leading to activation of acquired immunity and memory responses[473] .

(c) Dispose of apoptotic cells[474] , misfolded proteins, and altered cells:
safe removal of dying cells is a fundamental process necessary for the entire life of an organism. Decoration of dying cell surface membranes with soluble innate immune molecules such as complement C1q and mannose-binding lectin promotes recognition by cells that initiate a process of phagocytosis called "efferocytosis."[475] .
IgM-dependent C1q deposition plays an important role in determining the efficiency of apoptotic cell clearance by macrophages. In the healthy individual, apoptotic cells pose no threat to the host because efferocytosis ensures rapid and efficient clearance of the cell corpse by macrophages and dendritic cells. Defects in efferocytosis, as postulated by Walport et al.[476] in the "waste disposal" hypothesis, may be linked to autoimmune diseases. A defect in apoptotic cell clearance can progress into secondary necrosis, which leads to the release of pathogenic factors such as heat shock protein, high mobility group 1 protein, and other components of dying cells.

Zhou ZH, Zhang Y, Hu YF, Wahl LM, Cisar JO, Notkins AL.
The broad antibacterial activity of the natural antibody repertoire is due to polyreactive antibodies.
Cell Host Microbe. 2007 Mar 15;1(1):51-61. doi: 10.1016/j.chom.2007.01.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2212603/

Stäger S, Alexander J, Kirby AC, Botto M, Rooijen NV, Smith DF, Brombacher F, Kaye PM.
Natural antibodies and complement are endogenous adjuvants for vaccine-induced $CD8^+$ T-cell responses.
Nat Med. 2003 Oct;9(10):1287-92. doi: 10.1038/nm933.
https://pubmed.ncbi.nlm.nih.gov/14502281/

Kohler H, Bayry J, Nicoletti A, Kaveri SV.
Natural autoantibodies as tools to predict the outcome of immune response?
Scand J Immunol. 2003 Sep;58(3):285-9. doi: 10.1046/j.1365-3083.2003.01314.x.
https://doi.org/10.1046/j.1365-3083.2003.01314.x

Jayasekera JP, Moseman EA, Carroll MC.
Natural antibody and complement mediate neutralization of influenza virus in the absence of prior immunity.
J Virol. 2007 Apr;81(7):3487-94. doi: 10.1128/JVI.02128-06.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1866020/

[473] Rapaka RR, Ricks DM, Alcorn JF, Chen K, Khader SA, Zheng M, Plevy S, Bengtén E, Kolls JK.
Conserved natural IgM antibodies mediate innate and adaptive immunity against the opportunistic fungus Pneumocystis murina.
J Exp Med. 2010 Dec 20;207(13):2907-19. doi: 10.1084/jem.20100034.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3005228/

Fernandez Gonzalez S, Jayasekera JP, Carroll MC.
Complement and natural antibody are required in the long-term memory response to influenza virus.
Vaccine. 2008 Dec 30;26 Suppl 8:I86-93. doi: 10.1016/j.vaccine.2008.11.057.
https://pubmed.ncbi.nlm.nih.gov/19388171/

[474] Gong S, Ruprecht RM.
Immunoglobulin M: An Ancient Antiviral Weapon - Rediscovered.
Front Immunol. 2020 Aug 11;11:1943. doi: 10.3389/fimmu.2020.01943.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7432194/

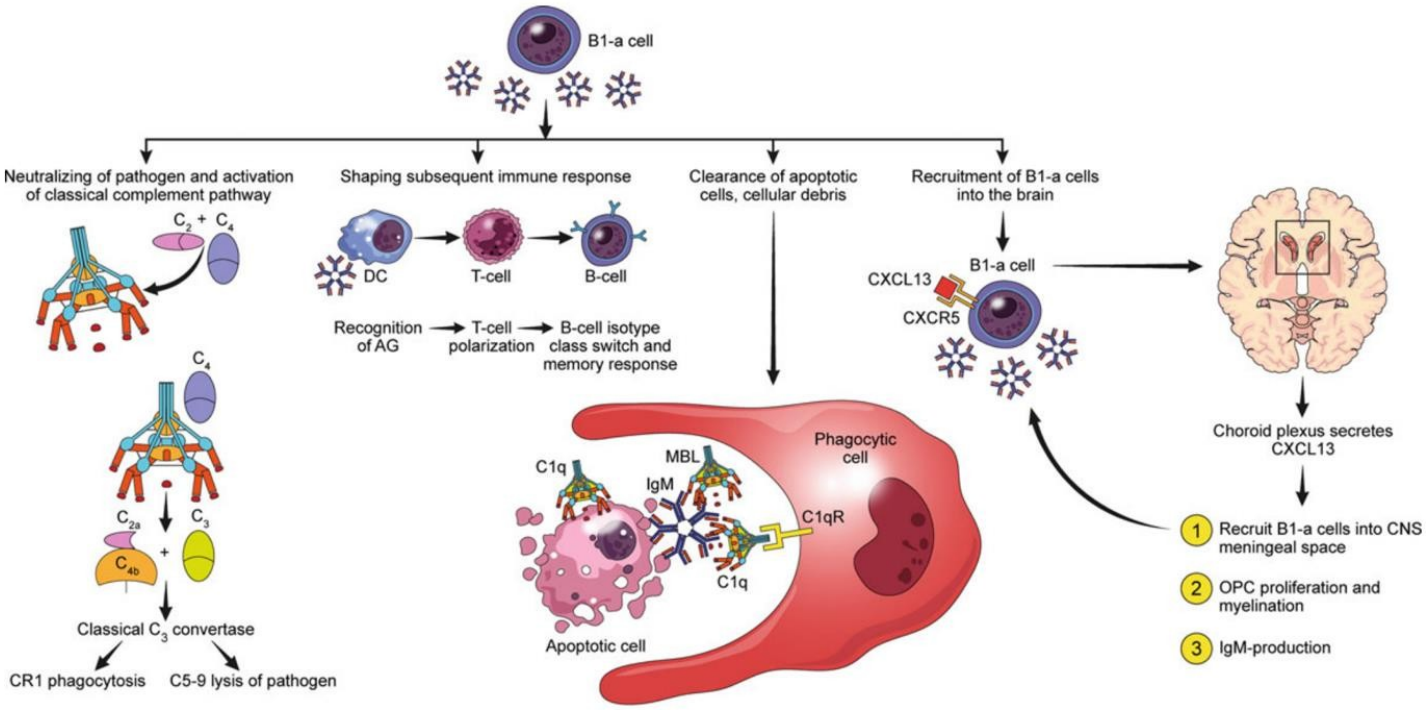
[475] Henson PM.
Cell Removal: Efferocytosis.
Annu Rev Cell Dev Biol. 2017 Oct 6;33:127-144. doi: 10.1146/annurev-cellbio-111315-125315.
https://pubmed.ncbi.nlm.nih.gov/28613937/

[476] Manderson AP, Botto M, Walport MJ.
The role of complement in the development of systemic lupus erythematosus.
Annu Rev Immunol. 2004;22:431-56. doi: 10.1146/annurev.immunol.22.012703.104549.
https://pubmed.ncbi.nlm.nih.gov/15032584/

These factors, together with new autoantigens released by necrotic cells, can activate pathogenic B and T cells, cause inflammatory responses and development of autoimmune disease in a susceptible individual[477] .

(d) They recruit B-1a cells to the developing brain:

cells in the peritoneal and pleural cavities express CXCL13, and the binding of this chemokine to its receptor (CXCR5) is a crucial step in the induction of IgM-NAA production[478] . CXCL13 is secreted from the choroid plexus and recruits B-1a cells to the meningeal space that subsequently lead to proliferation, myelination, and production in the brain of IgM-NAA by oligodendrocyte progenitor cells (OPCs)[479] .



https://pubmed.ncbi.nlm.nih.gov/30539466/

Physiological role of natural IgM autoantibodies in the body: natural IgM autoantibodies can act as the first line of defense against invading microbes by neutralizing the pathogen and activating complement pathways. Natural IgM can also shape the subsequent immune response to a pathogen by influencing T-lymphocyte polarization and B-lymphocyte class switching. They recognize apoptotic cell membranes, cell debris and decorate them with the complement system and mannose-binding lectin (MBL) to promote clearance by phagocytes such as dendritic cells (DCs) and macrophages. In addition, natural IgM secreted by B-1a cells in the CNS leads to proliferation and myelination of oligodendrocyte progenitor cells

Therapeutic use of preparations a. based on polyclonal pentameric IgM

As discussed above, therapeutic preparations of polyclonal IgG, administered as immunoglobulins for intravenously (IVIG) or subcutaneously (SCIG), are purified from the plasma of thousands of healthy donors. Over

[477] Kaveri SV, Silverman GJ, Bayry J.
Natural IgM in immune equilibrium and harnessing their therapeutic potential.
J Immunol. 2012 Feb 1;188(3):939-45. doi: 10.4049/jimmunol.1102107.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3266110/

[478] Ansel KM, Harris RB, Cyster JG.
CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity.
Immunity. 2002 Jan;16(1):67-76. doi: 10.1016/s1074-7613(01)00257-6.
https://doi.org/10.1016/S1074-7613(01)00257-6

[479] Tanabe S, Yamashita T.
B-1a lymphocytes promote oligodendrogenesis during brain development.
Nat Neurosci. 2018 Apr;21(4):506-516. doi: 10.1038/s41593-018-0106-4.
https://pubmed.ncbi.nlm.nih.gov/29507409/

to humoral immunodeficiency replacement therapy, IVIG/SCIGs are increasingly being used to treat a broad spectrum of inflammatory and autoimmune diseases with heterogeneous pathogenesis[480] .
After the introduction of replacement therapy for primary immunodeficiencies in 1952[481] , the demonstration of the potent effects of IVIGs on immune thrombocytopenia purpura (ITP)[482] opened the door to broad clinical applications of IVIGs as an immunomodulatory and anti-inflammatory drug.
Since 1981, IVIG has been registered for the treatment of Kawasaki disease[483] , Guillain- Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy (CIDP)[484] and multifocal motor neuropathy (MMN),[485] in addition to many other off-label indications[486] .
Regarding the route of administration, in general the subcutaneous route[487] has been shown to be convenient, as effective and ultimately safer than IVIG[488] .

---

[480] Kazatchkine MD, Kaveri SV.
Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin.
N Engl J Med. 2001 Sep 6;345(10):747-55. doi: 10.1056/NEJMra993360.
https://pubmed.ncbi.nlm.nih.gov/11547745/

Kerr J, Quinti I, Eibl M, Chapel H, Späth PJ, Sewell WA, Salama A, van Schaik IN, Kuijpers TW, Peter HH.
Is dosing of therapeutic immunoglobulins optimal? A review of a three-decade long debate in europe.
Front Immunol. 2014 Dec 12;5:629. doi: 10.3389/fimmu.2014.00629.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4263903/

[481] Bruton OC.
Agammaglobulinemia.
Pediatrics. 1952 Jun;9(6):722-8.
https://pubmed.ncbi.nlm.nih.gov/14929630/

Pashangzadeh S, Yazdani R, Nazari F, Azizi G, Abolhassani H, Aghamohammadi A.
Agammaglobulinemia: Epidemiology, Pathogenesis, Clinical Phenotype, Diagnosis, Prognosis and Management.
Endocr Metab Immune Disord Drug Targets. 2020;20(9):1434-1447. doi: 10.2174/1871530320666200508114349.
https://pubmed.ncbi.nlm.nih.gov/32384040/

[482] Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schöni M, Vest M, Wagner HP. High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood.
Lancet. 1981 Jun 6;1(8232):1228-31. doi: 10.1016/s0140-6736(81)92400-4.
https://pubmed.ncbi.nlm.nih.gov/6112565/

[483] Newburger JW, Takahashi M, Burns JC, Beiser AS, Chung KJ, Duffy CE, Glode MP, Mason WH, Reddy V, Sanders SP, et al.
The treatment of Kawasaki syndrome with intravenous gamma globulin.
N Engl J Med. 1986 Aug 7;315(6):341-7. doi: 10.1056/NEJM198608073150601.
https://pubmed.ncbi.nlm.nih.gov/2426590/

[484] Hughes RA, Donofrio P, Bril V, Dalakas MC, Deng C, Hanna K, Hartung HP, Latov N, Merkies IS, van Doorn PA; ICE Study Group.
Intravenous immune globulin (10% caprylate-chromatography purified) for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy (ICE study): a randomized placebo-controlled trial.
Lancet Neurol. 2008 Feb;7(2):136-44. doi: 10.1016/S1474-4422(07)70329-0. Erratum in: Lancet Neurol. 2008 Sep;7(9):771.
https://pubmed.ncbi.nlm.nih.gov/18178525/

[485] European Federation of Neurological Societies; Peripheral Nerve Society; van Schaik IN, et al.
European Federation of Neurological Societies/Peripheral Nerve Society guideline on management of multifocal motor neuropathy.
Eur J Neurol. 2006 Aug;13(8):802-8. doi: 10.1111/j.1468-1331.2006.01466.x.
https://pubmed.ncbi.nlm.nih.gov/16879289/

[486] Jolles S, Sewell WA, Misbah SA.
Clinical uses of intravenous immunoglobulin.
Clin Exp Immunol. 2005 Oct;142(1):1-11. doi: 10.1111/j.1365-2249.2005.02834.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1809480/

[487] Berger M, Cupps TR, Fauci AS.
Immunoglobulin replacement therapy by slow subcutaneous infusion.
Ann Intern Med. 1980 Jul;93(1):55-6. doi: 10.7326/0003-4819-93-1-55.
https://pubmed.ncbi.nlm.nih.gov/7396316/

[488] Haddad É, Barnes D, Kafal A.
Home therapy with subcutaneous immunoglobulins for patients with primary immunodeficiency diseases.
Transfus Apher Sci. 2012 Jun;46(3):315-21. doi: 10.1016/j.transci.2012.03.022.
https://pubmed.ncbi.nlm.nih.gov/22503304/

Ochs HD, Gupta S, Kiessling P, Nicolay U, Berger M;

The only hyperimmune preparations in clinical use not directed against pathogens are anti-Rhesus D (Rh(D)). These preparations prevent Rh(D) sensitization of Rh(D)-negative mothers by the fetus Rh(D)-positive, to avoid the risk of hemolytic disease of the newborn in subsequent pregnancies[489] .
Anti-Rh(D) has also found application for the treatment of PTI[490] .
The immunoglobulin preparations for human use that contain relevant amounts of isotypes other than sIgG such as IgA and/or IgM most widely used in clinical practice are Pentaglobin , Venimmun N and IgAbulin (Table 1)[491] .
Pentaglobin and Venimmun N are intravenous preparations that have clinical applications to date for supportive therapy of severe bacterial infections in combination with antibiotic therapy, immunoglobulin replacement therapy in immunosuppressed patients, and in patients with severe secondary antibody deficiency syndrome .
There is growing evidence to suggest that IgM in the natural antibody configuration may have anti-inflammatory, immunomodulatory, and tumor surveillance potential that could be relevant in a variety of clinical conditions. [492]

Subcutaneous IgG Study Group. Safety and efficacy of self-administered subcutaneous immunoglobulin in patients with primary immunodeficiency diseases.
J Clin Immunol. 2006 May;26(3):265-73. doi: 10.1007/s10875-006-9021-7.
https://pubmed.ncbi.nlm.nih.gov/16783465/

Samaan K, Levasseur MC, Decaluwe H, St-Cyr C, Chapdelaine H, Des Roches A, Haddad E.
SCIg vs. IVIg: let's give patients the choice!
J Clin Immunol. 2014 Aug;34(6):611-4. doi: 10.1007/s10875-014-0057-9.
https://pubmed.ncbi.nlm.nih.gov/24888599/

Wasserman RL.
Progress in gammaglobulin therapy for immunodeficiency: from subcutaneous to intravenous infusions and back again.
J Clin Immunol. 2012 Dec;32(6):1153-64. doi: 10.1007/s10875-012-9740-x.
https://pubmed.ncbi.nlm.nih.gov/22828788/

[489] MacKenzie IZ, Bichler J, Mason GC, Lunan CB, Stewart P, Al-Azzawi F, De Bono M, Watson N, Andresen I.
Efficacy and safety of a new, chromatographically purified rhesus (D) immunoglobulin.
Eur J Obstet Gynecol Reprod Biol. 2004 Dec 1;117(2):154-61. doi: 10.1016/j.ejogrb.2004.03.009.
https://pubmed.ncbi.nlm.nih.gov/15541850/

Pollack W, Gorman JG, Freda VJ, Ascari WQ, Allen AE, Baker WJ.
Results of clinical trials of RhoGAM in women.
Transfusion. 1968 May-Jun;8(3):151-3. doi: 10.1111/j.1537-2995.1968.tb04895.x.
https://pubmed.ncbi.nlm.nih.gov/4173363/

[490] Despotovic JM, Lambert MP, Herman JH, Gernsheimer TB, McCrae KR, Tarantino MD, Bussel JB.
RhIG for the treatment of immune thrombocytopenia: consensus and controversy (CME).
Transfusion. 2012 May;52(5):1126-36; quiz 1125. doi: 10.1111/j.1537-2995.2011.03384.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3427798/

[491] Späth PJ, Schneider C, von Gunten S.
Clinical Use and Therapeutic Potential of IVIG/SCIG, Plasma-Derived IgA or IgM, and Other Alternative Immunoglobulin Preparations.
Arch Immunol Ther Exp (Warsz). 2017 Jun;65(3):215-231. doi: 10.1007/s00005-016-0422-x..
https://link.springer.com/article/10.1007/s00005-016-0422-x

[492] Devarapu SK, Mamidi S, Plöger F, Dill O, Blixt O, Kirschfink M, Schwartz-Albiez R.
Cytotoxic activity against human neuroblastoma and melanoma cells mediated by IgM antibodies derived from peripheral blood of healthy donors.
Int J Cancer. 2016 Jun 15;138(12):2963-73. doi: 10.1002/ijc.30025.
https://doi.org/10.1002/ijc.30025

Díaz-Zaragoza M, Hernández-Ávila R, Viedma-Rodríguez R, Arenas-Aranda D, Ostoa-Saloma P.
Natural and adaptive IgM antibodies in the recognition of tumor-associated antigens of breast cancer (Review).
Oncol Rep. 2015 Sep;34(3):1106-14. doi: 10.3892/or.2015.4095.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4530904/

Gleissner CA, et al
Low levels of natural IgM antibodies against phosphorylcholine are independently associated with vascular remodeling in patients with coronary artery disease.
Clin Res Cardiol. 2015 Jan;104(1):13-22. doi: 10.1007/s00392-014-0750-y.
https://pubmed.ncbi.nlm.nih.gov/25103819/

Therefore, it is widely accepted that immunoglobulin preparations containing polyclonal IgM may contain a high potential for immunomodulatory, anti-inflammatory, and homeostatic effects on the fabrics. [493]
In particular, it was recently shown that treatment of diabetic mice with IgM from healthy donors resulted in the reversal of disease[494] . In human studies, passive transfer of pooled IgM from 2,500 healthy donors containing 90% pure IgM (IVIgM) suppressed IgG autoantibody activities from patients with autoimmune diseases *in vitro.* In addition, IVIgM has been shown to have a promising therapeutic effect in ameliorating inflammatory diseases such as myasthenia gravis and multiple sclerosis in experimental models due to its remyelinating action. [495]

Grönwall C, Silverman GJ.
Natural IgM: beneficial autoantibodies for the control of inflammatory and autoimmune disease.
J Clin Immunol. 2014 Jul;34 Suppl 1(0 1):S12-21. doi: 10.1007/s10875-014-0025-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4354681/

Nguyen TT, Elsner RA, Baumgarth N.
Natural IgM prevents autoimmunity by enforcing B cell central tolerance induction.
J Immunol. 2015 Feb 15;194(4):1489-502. doi: 10.4049/jimmunol.1401880.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4324358/

Tsiantoulas D, et al
Circulating microparticles carry oxidation-specific epitopes and are recognized by natural IgM antibodies.
J Lipid Res. 2015 Feb;56(2):440-8. doi: 10.1194/jlr.P054569.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4306697/

Villar LM, et al
Lipid-specific immunoglobulin M bands in cerebrospinal fluid are associated with a reduced risk of developing progressive multifocal leukoencephalopathy during treatment with natalizumab.
Ann Neurol. 2015 Mar;77(3):447-57. doi: 10.1002/ana.24345.
https://pubmed.ncbi.nlm.nih.gov/25581547/

Wootla B, et al
Naturally Occurring Monoclonal Antibodies and Their Therapeutic Potential for Neurologic Diseases.
JAMA Neurol. 2015 Nov;72(11):1346-53. doi: 10.1001/jamaneurol.2015.2188.
https://pubmed.ncbi.nlm.nih.gov/26389734/

[493] Ehrenstein MR, Notley CA.
The importance of natural IgM: scavenger, protector and regulator.
Nat Rev Immunol. 2010 Nov;10(11):778-86. doi: 10.1038/nri2849.
https://pubmed.ncbi.nlm.nih.gov/20948548/

Späth PJ, Schneider C, von Gunten S.
Clinical Use and Therapeutic Potential of IVIG/SCIG, Plasma-Derived IgA or IgM, and Other Alternative Immunoglobulin Preparations.
Arch Immunol Ther Exp (Warsz). 2017 Jun;65(3):215-231. doi: 10.1007/s00005-016-0422-x.
https://link.springer.com/article/10.1007/s00005-016-0422-x

[494] Wilson CS, Hoopes EM, Falk AC, Moore DJ.
A human IgM enriched immunoglobulin preparation, Pentaglobin, reverses autoimmune diabetes without immune suppression in NOD mice.
Sci Rep. 2022 Jul 11;12(1):11731. doi: 10.1038/s41598-022-15676-8.
https://www.nature.com/articles/s41598-022-15676-8

[495] Nguyen TT, Baumgarth N.
Natural IgM and the Development of B Cell-Mediated Autoimmune Diseases.
Crit Rev Immunol. 2016;36(2):163-177. doi: 10.1615/CritRevImmunol.2016018175.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5292326/

Hurez V, Kazatchkine MD, Vassilev T, Ramanathan S, Pashov A, Basuyaux B, de Kozak Y, Bellon B, Kaveri SV.
Pooled normal human polyspecific IgM contains neutralizing anti-idiotypes to IgG autoantibodies of autoimmune patients and protects from experimental autoimmune disease.
Blood. 1997 Nov 15;90(10):4004-13.
https://doi.org/10.1182/blood.V90.10.4004

Rieben R, Roos A, Muizert Y, Tinguely C, Gerritsen AF, Daha MR.
M-enriched human intravenous immunoglobulin prevents complement activation in vitro and in vivo in a rat model of acute inflammation.
Blood. 1999 Feb 1;93(3):942-51.
https://doi.org/10.1182/blood.V93.3.942

Regarding the warnings for use, please refer to the drug data sheet, while the mechanism of reactions will be detailed in the chapter below on monoclonal antibodies.

MONOCLONAL ANTIBODIES

Production of monoclonal antibodies

Monoclonal antibodies are antibodies generated mainly from lymphocytes of lymphocytes, and because these cells cannot propagate in cell culture for a long period of time, several methods used to produce them in stable cell lines have been studied.[496]

If the source of these cells are murine lymphocytes, the obtained murine monoclonal antibodies are mainly used for immunodiagnostic applications. [497]

In the case of using human-derived cells, obtained particularly from convalescent patients, such as patients with COVID-19, the human monoclonal antibodies produced are considered therapeutic antibodies targeting SARS-CoV-2.[498]

The classical method for the production of mouse monoclonal antibodies is based on cell fusion of splenic cells from immunized mice (e.g., by immunization with SARS-CoV-2 proteins) and lines

---

Vassilev T, Yamamoto M, Aissaoui A, Bonnin E, Berrih-Aknin S, Kazatchkine MD, Kaveri SV.
Normal human immunoglobulin suppresses experimental myasthenia gravis in SCID mice.
Eur J Immunol. 1999 Aug;29(8):2436-42. doi: 10.1002/(SICI)1521-4141(199908)29:08<2436::AID-IMMU2436>3.0.CO;2-9.
https://doi.org/10.1002/(SICI)1521-4141(199908)29:08<2436::AID-IMMU2436>3.0.CO;2-9

Bieber AJ, Warrington A, Asakura K, Ciric B, Kaveri SV, Pease LR, Rodriguez M.
Human antibodies accelerate the rate of remyelination following lysolecithin-induced demyelination in mice.
Glia. 2002 Mar 1;37(3):241-9. doi: 10.1002/glia.10033.
https://pubmed.ncbi.nlm.nih.gov/11857682/

Warrington AE, Asakura K, Bieber AJ, Ciric B, Van Keulen V, Kaveri SV, Kyle RA, Pease LR, Rodriguez M.
Human monoclonal antibodies reactive to oligodendrocytes promote remyelination in a model of multiple sclerosis.
Proc Natl Acad Sci U S A. 2000 Jun 6;97(12):6820-5. doi: 10.1073/pnas.97.12.6820
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC18751/

[496] de Moraes, Jane et al.
"Alternative Methods to Animal Use for Monoclonal Antibody Generation and Production."
Monoclonal Antibodies, edited by Nima Rezaei, IntechOpen, 2020. 10.5772/intechopen.95485.
https://www.intechopen.com/chapters/74640

Tabll AA, Shahein YE, Omran MM, Elnakib MM, Ragheb AA, Amer KE.
A review of monoclonal antibodies in COVID-19: Role in immunotherapy, vaccine development and viral detection.
Hum Antibodies. 2021;29(3):179-191. doi: 10.3233/HAB-200441.
https://cdn.who.int/media/docs/default-source/biologicals/mabs-manufacture-guideline-draft-for-1st-public-comment.pdf

[497] Tabll A, Abbas AT, El-Kafrawy S, Wahid A.
Monoclonal antibodies: Principles and applications of immunodiagnosis and immunotherapy for hepatitis C virus.
World J Hepatol. 2015 Oct 8;7(22):2369-83. doi: 10.4254/wjh.v7.i22.2369.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4598607/

Abdel Malak CA, Abelhafez TH, Tabll AA, Mashaly MM, El Shenawy R, El-Abd YS, Shaker MH, El-Awady MK.
Neutralizing activity and safety of human monoclonal antibodies against hepatitis C virus.
Hum Antibodies. 2017;26(3):127-134. doi: 10.3233/HAB-170330.
https://pubmed.ncbi.nlm.nih.gov/29036810/

[498] Henderson JP.
What are protective antibody responses to pandemic SARS-CoV-2?
J Clin Invest. 2020 Dec 1;130(12):6232-6234. doi: 10.1172/JCI143466.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7685743/

Valdez-Cruz NA, et al.
Integrative overview of antibodies against SARS-CoV-2 and their possible applications in COVID-19 prophylaxis and treatment.
Microb Cell Fact. 2021 Apr 22;20(1):88. doi: 10.1186/s12934-021-01576-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8061467/

mouse myeloma cells, with fusion media such as polyethylene glycol (PEG), and subsequent cell subcloning (hybridoma technique). [499]
In contrast, the generation of potential therapeutic recombinant human mAbs includes the cloning of cDNAs coding for the variable regions of the human IgG1 heavy chain and the constant regions of the Ig kappa light chain into expression plasmids.
Both plasmids contain the interleukin-2 signal sequence to enable efficient secretion of the recombinant antibodies.
Recombinant human antibodies are then produced in HEK-293T cells after transfection with IgG1 heavy and light chain expression plasmid sets and purified by protein A affinity chromatography[500] .
Since the hybridoma technique was introduced in 1975 as an effective approach to have sufficient quantities of pure mAbs, therapeutic studies have been conducted to neutralize viral surface epitopes, as in SARS-CoV[501] and MERS[502] . These principles are now used to generate human monoclonal antibodies to neutralize SARS-CoV-2.

Mouse **hybridoma technology** is a multistep process that takes advantage of the host animal's natural ability to produce highly specific, high-affinity, fully functional mAbs.
It involves the development and optimization of the specific immunogenic antigen (Ag). After optimization, a host animal is immunized with the Ag along with the adjuvant for several weeks.
Sera from immunized animals are tested for their reactivity and specificity to the immunizing antigen, while animals with high titers of binding antibodies are further selected for splenocyte isolation. Spleen cells are fused with immortalized myeloma cells in the presence of fusogenic agents such as viruses, chemicals and electrical impulses.
The most common myeloma fusion cell lines are X63-Ag 8.6539 and Sp2/0-Ag 1410, with origin from BALB/c mice. The fused cells are then selected on hypoxanthine-aminopterin-thymidine (HAT) medium. Myeloma cells are sensitive to HAT medium, as they lack the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene required for nucleotide synthesis from *de novo* or salvage pathways, while nonfused B cells die as short-lived. In this process, only the hybrid (B-cell myeloma) survives, as it harbors the functional HGPRT gene from B cells.
However, hybrid cells retain dual properties, the property of secreting B-cell antibodies and the continuously growing property (immortality) from myeloma cells.

---

[499] Zhang C.
Hybridoma technology for the generation of monoclonal antibodies.
Methods Mol Biol. 2012;901:117-35. doi: 10.1007/978-1-61779-931-0_7.
https://pubmed.ncbi.nlm.nih.gov/22723097/

Muhsin A, Rangel R, Vien L, Bover L.
Monoclonal Antibodies Generation: Updates and Protocols on Hybridoma Technology. Methods Mol Biol. 2022;2435:73-93. doi: 10.1007/978-1-0716-2014-4_6
https://pubmed.ncbi.nlm.nih.gov/34993940/

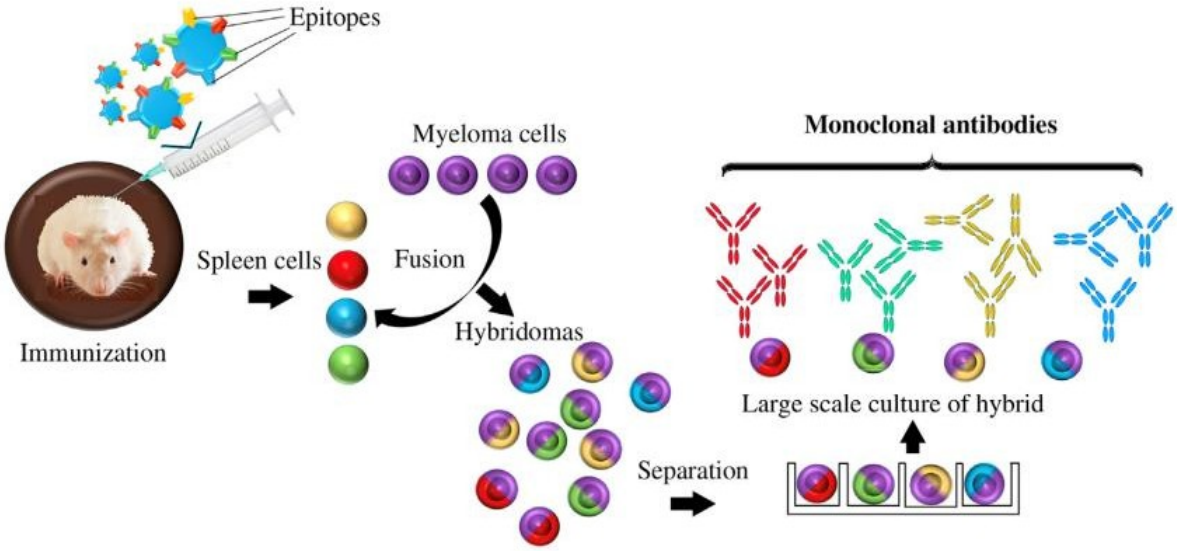
[500] Wang C, Li W, Drabek D, Okba NMA, van Haperen R, Osterhaus ADME, van Kuppeveld FJM, Haagmans BL, Grosveld F, Bosch BJ.
A human monoclonal antibody blocking SARS-CoV-2 infection.
Nat Commun. 2020 May 4;11(1):2251. doi: 10.1038/s41467-020-16256-y. Erratum in: Nat Commun. 2020 May 14;11(1):2511.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198537/

Monoclonal Antibody Production
https://www.moleculardevices.com/applications/monoclonal-antibody-production#gref

[501] Rockx B, Corti D, Donaldson E, Sheahan T, Stadler K, Lanzavecchia A, Baric R.
Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and zoonotic severe acute respiratory syndrome coronavirus challenge.
J Virol. 2008 Apr;82(7):3220-35. doi: 10.1128/JVI.02377-07.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2268459/

[502] Goo J, Jeong Y, Park YS, Yang E, Jung DI, Rho S, Park U, Sung H, Park PG, Choi JA, Seo SH, Cho NH, Lee H, Lee JM, Kim JO, Song M.
Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein.
Virus Res. 2020 Mar;278:197863. doi: 10.1016/j.virusres.2020.197863.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114870/

The fused or hybrid cells are then screened by the "limited dilution cloning" method or by a semi-solid selective medium to select only those hybridomas that produce antibodies of appropriate specificity. [503]



https://doi.org/10.1016/B978-0-12-804659-3.00016-6
Hybridoma technology used to produce mAb: generation of mAb by immunizing laboratory animals with any target antigen. Hybridoma cells generated by fusion between B cells from an immunized animal (usually a rat, mouse, rabbit, or monkey) and myeloma cells. The hybridoma cells are selected in the HAT media, and finally, cells secreting the desired antibodies are screened.

Potential approaches for the production of monoclonal antibodies against SARS-Cov-2 are:

1. Use of classical hybridoma technology to produce monoclonal antibodies against the specific surface epitopes of the SARS spike protein. This model was made by Wang et al.[504] to generate the human monoclonal antibody 47D11. The antibody showed its neutralizing ability against common epitopes of SARS-CoV and SARS-CoV-2 viruses in Vero cell culture.

2. The use of peripheral blood mononuclear cells (PBMCs), from convalescent patients with COVID-19, as a source of B-cell lymphocytes. From peripheral blood mononuclear cells (PMBCs), CD22 B cells^{+} are separated by Magnetic-activated cell sorting (MACS) and immortalized by EBV[505] .

---

[503] Parray HA, Shukla S, Samal S, Shrivastava T, Ahmed S, Sharma C, Kumar R. Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives.
Int Immunopharmacol. 2020 Aug;85:106639. doi: 10.1016/j.intimp.2020.106639.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7255167/

[504] Wang C, Li W, Drabek D, Okba NMA, van Haperen R, Osterhaus ADME, van Kuppeveld FJM, Haagmans BL, Grosveld F, Bosch BJ.
A human monoclonal antibody blocking SARS-CoV-2 infection.
Nat Commun. 2020 May 4;11(1):2251. doi: 10.1038/s41467-020-16256-y. Erratum in: Nat Commun. 2020 May 14;11(1):2511.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198537/

https://upbiotech.wordpress.com/2018/08/28/produzione-di-anticorpi-monoclonali/

[505] Fraussen J, Vrolix K, Martinez-Martinez P, Losen M, Meulemans E, De Baets MH, Stinissen P, Somers V.
A novel method for making human monoclonal antibodies.
J Autoimmun. 2010 Sep;35(2):130-4. doi: 10.1016/j.jaut.2010.05.001.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7126289/

Tabll A, El Abd Y, El Din NG, El Shenawy R, Abdelhafez TH, El Awady M, El-Mohamady H, Viazov S.
Establishment of human clones producing neutralizing human monoclonal antibodies to the envelope E1/E2 protein of hepatitis C virus by EBV Immortalization of immune CD22^{+} B cells.
Hum Antibodies. 2013;22(3-4):55-65. doi: 10.3233/HAB-140271.
https://pubmed.ncbi.nlm.nih.gov/24961863/

3. The use of PBMCs from COVID-19 convalescent patients, for the separation of CD27 B cells^{+} and subsequent cell fusion procedure with human cell lines. These approaches were employed by Puligedda et al.[506] for the production of mAb with potent neutralizing activity against poliovirus.

4. Using Phage display technology[507] (Tomlinson I library) to select the receptor binding domain (RBD) of SARS-CoV-2. In COVID-19 patients[508] , 3 types of antibodies (scFv, scFv- Fc and IgG1) were identified with high binding specificity for the RBD of the trimethyl spike glycoprotein.
Another Phage Display protocol uses a native VHH Llama library and a synthetic humanized VHH Llama library[509] . The approach is based on the generation of bi- or tri-specific antibodies that can target several antigens simultaneously to block the S/ACE2 interaction site. [495].

An alternative method to hybridoma is phage display or **phage display** libraries, a strategy that allows a peptide or polypeptide to be exhibited on the surface of a bacteriophage.
Usually M13, f1 or fd phage vectors that have an outer protein coating are selected.
All phages listed above are filamentous, nonlytic, and capable of incorporating single-stranded circular DNA.
Although the phage families that can be used are diverse, actually the one that is most widely used in phage display is the M13 family. The choice is dictated by essentially practical reasons. M13 is a phage that, unlike the others, can be easily purified and manipulated.
M13 must infect bacterial cells to replicate; however, it does not induce lysis of the bacterium but a lysogenic pathway that guides toward the production of phage particles. The phage genome also contains the information to encode proteins involved in replication, morphogenetic proteins, and finally structural proteins. Structural proteins are defined as those proteins that constitute the envelope of phage particles. These are divided into: major proteins **(**pVIII), present in copies of five on the surface of the Phage, and minor proteins (PIII **and** pVI) present in copies of 2800.
M13 is genetically modified in a way that exposes small peptides fused together with pIII and pVIII proteins on its surface. In this way, a large and diverse group of phage clones can be obtained, each expressing random sequences. At this point, the population of different bacteriophages,

---

Esmaeilzadeh A, Elahi R.
Immunobiology and immunotherapy of COVID-19: A clinically updated overview.
J Cell Physiol. 2021 Apr;236(4):2519-2543. doi: 10.1002/jcp.30076.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7675260/

[506] Puligedda RD, Vigdorovich V, Kouiavskaia D, Kattala CD, Zhao JY, Al-Saleem FH, Chumakov K, Sather DN, Dessain SK.
Human IgA Monoclonal Antibodies That Neutralize Poliovirus, Produced by Hybridomas and Recombinant Expression.
Antibodies (Basel). 2020 Feb 28;9(1):5. doi: 10.3390/antib9010005.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7148538/

[507] Gülay Büyükköroğlu, Behiye Şenel,
Chapter 16 - Engineering Monoclonal Antibodies: Production and Applications, Editor(s): Debmalya Barh, Vasco Azevedo,
Omics Technologies and Bio-Engineering, Academic Press, 2018, Pages 353-389,ISBN 9780128046593,
https://doi.org/10.1016/B978-0-12-804659-3.00016-6.
https://www.sciencedirect.com/science/article/pii/B9780128046593000166

Anand T, Virmani N, Bera BC, Vaid RK, Vashisth M, Bardajatya P, Kumar A, Tripathi BN.
Phage Display Technique as a Tool for Diagnosis and Antibody Selection for Coronaviruses.
Curr Microbiol. 2021 Apr;78(4):1124-1134. doi: 10.1007/s00284-021-02398-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7941128/

[508] Parray HA, et al
Identification of an anti-SARS-CoV-2 receptor-binding domain-directed human monoclonal antibody from a naïve semisynthetic library.
J Biol Chem. 2020 Sep 4;295(36):12814-12821. doi: 10.1074/jbc.AC120.014918.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7476711/

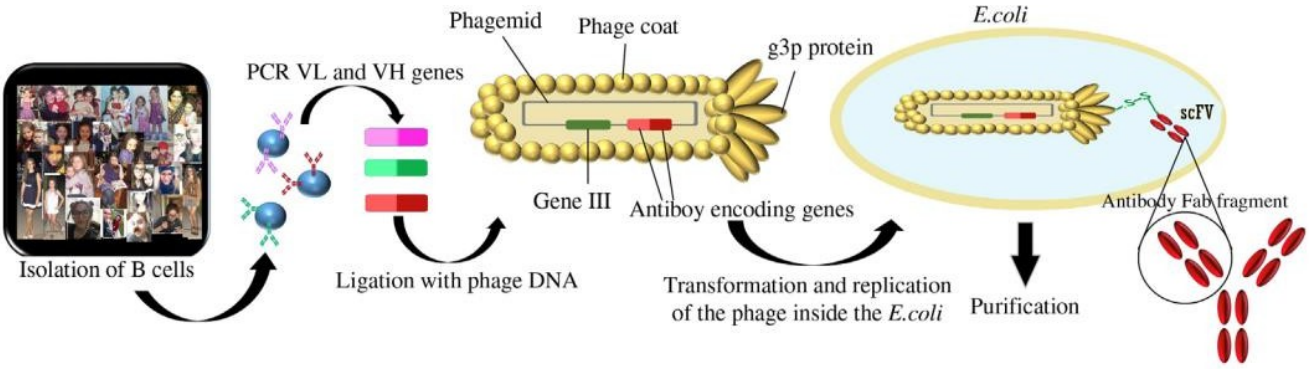
[509] Dong J, Huang B, Jia Z, Wang B, Gallolu Kankanamalage S, Titong A, Liu Y.
Development of multi-specific humanized llama antibodies blocking SARS-CoV-2/ACE2 interaction with high affinity and avidity.
Emerg Microbes Infect. 2020 Dec;9(1):1034-1036. doi: 10.1080/22221751.2020.1768806.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8284970/

each with a distinct phenotype is exposed to the specific molecular target. Only those bacteriophages with high affinity for the target molecules remain bound to them (after the different washing steps) and can be amplified using *E. coli*.

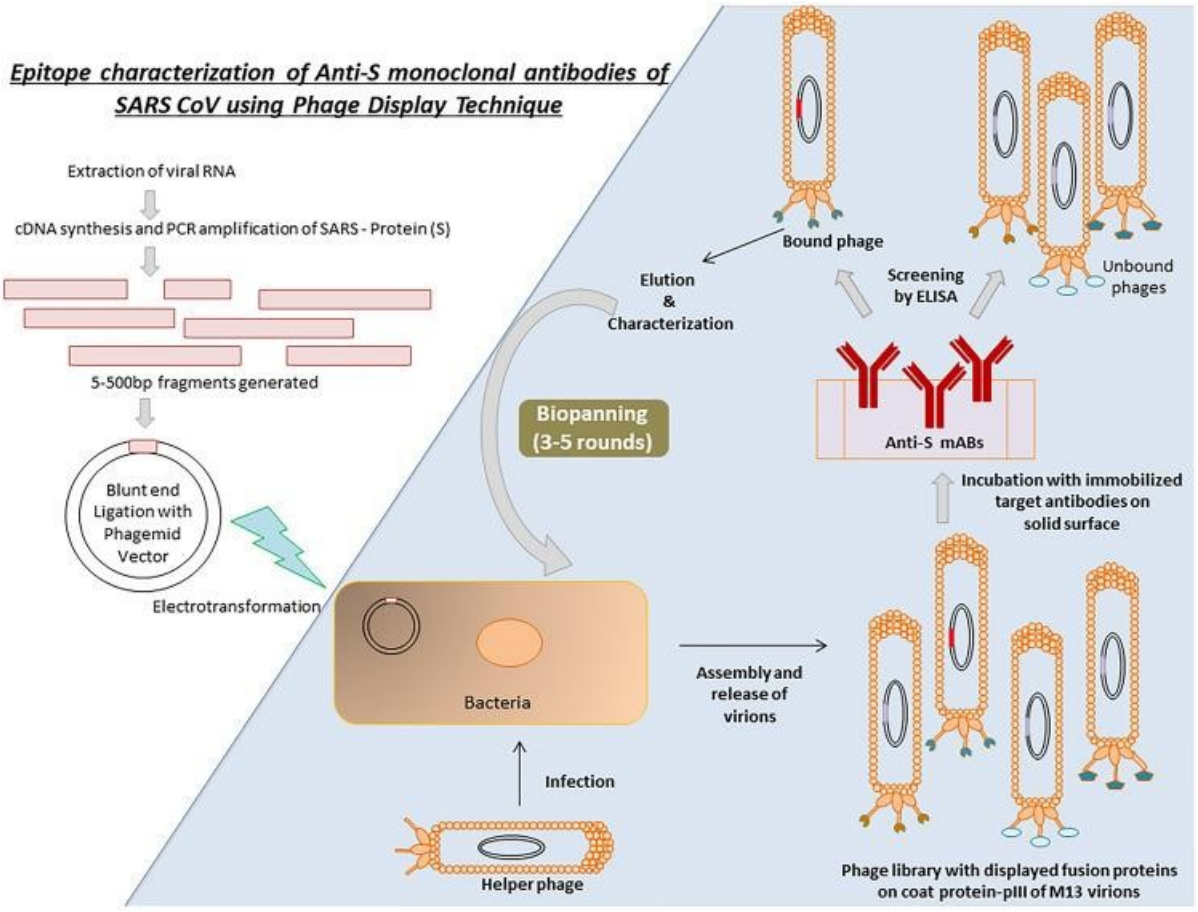
The clones thus amplified are sequenced to trace the amino acid sequence of the peptide exposed on the surface of the Phage, and to confirm the specificity of the peptide to the target, an ELISA assay is performed.

The potentials of phage display are mainly two: 1) It allows a direct connection to be made between the phenotype (the peptide exposed on the phage surface) and the genotype (the DNA sequence coding for the peptide) within the same viral particle, and 2) The production of a large and diverse library of peptides that is exposed on the phage surface.

The first antibody produced by exploiting this technique is adalimumab used to treat rheumatoid arthritis and other inflammatory diseases. The importance of this technique has been unequivocally recognized by the scientific community, so much so that the Nobel Prize in Chemistry in 2018 was awarded to the two pioneers in the development of this technique-Smith and Winter. [510]



https://doi.org/10.1016/B978-0-12-804659-3.00016-6



[510] https://upbiotech.wordpress.com/2019/05/15/phage-display/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7941128/
An illustration of the phage display technique for characterizing monoclonal antibody epitopes that bind to the spike protein of MERS-CoV using the phage display technique. The whole RNA can be extracted and the cDNA amplified. The gene encoding for the spike protein can be amplified by PCR. Gene segments can be obtained by fragmentation using restriction enzymes and segments cloned into the phagemid vector. Transformation of phagemids into E. coli and infection with the helper phage can result in the production of phages that exhibit proteins encoded by the cloned genomic segment. The phage library thus formed can be screened using Anti-S- mAB by ELISA, and phage-specific binding can be selected by repeated cycles of biopanning (elution, amplification, binding and washing). DNA sequencing of the bound phages leads to mapping of the epitopes

CHO cells as producers of anti-SARS-CoV-2 mAb

The tetrameric nature of an IgG molecule and its glycosylation are essential for its function, making it a difficult protein to express. In this sense, mammalian cells, such as CHO (Chinese Hamster Ovary) cells, have become one of the most widely used cell factories for the industrial production of mAb[511] .
However, compared with bacteria and yeast, the yields and productivity of mammalian cell-based processes are low due to slow cell growth, their tendency to undergo apoptosis, and low throughput per cell.[512]
For this reason, the development of cells with superior production characteristics has been an important goal of the field, and thanks to cell engineering, the time to create productive cell lines of fully humanized mAbs has been dramatically reduced to a few months, with increased productivity (up to 100 pg/cell per day, representing bioreactor titers of nearly 10 g/L), which is currently critical for the production of anti-SARS-CoV-2 mAbs. [513]

---

[511] Walsh G.
Biopharmaceutical benchmarks 2018.
Nat Biotechnol. 2018 Dec 6;36(12):1136-1145. doi: 10.1038/nbt.4305
https://pubmed.ncbi.nlm.nih.gov/30520869/

Jayapal, Karthik P., Katie F Wlaschin, W. -S. Hu and Miranda Gek Sim Yap.
"Recombinant protein therapeutics from CHO cells : 20 years and counting." Chemical Engineering Progress 103 (2007): 40-47.
https://www.aiche.org/sites/default/files/docs/pages/CHO.pdf

Sharker SM, Rahman A.
A Review on the Current Methods of Chinese Hamster Ovary (CHO) Cells Cultivation for the Production of Therapeutic Protein.
Curr Drug Discov Technol. 2021;18(3):354-364. doi: 10.2174/1570163817666200312102137
https://pubmed.ncbi.nlm.nih.gov/32164511/

[512] Al-Rubeai M.
Antibody expression and production. Cell engineering, vol. 7; 2007.
https://doi.org/10.1007/978-94-007-1257-7
https://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=75D6B4B5FC03D7B718EDD9A5011C2505?doi=10.1.1.472.1352&rep=rep1&type=pdf

Bandaranayake AD, Almo SC.
Recent advances in mammalian protein production.
FEBS Lett. 2014 Jan 21;588(2):253-60. doi: 10.1016/j.febslet.2013.11.035.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3924552/

[513] Kuo CC, Chiang AW, Shamie I, Samoudi M, Gutierrez JM, Lewis NE.
The emerging role of systems biology for engineering protein production in CHO cells.
Curr Opin Biotechnol. 2018 Jun;51:64-69. doi: 10.1016/j.copbio.2017.11.015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5988649/

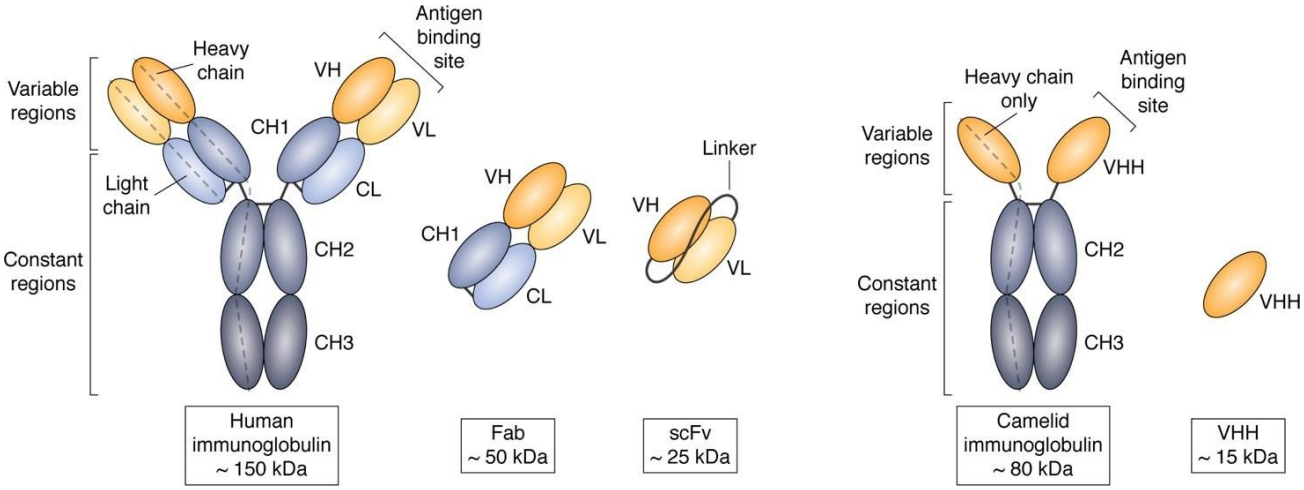
Sarsaiya S, Shi J, Chen J.
Bioengineering tools for the production of pharmaceuticals: current perspective and future outlook.
Bioengineered. 2019 Dec;10(1):469-492. doi: 10.1080/21655979.2019.1682108.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6844412/

Pérez-Rodriguez S, Ramírez-Lira MJ, Trujillo-Roldán MA, Valdez-Cruz NA.
Nutrient supplementation strategy improves cell concentration and longevity, monoclonal antibody production and lactate metabolism of Chinese hamster ovary cells.
Bioengineered. 2020 Dec;11(1):463-471. doi: 10.1080/21655979.2020.1744266.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7161567/

Golabgir A, Gutierrez JM, Hefzi H, Li S, Palsson BO, Herwig C, Lewis NE.
Quantitative feature extraction from the Chinese hamster ovary bioprocess bibliome using a novel meta-analysis workflow.

In the search for tools to cope with the COVID-19 pandemic, the production of Nbs (nanobodies) has become an alternative, as Nbs are smaller, non-glycosylated proteins, can be produced in cell factories such as bacteria or yeast, at lower cost, with a larger production scale [514].

A **single-domain antibody** (**sdAb**), also known as a **nanobody (nanobody)**, is an antibody fragment consisting of a single variable monomeric antibody domain. Like a whole antibody, it is capable of selectively binding to a specific antigen. With a molecular weight of only 12-15 kDa, single-domain antibodies are much smaller than common antibodies (150-160 kDa), which are composed of two heavy and two light protein chains, and also smaller than Fab fragments (~50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (~25 kDa, two variable domains, one from a light chain and one from a heavy chain). [515]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7650250/
Structures of human and camelid Igs and fragments. Conventional human Igs (i.e., IgG) have been truncated to provide functional fragments (Fab and scFv) that contain variable regions from the light and heavy chains. In the case of scFv, a linker is required to facilitate appropriate matching of heavy- and light-chain variable regions. A subset of camelid antibodies consists only of the heavy chains. Expression of the variable region isolated from the heavy chain-only antibodies provides functional single-domain antibodies (VHH/nanocorps).

Moreover, monomeric or multimeric VHHs can be produced without involving significant changes in the operations of the bioprocessing unit. However, since these are new molecules with structural features

Biotechnol Adv. 2016 Sep-Oct;34(5):621-633. doi: 10.1016/j.biotechadv.2016.02.011.
https://pubmed.ncbi.nlm.nih.gov/26948029/

Kelley B.
Industrialization of mAb production technology: the bioprocessing industry at a crossroads.
MAbs. 2009 Sep-Oct;1(5):443-52. doi: 10.4161/mabs.1.5.9448.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2759494/

Welch JT, Arden NS.
Considering "clonality": A regulatory perspective on the importance of the clonal derivation of mammalian cell banks in biopharmaceutical development.
Biologicals. 2019 Nov;62:16-21. doi: 10.1016/j.biologicals.2019.09.006.
https://pubmed.ncbi.nlm.nih.gov/31588011/

[514] Wu Y, Jiang S, Ying T.
Single-Domain Antibodies As Therapeutics against Human Viral Diseases.
Front Immunol. 2017 Dec 13;8:1802. doi: 10.3389/fimmu.2017.01802.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5733491/

[515] Cheloha RW, Harmand TJ, Wijne C, Schwartz TU, Ploegh HL.
Exploring cellular biochemistry with nanobodies.
J Biol Chem. 2020 Nov 6;295(45):15307-15327. doi: 10.1074/jbc.REV120.012960.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7650250/

complex and novel, testing for quality, safety, and efficacy must be very rigorous, and regulatory approval could be longer and more intense than for a full mAb.[516]
CHO cells were created and considered "immortal" in the late 1950s[517] and have several advantages over other cell types for mAb production:

(a) the ability to perform complex post-translational modifications (PTMs), such as "human-like" glycosylations, protein processing (e.g., phosphorylation) and folding,

(b) robust cell culture in chemically defined serum-free media that facilitates scaling-up,

> ***Scaling-up**: Increased production of a new drug to meet the needs of the later stages of clinical trials. It is a quantitative scale-up that occurs just prior to actual industrial-scale production for market launch.* [518]

(c) a safe host with a high rate of regulatory approval and

(d) Optimized transfection/selection systems that enable stable expression of heterologous genes.[519]

Mechanism of action and safety of monoclonal antibodies

Monoclonal antibodies act through several mechanisms[520] .

- When the Fab part of an antibody binds to the antigen, it blocks its interaction with a ligand, whereas signaling occurs when binding of the antibody to a receptor provides an agonist signal. These functions may be independent of the Fc part of the molecule (although interactions of the Fc part with other molecules may enhance these mechanisms).
  In addition, the antibody can exert actions through its Fc region: these include antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cell phagocytosis.
- Constant heavy chain domain regions ($_{CH2}$ and $_{CH3}$) of Fc on immunoglobulin G (IgG) interact with the neonatal Fc receptor (FcR) to influence IgG transport across cellular barriers and regulate circulating levels of the antibody, prolonging its half-life[521] . The recruitment of these

[516] Steeland S, Vandenbroucke RE, Libert C.
Nanobodies as therapeutics: big opportunities for small antibodies.
Drug Discov Today. 2016 Jul;21(7):1076-113. doi: 10.1016/j.drudis.2016.04.003.
https://pubmed.ncbi.nlm.nih.gov/27080147/

[517] Wurm, Florian M., and Maria João Wurm. 2017.
"Cloning of CHO Cells, Productivity and Genetic Stability-A Discussion,"
Processes 5, no. 2: 20. https://doi.org/10.3390/pr5020020
https://www.mdpi.com/2227-9717/5/2/20/htm

[518] https://www.molecularlab.it/principi/dizionario/definizione.asp?w=Scale-up

From laboratory chemistry to industrial plant. the science of scale-up
Ferruccio Trifirò
http://www.chim.it/sites/default/files/chimind/pdf/2018_2_4476_on.pdf

[519] Mead EJ, Chiverton LM, Smales CM, von der Haar T.
Identification of the limitations on recombinant gene expression in CHO cell lines with varying luciferase production rates.
Biotechnol Bioeng. 2009 Apr 15;102(6):1593-602. doi: 10.1002/bit.22201.
https://pubmed.ncbi.nlm.nih.gov/19090535/

[520] Lutterotti A, Martin R.
Getting specific: monoclonal antibodies in multiple sclerosis.
Lancet Neurol. 2008 Jun;7(6):538-47. doi: 10.1016/S1474-4422(08)70110-8.
https://pubmed.ncbi.nlm.nih.gov/18485317/

[521] Yeung YA, Leabman MK, Marvin JS, Qiu J, Adams CW, Lien S, Starovasnik MA, Lowman HB.
Engineering human IgG1 affinity to human neonatal Fc receptor: impact of affinity improvement on pharmacokinetics in primates.
J Immunol. 2009 Jun 15;182(12):7663-71. doi: 10.4049/jimmunol.0804182. https://www.jimmunol.org/content/182/12/7663.long

effectors depends on the isotype of the antibody and its ability to recruit complement or effector cells.

- IgG1 is the most commonly used subclass of Ig to trigger cell death, and in cases where cytotoxicity is not desired, IgG4, whose Fc region is poorly able to induce antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity, is more commonly used.
- It is also possible to modify the Fc region (e.g., by removing carbohydrates) to further reduce complement or effector cell recruitment. Omalizumab (Xolair; Genentech, Novartis) is a humanized IgE-specific mAb for severe allergic asthma, developed to target free IgE and membrane-bound IgE, but not to target IgE bound to the FcRs of IgE on mast cells and thus not to trigger mast cell degranulation.[522]
- In the development of therapeutic mAbs, the choice of IgG subclass is important, especially in oncology. In this case, IgG1 has the highest potential for antibody-dependent cell-mediated cytotoxicity and is therefore ideal for eliminating tumor cells.
  In contrast, IgG3 is rarely used for therapeutic mAbs because the long hinge region is prone to proteolysis and causes a shorter half-life[523] .
- Glycosylation of the Fc portion of mAb IgG is essential to activate certain effector functions, and cell engineering can be used to generate selected glycoforms of antibodies[524] .
- Interestingly, IgG4 may have the potential to activate inflammatory reactions through FcRs[525] and that IgG4 may exhibit dynamic dissociation and Fab arm exchange[526] .

https://www.nature.com/articles/nrd3003
(a) Schematic structure of a monoclonal immunoglobulin G (IgG) antibody (mAb). There has been a progressive development from murine mAbs, to chimeric mAbs (with variable murine regions (V) grafted onto constant human regions (C)), to humanized (consisting of a human Ig scaffold with only the complementarity determinant regions (CDRs) being of murine origin), to the recent generation fully human mAbs. CDRs within the Fab region of a mAb bind to specific targets and cause antagonism or signaling. The Fc region of a mAb is composed of the hinge and constant heavy chain domains ($_{CH2}$ and $_{CH3}$) and has other functions, such as complement fixation or binding to Fc receptors. The nomenclature of mAbs reflects the type of mAb; for example, 'xi' in rituximab indicates that it is a chimeric mAb. **b)** The functions of mAbs, w h i c h include antagonism and signaling, are controlled by specific CDRs within the Fab region. Some mAbs can bind specifically to a ligand, e.g. infliximab and omalizumab, or to a receptor, e.g. natalizumab and daclizumab, and thus prevent stimulation. In contrast, other mAbs can specifically induce signal transduction by binding to a receptor. TGN1412 is a CD28 superagonist (CD28SA), which means that binding to the T lymphocyte receptor is not required for T lymphocyte activation. The functions of mAbs controlled by the Fc region include complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (not shown). Some mAbs can lyse cells (e.g., T cells or B cells) through complement activation, while other mAbs can bind to Fc receptors and mediate cell lysis. Neonatal Fc receptor binding controls IgG transport across cell barriers and influences the half-life of a mAb. CL, constant light region; VH, variable heavy region; VL, variable light region

---

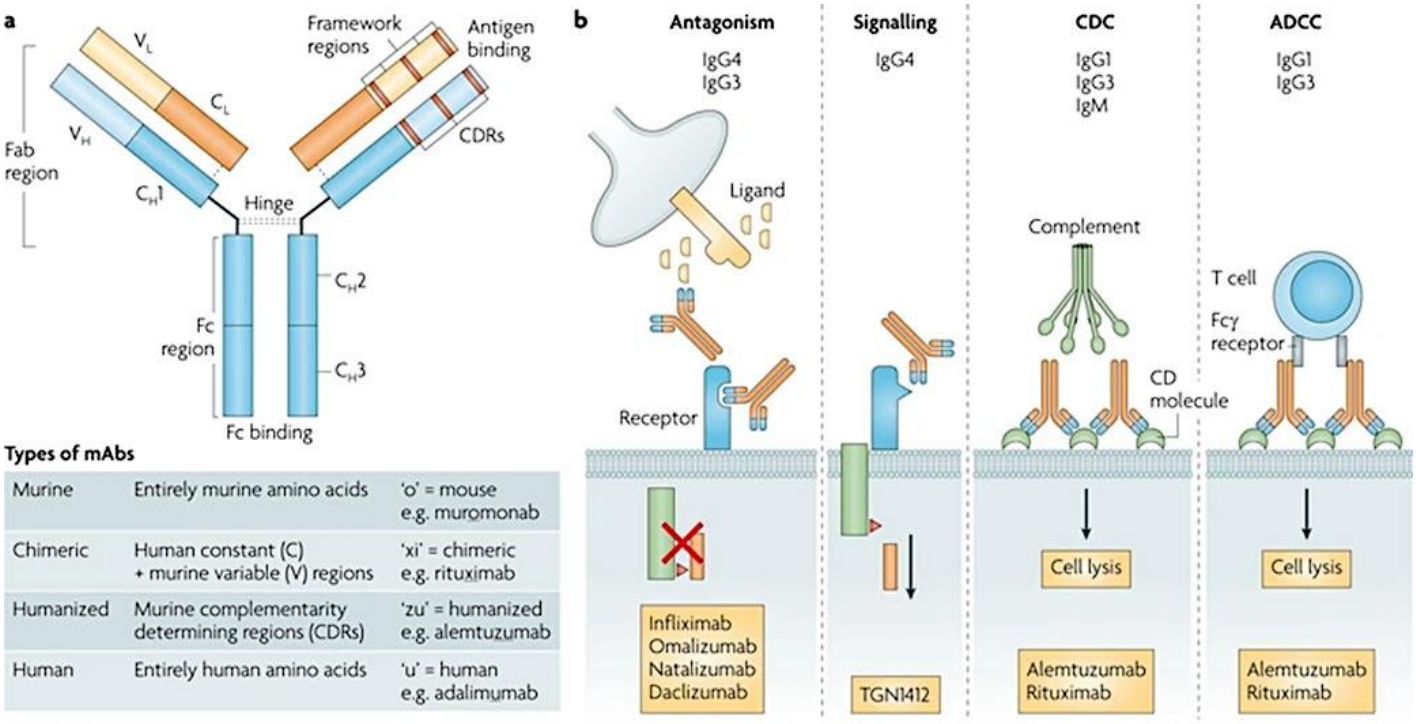
[522] Chang TW.
Developing antibodies for targeting immunoglobulin and membrane-bound immunoglobulin E.
Allergy Asthma Proc. 2006 Mar-Apr;27(2 Suppl 1):S7-14.
https://pubmed.ncbi.nlm.nih.gov/16722326/

[523] Hassan MS, Abedi-Valugerdi M, Lefranc G, Hammarström L, Smith CI.
Biological half-life of normal and truncated human IgG3 in scid mice.
Eur J Immunol. 1991 May;21(5):1319-22. doi: 10.1002/eji.1830210534.
https://pubmed.ncbi.nlm.nih.gov/2037016/

[524] Jefferis R.
Recombinant antibody therapeutics: the impact of glycosylation on mechanisms of action.
Trends Pharmacol Sci. 2009 Jul;30(7):356-62. doi: 10.1016/j.tips.2009.04.007.
https://pubmed.ncbi.nlm.nih.gov/19552968/

[525] Holland M, Hewins P, Goodall M, Adu D, Jefferis R, Savage CO.
Anti-neutrophil cytoplasm antibody IgG subclasses in Wegener's granulomatosis: a possible pathogenic role for the IgG4 subclass.
Clin Exp Immunol. 2004 Oct;138(1):183-92. doi: 10.1111/j.1365-2249.2004.02566.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1809192/

[526] van der Neut Kolfschoten M, et al.
Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange.
Science. 2007 Sep 14;317(5844):1554-7. doi: 10.1126/science.1144603.
https://pubmed.ncbi.nlm.nih.gov/17872445/



Types of mAbs

Murine	Entirely murine amino acids	'o' = mouse e.g. muromonab
Chimeric	Human constant (C) + murine variable (V) regions	'xi' = chimeric e.g. rituximab
Humanized	Murine complementarity determining regions (CDRs)	'zu' = humanized e.g. alemtuzumab
Human	Entirely human amino acids	'u' = human e.g. adalimumab

The adverse effects encountered with approved mAb therapy will be discussed below (Table below), as well as examples of side effects encountered during exploratory clinical trials with mAbs. [527] Of particular concern is the fact that some of the serious adverse effects of biologics found recently were not predicted by currently available preclinical screening tools[528] and animal models[529] .

---

[527] Tabrizi MA, Roskos LK.
Preclinical and clinical safety of monoclonal antibodies.
Drug Discov Today. 2007 Jul;12(13-14):540-7. doi: 10.1016/j.drudis.2007.05.010.
https://pubmed.ncbi.nlm.nih.gov/17631248/

Descotes J.
Immunotoxicity of monoclonal antibodies.
MAbs. 2009 Mar-Apr;1(2):104-11. doi: 10.4161/mabs.1.2.7909.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2725414/

Brennan FR, Kiessling A.
Translational immunotoxicology of immunomodulatory monoclonal antibodies.
Drug Discov Today Technol. 2016 Sep-Dec;21-22:85-93. doi: 10.1016/j.ddtec.2016.08.002.
https://pubmed.ncbi.nlm.nih.gov/27978992/

Brennan FR, Morton LD, Spindeldreher S, et al.
Safety and immunotoxicity assessment of immunomodulatory monoclonal antibodies.
MAbs. 2010;2(3):233-255. doi:10.4161/mabs.2.3.11782
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2881251/pdf/mabs0203_0233.pdf

[528] Cavagnaro, J. A. (ed.) Preclinical Safety Evaluation of Biopharmaceuticals: A Science Based Approach to Facilitating Clinical Trials (Wiley, London, 2008).

Longstaff C, Whitton CM, Stebbings R, Gray E.
How do we assure the quality of biological medicines?
Drug Discov Today. 2009 Jan;14(1-2):50-5. doi: 10.1016/j.drudis.2008.09.010.
https://pubmed.ncbi.nlm.nih.gov/18951998/

[529] Loisel S, Ohresser M, Pallardy M, Daydé D, Berthou C, Cartron G, Watier H.
Relevance, advantages and limitations of animal models used in the development of monoclonal antibodies for cancer treatment. Crit Rev Oncol Hematol. 2007 Apr;62(1):34-42. doi: 10.1016/j.critrevonc.2006.11.010.
https://pubmed.ncbi.nlm.nih.gov/17197192/

Chapman K, Pullen N, Graham M, Ragan I.
Preclinical safety testing of monoclonal antibodies: the significance of species relevance.

Target	mAb	Type	FDA approval	Indications*	Selected side effects
Platelet glycoprotein IIb/IIIa	Abciximab (ReoPro; Centocor Ortho Biotech, Eli Lilly)	Chimeric antibody fragment: c7E3 Fab	1994	• Prevention of ischaemic cardiac complications of percutaneous coronary interventions and unstable angina	• Hypersensitivity and immunogenicity • Increased risk of bleeding • Thrombocytopaenia
Tumour necrosis factor-α	Adalimumab (Humira; Abbott)	Fully human	2002	• Rheumatoid arthritis • Ankylosing spondylitis • Psoriasis • Psoriatic arthritis • Crohn's disease • Ulcerative colitis	• Infusion reactions and immunogenicity • Hypersensitivity reactions • Immunosuppression and infections (tuberculosis) • Anaemia, leukopaenia and thrombocytopaenia • Worsening heart failure • Malignancy, lymphoma and lympho-proliferative disorders • Elevated liver transaminases • Increased nuclear-specific antibodies
	Certolizumab (Cimzia; UCB)	Humanized pegylated	2008		
	Infliximab (Remicade; Centocor Ortho Biotech)	Chimeric	1998		
CD52 on mature B, T and natural killer cells	Alemtuzumab (Campath; Genzyme)	Humanized	2001	• B cell chronic lymphocytic leukaemia • Graft-versus-host disease • Multiple myeloma • Multiple sclerosis • Vasculitis • Behçet's disease	• Infusion reactions • Hypersensitivity and immunogenicity • CRS • Tumour lysis syndrome • Immunosuppression and opportunistic infections • Cytopaenias: pancytopaenia, lymphopaenia and thrombocytopaenia • Autoimmune haemolytic anaemia • Thyroid disorders • Cardiotoxicity
Interleukin-2 receptor-α on activated lymphocytes	Basiliximab (Simulect; Novartis)	Chimeric	1998	• Prophylaxis of renal transplant allograft rejection	• Severe acute hypersensitivity reactions • CRS and immunogenicity • Immunosuppression and infections • Local skin reactions • Warnings when combined with other immunosuppressives
	Daclizumab (Zenapax; Roche)	Humanized	1997 Discontinued in Europe		
Vascular endothelial growth factor	Bevacizumab (Avastin; Genentech)	Humanized	2004	• Metastatic colorectal cancer • Non-small-cell lung carcinoma • Metastatic breast carcinoma • Metastatic renal carcinoma	• Infusion reactions and immunogenicity • Local complications at tumour site • Arterial and venous thromboembolic events • Haemorrhage • Severe hypertension • Cardiac failure • Reversible posterior leukoencephalopathy syndrome • Slower wound healing and GI perforation
	Ranibizumab (Lucentis; Genentech, Novartis)	Humanized (Fab fragment from bevacizumab)	2006	• Injected intravitreally for neovascular (wet) age-related macular degeneration	• Conjunctival haemorrhage • Intraocular inflammation • Increased intraocular pressure • Retinal detachment • Endophthalmitis
Complement C5	Eculizumab (Soliris; Alexion)	Humanized	2007	• Paroxysmal nocturnal haemoglobinuria	• Meningococcal and *Neisseria* infection • Intravascular haemolysis
CD11a	Efalizumab (Raptiva; Genentech)	Humanized	2003 Recently discontinued	• No longer licensed for chronic plaque psoriasis	• First-dose reaction complex • Immunosuppression • Serious opportunistic infections • PML • Guillain–Barré syndrome, encephalitis, meningitis • Immune haemolytic anaemia • Immune thrombocytopaenia
CD3 antigen on T cells	Muromonab-CD3 (Orthoclone OKT3; Ortho Biotech)	Mouse	1986 (no European Medicines Authority authorization)	• Acute resistant allograft rejection in renal, cardiac and hepatic transplant patients	• Severe acute infusion reactions • Immunosuppression and infections • Immunogenicity • Cardiovascular side effects • Hepatitis

https://www.nature.com/articles/nrd3003

Nat Rev Drug Discov. 2007 Feb;6(2):120-6. doi: 10.1038/nrd2242.
https://pubmed.ncbi.nlm.nih.gov/17268483/

Immune reactions

mAbs, are generally tolerated in humans; however, they contain elements that may be recognized by the recipient as foreign and may therefore cause the activation of immune and innate reactions[530] .

Acute reactions following mAb infusion can be caused by various mechanisms, including acute anaphylactic (IgE-mediated) and anaphylactoid reactions against mAb, serum sickness, tumor lysis syndrome (TLS), and cytokine release syndrome (CRS).

Clinical manifestations can range from local skin reactions at the injection site, pyrexia, and flu-like syndrome to acute anaphylaxis and systemic inflammatory response syndrome, which can be fatal.

Infusion reactions commonly occur after initial administration[531] and may combine TLS, CRS, and systemic inflammatory response syndrome, as exemplified by rituximab (Rituxan/MabThera; Genentech, Biogen Idec), a chimeric mAb specific for CD20. [532]

Infections

Infectious diseases are a well-described side effect of some mAbs and are a reflection of acquired immunodeficiency, usually due to removal of that mAb's target ligand.

Indeed, particular types of infections illustrate the protective function of the target ligand in the normal immune system and provide insights into the function of this molecule to combat particular pathogens.

Reactivation of tuberculosis. Therapy directed against the pro-inflammatory cytokine TNFα has contributed greatly to the management of severe rheumatoid arthritis and other arthritis[533] .

---

[530] Lend LG.
Engineering of therapeutic antibodies to minimize immunogenicity and optimize function.
Adv Drug Deliv Rev. 2006 Aug 7;58(5-6):640-56. doi: 10.1016/j.addr.2006.01.026.
https://pubmed.ncbi.nlm.nih.gov/16904789/

[531] Chung CH.
Managing premedications and the risk for reactions to infusional monoclonal antibody therapy.
Oncologist. 2008 Jun;13(6):725-32. doi: 10.1634/theoncologist.2008-0012.
https://doi.org/10.1634/theoncologist.2008-0012

Klastersky J.
Adverse effects of the humanized antibodies used as cancer therapeutics.
Curr Opin Oncol. 2006 Jul;18(4):316-20. doi: 10.1097/01.cco.0000228734.32261.62.
https://pubmed.ncbi.nlm.nih.gov/16721123/

Kang SP, Saif MW.
Infusion-related and hypersensitivity reactions of monoclonal antibodies used to treat colorectal cancer--identification, prevention, and management.
J Support Oncol. 2007 Oct;5(9):451-7.
https://pubmed.ncbi.nlm.nih.gov/18019853/

Lenz HJ.
Management and preparedness for infusion and hypersensitivity reactions.
Oncologist. 2007 May;12(5):601-9. doi: 10.1634/theoncologist.12-5-601.
https://doi.org/10.1634/theoncologist.12-5-601

[532] Coiffier B,et al
CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma.
N Engl J Med. 2002 Jan 24;346(4):235-42. doi: 10.1056/NEJMoa011795. PMID: 11807147.
https://www.nejm.org/doi/10.1056/NEJMoa011795?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200www.ncbi.nlm.nih.gov

[533] Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP.
Tumor necrosis factor antagonist mechanisms of action: a comprehensive review.
Pharmacol Ther. 2008 Feb;117(2):244-79. doi: 10.1016/j.pharmthera.2007.10.001
https://pubmed.ncbi.nlm.nih.gov/18155297/

Taylor PC, Feldmann M.
Anti-TNF biologic agents: still the therapy of choice for rheumatoid arthritis.

However, the tendency for reactivation of latent tuberculosis (presumably due to the key role of TNFα in immunity to *Mycobacterium tuberculosis*) is a serious and limiting side effect[534] .
In a meta-analysis, TNF-specific mAb therapy was associated with an increased risk of serious infections and malignancies.[535]

Progressive multifocal leukoencephalopathy. Progressive multifocal leukoencephalopathy (PML) is a rapidly progressive, often fatal, demyelinating disease usually due to reactivation of a latent infection in the central nervous system with John Cunningham polyoma virus (JCV).
Most healthy people are seropositive for JCV, and JCV reactivation can occur after immunosuppression[536] . Reactivation has also been reported after the use of natalizumab to counteract T-cell trafficking and adhesion in multiple sclerosis[537] .

Nat Rev Rheumatol. 2009 Oct;5(10):578-82. doi: 10.1038/nrrheum.2009.181.
https://pubmed.ncbi.nlm.nih.gov/19798034/

Feldmann M, Maini SR.
Role of cytokines in rheumatoid arthritis: an education in pathophysiology and therapeutics.
Immunol Rev. 2008 Jun;223:7-19. doi: 10.1111/j.1600-065X.2008.00626.x..
https://pubmed.ncbi.nlm.nih.gov/18613827/

Moss ML, Sklair-Tavron L, Nudelman R.
Drug insight: tumor necrosis factor-converting enzyme as a pharmaceutical target for rheumatoid arthritis.
Nat Clin Pract Rheumatol. 2008 Jun;4(6):300-9. doi: 10.1038/ncprheum0797.
https://pubmed.ncbi.nlm.nih.gov/18414459/

[534] Keane J.
TNF-blocking agents and tuberculosis: new drugs illuminate an old topic.
Rheumatology (Oxford). 2005 Jun;44(6):714-20. doi: 10.1093/rheumatology/keh567.
https://pubmed.ncbi.nlm.nih.gov/15741198/

Askling J, et al
Risk and case characteristics of tuberculosis in rheumatoid arthritis associated with tumor necrosis factor antagonists in Sweden.
Arthritis Rheum. 2005 Jul;52(7):1986-92. doi: 10.1002/art.21137.
https://onlinelibrary.wiley.com/doi/epdf/10.1002/art.21137

[535] Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V.
Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials.
JAMA. 2006 May 17;295(19):2275-85. doi: 10.1001/jama.295.19.2275. Erratum in: JAMA. 2006 Jun 7;295(21):2482.
https://pubmed.ncbi.nlm.nih.gov/16705109/

[536] Major EO.
Progressive multifocal leukoencephalopathy in patients on immunomodulatory therapies.
Annu Rev Med. 2010;61:35-47. doi: 10.1146/annurev.med.080708.082655.
https://pubmed.ncbi.nlm.nih.gov/19719397/

Carson KR, Focosi D, Major EO, Petrini M, Richey EA, West DP, Bennett CL.
Monoclonal antibody-associated progressive multifocal leukoencephalopathy in patients treated with rituximab, natalizumab, and efalizumab: a Review from the Research on Adverse Drug Events and Reports (RADAR) Project.
Lancet Oncol. 2009 Aug;10(8):816-24. doi: 10.1016/S1470-2045(09)70161-5.
https://pubmed.ncbi.nlm.nih.gov/19647202/

[537] Lutterotti A, Martin R.
Getting specific: monoclonal antibodies in multiple sclerosis.
Lancet Neurol. 2008 Jun;7(6):538-47. doi: 10.1016/S1474-4422(08)70110-8
https://pubmed.ncbi.nlm.nih.gov/18485317/

Ransohoff RM.
Natalizumab for multiple sclerosis.
N Engl J Med. 2007 Jun 21;356(25):2622-9. doi: 10.1056/NEJMct071462.
https://pubmed.ncbi.nlm.nih.gov/17582072/

Lopez-Diego RS, Weiner HL.
Novel therapeutic strategies for multiple sclerosis--a multifaceted adversary.
Nat Rev Drug Discov. 2008 Nov;7(11):909-25. doi: 10.1038/nrd2358.
https://pubmed.ncbi.nlm.nih.gov/18974749/

PML occurring in patients with multiple sclerosis is remarkable in that they are both demyelinating diseases, but with very different origins and pathological features. [538]

Platelet and thrombotic disorders

Drug-induced immune thrombocytopenia can be caused by many drugs, including mAb[539] . Acute, severe, self-limiting thrombocytopenia can be caused by infliximab (specific for TNFα), efalizumab (specific for CD11a), and rituximab (specific for CD20); however, the mechanisms of action remain unclear.

Autoimmune diseases

mAbs have the ability, through their immunomodulatory actions, including immunosuppression, to cause several autoimmune conditions[540] , some of which are described below.

Lupus-like syndromes and drug-related lupus. The use of TNF-specific mAbs for rheumatic diseases has been associated with the development of antinuclear antibodies and antibodies against double-stranded DNA, as well as lupus-like syndromes[541] .

Although the development of autoantibodies is common, the development of musculoskeletal manifestations and lupus-like syndromes is rare and often subsides with discontinuation of therapy[542] . Other autoimmune complications include cutaneous or systemic vasculitis, nephritis, and demyelinating syndromes.

Thyroid diseases. Alemtuzumab is a potent immunosuppressive mAb used in multiple sclerosis, but it can also cause antibody-mediated thyroid autoimmunity[543] , probably mediated by lymphopenia following treatment.

In an initial study of 27 patients with multiple sclerosis, 9 patients developed autoantibodies against the thyrotropin receptor and autoimmune hyperthyroidism that responded to carbimazole[544] .

Sadiq SA, Puccio LM, Brydon EW.
JCV detection in multiple sclerosis patients treated with natalizumab.
J Neurol. 2010 Jun;257(6):954-8. doi: 10.1007/s00415-009-5444-4.
https://pubmed.ncbi.nlm.nih.gov/20052484/

[538] Major EO.
Reemergence of PML in natalizumab-treated patients--new cases, same concerns.
N Engl J Med. 2009 Sep 10;361(11):1041-3. doi: 10.1056/NEJMp0906248.
https://pubmed.ncbi.nlm.nih.gov/19741226/

[539] Aster RH, Bougie DW.
Drug-induced immune thrombocytopenia.
N Engl J Med. 2007 Aug 9;357(6):580-7. doi: 10.1056/NEJMra066469.
https://pubmed.ncbi.nlm.nih.gov/17687133/

[540] Mongey AB, Hess EV.
Drug insight: autoimmune effects of medications-what's new?
Nat Clin Pract Rheumatol. 2008 Mar;4(3):136-44. doi: 10.1038/ncprheum0708.
https://pubmed.ncbi.nlm.nih.gov/18200008/

[541] Ramos-Casals M, Brito-Zerón P, Muñoz S, Soria N, Galiana D, Bertolaccini L, Cuadrado MJ, Khamashta MA.
Autoimmune diseases induced by TNF-targeted therapies: analysis of 233 cases.
Medicine (Baltimore). 2007 Jul;86(4):242-251. doi: 10.1097/MD.0b013e3181441a68.
https://journals.lww.com/md-journal/Fulltext/2007/07000/Autoimmune_Diseases_Induced_by_TNF_Targeted.7.aspx

[542] Haraoui B, Keystone E.
Musculoskeletal manifestations and autoimmune diseases related to new biologic agents.
Curr Opin Rheumatol. 2006 Jan;18(1):96-100. doi: 10.1097/01.bor.0000198007.73320.6e.
https://pubmed.ncbi.nlm.nih.gov/16344625/

[543] CAMMS223 Trial Investigators, Coles AJ, Compston DA, Selmaj KW, Lake SL, Moran S, Margolin DH, Norris K, Tandon PK.
Alemtuzumab vs. interferon beta-1a in early multiple sclerosis.
N Engl J Med. 2008 Oct 23;359(17):1786-801. doi: 10.1056/NEJMoa0802670.
https://www.nejm.org/doi/10.1056/NEJMoa0802670?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub 0www.ncbi.nlm.nih.gov

[544] Coles AJ, Wing M, Smith S, Coraddu F, Greer S, Taylor C, Weetman A, Hale G, Chatterjee VK, Waldmann H, Compston A.

This thyroid disease associated with autoantibodies also occurred in about 25% of subjects in a more recent study of 334 patients[534] , suggesting a predisposition to this adverse effect in patients with multiple sclerosis[545] .
Previous interferon-β treatment in many of these subjects may have contributed to the autoimmune responses.

Autoimmune colitis. Cytotoxic T lymphocyte antigen 4 (CTLA4) is a key regulator of adaptive immune responses, and CTLA4-specific mAbs (ipilimumab and tremelimumab) act as immunomodulatory agents[546] .
Ipilimumab has been found to cause suppression of T cells and tumor cells, but also autoimmune enterocolitis sometimes requiring colectomy[547] .
In addition to colitis, CTLA4 inhibition causes a number of other immune-related adverse events, such as rash and hepatitis. These immune-related adverse events may be part of the action of mAb in causing tumor regression and immunosuppression in patients with metastatic melanoma and renal cell cancer[548] .

Cancer
Instead of excessive acute removal of malignant cells, some mAbs may contribute to tumor progression in a manner similar to other immunosuppressive agents.
The association of TNF-specific mAb therapy (infliximab) with an increased risk of malignancy remains controversial[549] .

Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis.
Lancet. 1999 Nov 13;354(9191):1691-5. doi: 10.1016/S0140-6736(99)02429-0.
https://pubmed.ncbi.nlm.nih.gov/10568572/

[545]Hauser SL.
Multiple lessons for multiple sclerosis.
N Engl J Med. 2008 Oct 23;359(17):1838-41. doi: 10.1056/NEJMe0806738.
https://pubmed.ncbi.nlm.nih.gov/18946071/

[546] Fong L, Small EJ.
Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment.
J Clin Oncol. 2008 Nov 10;26(32):5275-83. doi: 10.1200/JCO.2008.17.8954.
https://pubmed.ncbi.nlm.nih.gov/18838703/

[547] Peggs KS, Quezada SA, Korman AJ, Allison JP.
Principles and use of anti-CTLA4 antibody in human cancer immunotherapy.
Curr Opin Immunol. 2006 Apr;18(2):206-13. doi: 10.1016/j.coi.2006.01.011.
https://pubmed.ncbi.nlm.nih.gov/16464564/

Weber J.
Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events.
Oncologist. 2007 Jul;12(7):864-72. doi: 10.1634/theoncologist.12-7-864.
https://doi.org/10.1634/theoncologist.12-7-864

[548] Kaufman HL, Wolchok JD.
Is tumor immunity the same thing as autoimmunity? Implications for cancer immunotherapy.
J Clin Oncol. 2006 May 20;24(15):2230-2. doi: 10.1200/JCO.2006.05.6952.
https://pubmed.ncbi.nlm.nih.gov/16710020/

[549] Askling J, Bongartz T.
Malignancy and biologic therapy in rheumatoid arthritis.
Curr Opin Rheumatol. 2008 May;20(3):334-9. doi: 10.1097/BOR.0b013e3282f7c706.
https://pubmed.ncbi.nlm.nih.gov/18388527/

Scott DL, Kingsley GH.
Tumor necrosis factor inhibitors for rheumatoid arthritis.
N Engl J Med. 2006 Aug 17;355(7):704-12. doi: 10.1056/NEJMct055183.
https://pubmed.ncbi.nlm.nih.gov/16914706/

Dixon W, Silman A.
Is there an association between anti-TNF monoclonal antibody therapy in rheumatoid arthritis and risk of malignancy and serious infection?
Commentary on the meta-analysis by Bongartz et al.

A mAb specific for interleukin-12/23 (IL-12/23) has been shown to be effective in moderate to severe plaque psoriasis[550] and Crohn's disease[551] , and beneficial effects have been demonstrated in multiple sclerosis
[552]. However, there are concerns about potential tumorigenicity, as IL-12 plays a role in tumor immunity by promoting the infiltration of cytotoxic T cells[553] .
The issue is complicated by IL-23, which is suspected of inducing tumor-promoting pro-inflammatory processes[554] . Radioimmunotherapy with labeled tositumomab (Bexxar; GlaxoSmithKline) and ibritumomab (Zevalin; Biogen Idec) has also raised concerns regarding neoplasms[555] .

Dermatitis

A well-known example of adverse events related to the target rather than to mAbs involves human epidermal growth factor receptor 1 (EGFR; also known as HER1, ERBB1).
EGFR is a promising target for many solid tumors. EGFR-specific mAbs, cetuximab (a chimeric mAb) and panitumumab (Vectibix; Amgen, a fully humanized mAb), are effective therapies for refractory metastatic colorectal cancer[556] .
These mAbs (along with small-molecule EGFR inhibitors) commonly cause a skin rash on the face and upper torso, although dermatitis may present as dry skin, itching, and erythema.[557] The rash is usually mild to moderate and occurs within the first 15 days of therapy.

Arthritis Res Ther. 2006;8(5):111. doi: 10.1186/ar2026.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1779433/

[550] Krueger GG, Langley RG, Leonardi C, Yeilding N, Guzzo C, Wang Y, Dooley LT, Lebwohl M; CNTO 1275 Psoriasis Study Group.
A human interleukin-12/23 monoclonal antibody for the treatment of psoriasis.
N Engl J Med. 2007 Feb 8;356(6):580-92. doi: 10.1056/NEJMoa062382.
https://www.nejm.org/doi/10.1056/NEJMoa062382?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub 0www.ncbi.nlm.nih.gov

[551] Sandborn WJ.
Current directions in IBD therapy: what goals are feasible with biological modifiers?
Gastroenterology. 2008 Nov;135(5):1442-7. doi: 10.1053/j.gastro.2008.09.053.
https://pubmed.ncbi.nlm.nih.gov/18848556/

[552] Segal BM, Constantinescu CS, Raychaudhuri A, Kim L, Fidelus-Gort R, Kasper LH; Ustekinumab MS Investigators.
Repeated subcutaneous injections of IL12/23 p40 neutralising antibody, ustekinumab, in patients with relapsing-remitting multiple sclerosis: a phase II, double-blind, placebo-controlled, randomized, dose-ranging study.
Lancet Neurol. 2008 Sep;7(9):796-804. doi: 10.1016/S1474-4422(08)70173-X.
https://pubmed.ncbi.nlm.nih.gov/18703004/

[553] Weiss JM, Subleski JJ, Wigginton JM, Wiltrout RH.
Immunotherapy of cancer by IL-12-based cytokine combinations.
Expert Opin Biol Ther. 2007 Nov;7(11):1705-21. doi: 10.1517/14712598.7.11.1705.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2430051/

[554] Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, Basham B, McClanahan T, Kastelein RA, Oft M.
IL-23 promotes tumor incidence and growth.
Nature. 2006 Jul 27;442(7101):461-5. doi: 10.1038/nature04808
https://pubmed.ncbi.nlm.nih.gov/16688182/

[555] Knox SJ, Goris ML, Trisler K, Negrin R, Davis T, Liles TM, Grillo-López A, Chinn P, Varns C, Ning SC, Fowler S, Deb N, Becker M, Marquez C, Levy R.
Yttrium-90-labeled anti-CD20 monoclonal antibody therapy of recurrent B-cell lymphoma.
Clin Cancer Res. 1996 Mar;2(3):457-70.
https://pubmed.ncbi.nlm.nih.gov/9816191/

[556] Jean GW, Shah SR.
Epidermal growth factor receptor monoclonal antibodies for the treatment of metastatic colorectal cancer.
Pharmacotherapy. 2008 Jun;28(6):742-54. doi: 10.1592/phco.28.6.742.
https://pubmed.ncbi.nlm.nih.gov/18503402/

[557] Peréz-Soler R, Saltz L.
Cutaneous adverse effects with HER1/EGFR-targeted agents: is there a silver lining?
J Clin Oncol. 2005 Aug 1;23(22):5235-46. doi: 10.1200/JCO.2005.00.6916.
https://pubmed.ncbi.nlm.nih.gov/16051966/

Although it is often described as similar to acne, the histology of the lesions is different from that of acne; for example, topical medications used for acne tend to worsen the rash.
Dermatitis is thought to be part of the pharmacodynamic action of this agent, as EGFR is a transmembrane glycoprotein widely expressed on epithelial cells, and there is a correlation between the presence of rash and a positive response to the drug. [558]

Cardiotoxicity
Trastuzumab (Herceptin; Genentech) is a humanized mAb directed against human ERBB2 (also known as HER2/neu) and has been used successfully in women with ERBB2- positive metastatic breast cancer[559] .
However, an unexpected adverse event in women treated with trastuzumab in clinical trials was cardiotoxicity[560] . The antitumor and cytotoxic effects are related to the effects of trastuzumab on mitochondrial outer membrane permeabilization (MOMP), and the cardiac dysfunction caused by treatment is manifested by an asymptomatic decrease in left ventricular ejection fraction that tends to be reversible.

The target of trastuzumab, ERBB2, is a membrane receptor tyrosine kinase with an extracellular ligand-binding domain and an intracellular kinase domain,[561] and the cardiotoxicity of the drug is an on-target effect due to the blockade of all downstream signaling of ERBB2 causing MOMP, cytochrome c release and caspase activation, resulting in apoptosis of cardiac muscle cells, impaired contractility and ventricular function.[562]

---

[558] Bianchini D, Jayanth A, Chua YJ, Cunningham D.
Epidermal growth factor receptor inhibitor-related skin toxicity: mechanisms, treatment, and its potential role as a predictive marker.
Clin Colorectal Cancer. 2008 Jan;7(1):33-43. doi: 10.3816/CCC.2008.n.005.
https://pubmed.ncbi.nlm.nih.gov/18279575/

Saif MW, Longo WL, Israel G.
Correlation between rash and a positive drug response associated with bevacizumab in a patient with advanced colorectal cancer.
Clin Colorectal Cancer. 2008 Mar;7(2):144-8. doi: 10.3816/CCC.2008.n.020.
https://pubmed.ncbi.nlm.nih.gov/18501075/

[559] Hudis CA.
Trastuzumab--mechanism of action and use in clinical practice.
N Engl J Med. 2007 Jul 5;357(1):39-51. doi: 10.1056/NEJMra043186.
https://pubmed.ncbi.nlm.nih.gov/17611206/

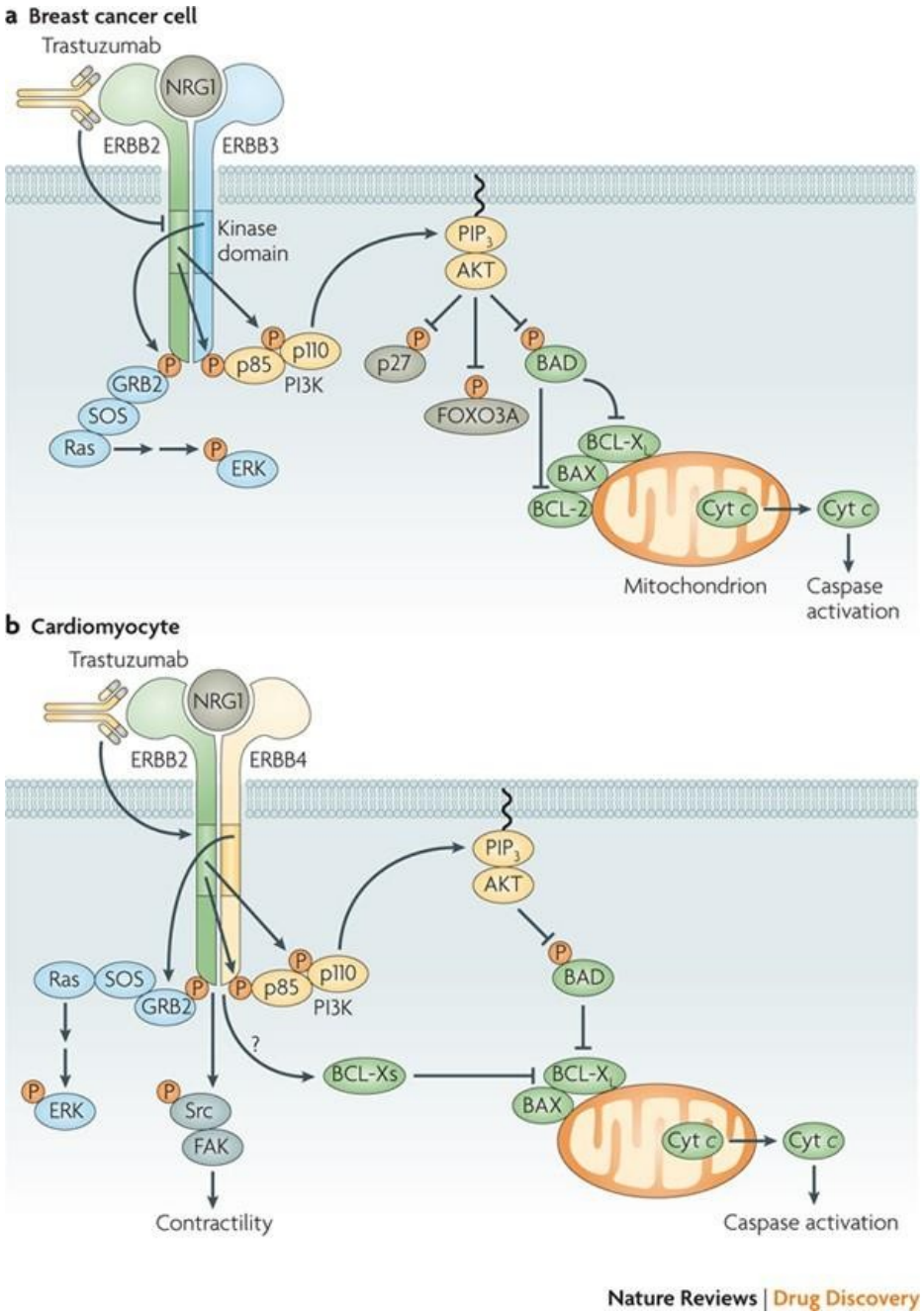
[560] Force T, Krause DS, Van Etten RA.
Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition.
Nat Rev Cancer. 2007 May;7(5):332-44. doi: 10.1038/nrc2106.
https://pubmed.ncbi.nlm.nih.gov/17457301/

Guglin M, Cutro R, Mishkin JD.
Trastuzumab-induced cardiomyopathy.
J Card Fail. 2008 Jun;14(5):437-44. doi: 10.1016/j.cardfail.2008.02.002.
https://pubmed.ncbi.nlm.nih.gov/18514938/

[561] Force T, Kerkelä R.
Cardiotoxicity of the new cancer therapeutics--mechanisms of, and approaches to, the problem.
Drug Discov Today. 2008 Sep;13(17-18):778-84. doi: 10.1016/j.drudis.2008.05.011
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735339/

Chen MH, Kerkelä R, Force T.
Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics.
Circulation. 2008 Jul 1;118(1):84-95. doi: 10.1161/CIRCULATIONAHA.108.776831.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2735334/

[562] Kuramochi Y, Guo X, Sawyer DB.
Neuregulin activates erbB2-dependent src/FAK signaling and cytoskeletal remodeling in isolated adult rat cardiac myocytes.
J Mol Cell Cardiol. 2006 Aug;41(2):228-35. doi: 10.1016/j.yjmcc.2006.04.007.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1847613/



https://www.nature.com/articles/nrd3003

(a) Oncogenic signaling in a breast cancer cell can be mediated by members of the epidermal growth factor receptor (EGFR) family. Amplification of the gene encoding for ERBB2 (also known as HER2/neu) tyrosine kinase is crucial for the progression of some forms of human breast cancer. ERBB2 - ERBB3 kinase then activates the Ras - extracellular signal-regulated kinase (ERK) pathway and the phosphatidylinositol 3-kinase (PI3K) - AKT pathway. AKT has a central oncogenic role, in part through inhibition of B-cell lymphoma 2 (BCL-2) and cell death antagonist (BAD). Trastuzumab (Herceptin; Genentech) binds to the extracellular domain of ERBB2 and inhibits proliferation and survival of ERBB2-dependent breast cancer cells. Trastuzumab also reverses inhibition of BAD, which leads to oligomerization of BCL-2-associated protein X (BAX) on the mitochondrial membrane, release of cytochrome c (Cyt c), and activation of caspase to cause apoptosis of cancer cells. In addition to inhibiting ERBB2 signaling, trastuzumab could also exert effects through antibody-dependent cell-mediated cytotoxicity (not shown). **b**) Signaling in cardiomyocytes through ERBB2-ERBB4 heterodimers is essential for cardiomyocyte proliferation during cardiac growth and development and for contractile function in adults. Although many of the same signaling pathways (such as Ras-ERK and PI3K-AKT) are activated in cardiomyocytes and breast cancer cells, an increase in the ratio of BCL-X to BCL-XL induced by ERBB2-specific antibodies could trigger BAX oligomerization, mitochondrial membrane depolarization, ATP depletion, and contractile dysfunction. In addition, antibody-dependent cell-mediated cytotoxicity could contribute to trastuzumab cardiotoxicity. Trastuzumab also blocks neuregulin 1 (NRG1)-mediated activation of Src and focal adhesion kinase (FAK), and this appears to worsen left ventricular dysfunction. GRB2, growth factor receptor-related protein 2; PIP3, phosphatidylinositol triphosphate.

The cytokine storm

Several mAbs trigger the release of a range of cytokines, causing a cytokine storm or CRS[563] .
SRC is an important feature in the context of therapy with CD3-specific mAb (muromonab)[564] , CD52-specific (alemtuzumab)[565] and CD20-specific (rituximab)[566] .
In 2006, when the fully humanized mAb TGN1412-a CD28 superagonist (CD28SA)-was first administered to six healthy male volunteers, it triggered an immediate and severe cytokine storm[567] .
The clinical, laboratory, and immunologic events that followed the rapid intravenous infusion of TGN1412 were dramatic and were divided into four phases[568] . *First*, a systemic inflammatory response consisting of high levels of cytokines in the blood, accompanied by headache, myalgias, nausea, diarrhea, erythema, vasodilation, and hypotension. *Second*, pulmonary infiltrates and lung injury, renal failure, and disseminated intravascular coagulation. *Third*, severe lymphopenia and monocytopenia. *Fourth*, prolonged cardiovascular shock and acute respiratory distress syndrome.

---

[563] Clark IA.
The advent of the cytokine storm.
Immunol Cell Biol. 2007 Jun;85(4):271-3. doi: 10.1038/sj.icb.7100062.
https://pubmed.ncbi.nlm.nih.gov/17551531/

Wing M.
Monoclonal antibody first dose cytokine release syndromes-mechanisms and prediction.
J Immunotoxicol. 2008 Jan;5(1):11-5. doi: 10.1080/15476910801897433.
https://pubmed.ncbi.nlm.nih.gov/18382853/

[564] Plevy S, Salzberg B, Van Assche G, Regueiro M, Hommes D, Sandborn W, Hanauer S, Targan S, Mayer L, Mahadevan U, Frankel M, Lowder J.
A phase I study of visilizumab, a humanized anti-CD3 monoclonal antibody, in severe steroid-refractory ulcerative colitis.
Gastroenterology. 2007 Nov;133(5):1414-22. doi: 10.1053/j.gastro.2007.08.035.
https://pubmed.ncbi.nlm.nih.gov/17920064/

[565] Wing MG, Waldmann H, Isaacs J, Compston DA, Hale G.
Ex-vivo whole blood cultures for predicting cytokine-release syndrome: dependence on target antigen and antibody isotype.
Ther Immunol. 1995 Aug;2(4):183-90.
https://pubmed.ncbi.nlm.nih.gov/9358610/

Wing MG, Moreau T, Greenwood J, Smith RM, Hale G, Isaacs J, Waldmann H, Lachmann PJ, Compston A.
Mechanism of first-dose cytokine-release syndrome by CAMPATH 1-H: involvement of CD16 (FcgammaRIII) and CD11a/CD18 (LFA-1) on NK cells.
J Clin Invest. 1996 Dec 15;98(12):2819-26. doi: 10.1172/JCI119110.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC507749/

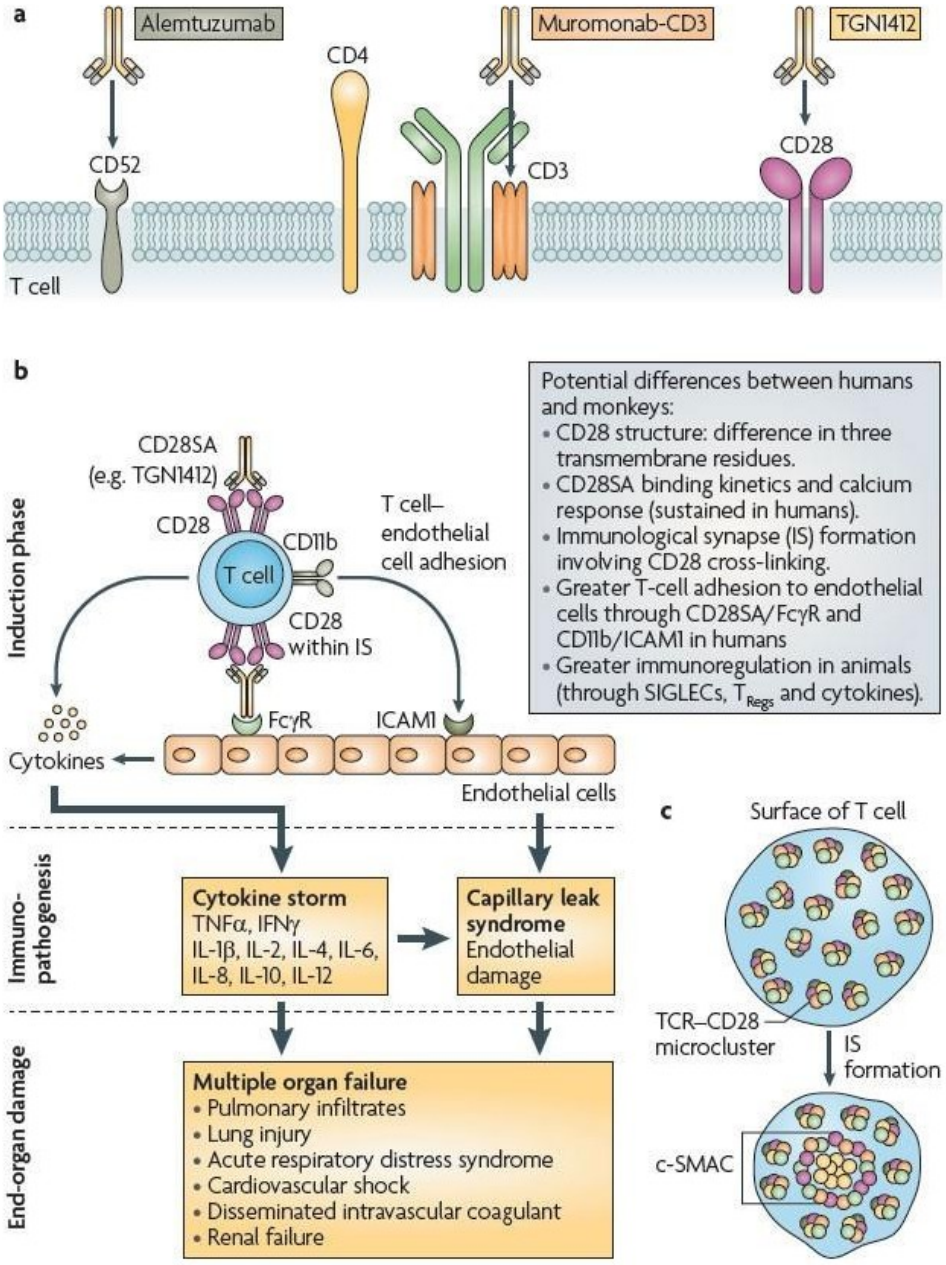
[566] Winkler U, Jensen M, Manzke O, Schulz H, Diehl V, Engert A.
Cytokine-release syndrome in patients with B-cell chronic lymphocytic leukemia and high lymphocyte counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8).
Blood. 1999 Oct 1;94(7):2217-24.
https://doi.org/10.1182/blood.V94.7.2217.419k02_2217_2224

[567] Ransohoff RM.
Natalizumab for multiple sclerosis.
N Engl J Med. 2007 Jun 21;356(25):2622-9. doi: 10.1056/NEJMct071462
https://pubmed.ncbi.nlm.nih.gov/17582072/

Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltsis N.
Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412.
N Engl J Med. 2006 Sep 7;355(10):1018-28. doi: 10.1056/NEJMoa063842.
https://www.nejm.org/doi/10.1056/NEJMoa063842?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub 0www.ncbi.nlm.nih.gov

Kenter MJ, Cohen AF.
Establishing risk of human experimentation with drugs: lessons from TGN1412.
Lancet. 2006 Oct 14;368(9544):1387-91. doi: 10.1016/S0140-6736(06)69562-7.
https://pubmed.ncbi.nlm.nih.gov/17046471/

[568] Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltsis N.
Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412.
N Engl J Med. 2006 Sep 7;355(10):1018-28. doi: 10.1056/NEJMoa063842.
https://www.nejm.org/doi/10.1056/NEJMoa063842?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub 0www.ncbi.nlm.nih.gov



https://www.nature.com/articles/nrd3003

(a) Surface receptors on T lymphocytes can cause a cytokine storm when activated by therapeutic monoclonal antibodies (mAbs). Three mAbs that cause cytokine release by infusion in humans are alemtuzumab (Campath; Genzyme), muromonab-CD3 (Orthoclone OKT3), and TGN1412. Alemtuzumab recognizes the CD52 molecule on T lymphocytes and confers efficient complement-dependent lysis of lymphocytes. Muromonab targets CD3, a part of the T lymphocyte receptor (TCR) complex. TGN1412 is an example of a CD28 superagonist (CD28SA); i.e., a co-stimulatory molecule that contributes to the activation of naive T cells. **b)** TGN1412 can directly cause cytokine release, since CD28 is expressed on a variety of cells in the normal immune system. TGN1412 is more potent on human T lymphocytes than on monkey T lymphocytes. This is probably because human CD28 has three different transmembrane amino acids, which could cause a prolonged calcium response within human T cells. The cross-linking of human CD28 may contribute to the formation of an activated immunological synapse (IS) on the surface of T lymphocytes, and the binding of CD28SA to Fcγ receptors (FcγRs) on endothelial cells and other leukocytes could cause further cytokine release. CD28 activation can also cause the upregulation of adhesion molecules such as CD11b on the surface of T lymphocytes or other cells of the innate immune system, which can then bind to intracellular adhesion molecule 1 (ICAM1) on endothelial cells. T-lymphocyte-endothelial complexes have the ability to cause amplified cytokine production and local endothelial damage. Thus, the cytokine storm and neutrophil infiltration could mediate the capillary leak syndrome resulting in multiorgan failure. **c)** IS is formed in a dynamic process on the plasma membrane of T lymphocytes, in which the five components of the TCR-CD28 microcluster aggregate to form a central supramolecular activating cluster (c-SMAC). The latter consists of a core of TCR and CD3 molecules, surrounded by a ring of CD28 molecules with associated protein kinase Cθ, which causes prolonged activation of T lymphocytes.

Regulations

There is a series of guidance documents that support first-in-human clinical trials with mAbs[569] . As an immediate response to the TGN1412 disaster, the EMA published guidance on identifying and reducing risks associated with new medicines studied in first-in-human clinical trials[570] . In addition, detailed regulatory guidance is available on the preclinical safety evaluation of drugs[571] and biologics.[572]

Microdosing is a method of studying the action of drugs in humans with doses so low that they do not cause effects on the whole organism, but have cellular responses[573] . A microdosing study is performed early in the development of a drug, before the start of Phase I clinical trials, and uses a dose equal to a small fraction of the intended drug dose. The EMA has published a position paper on nonclinical safety studies to support clinical trials with a single microdose.[574]

The EMA *guideline "Guideline on development, production, characterization and specification for monoclonal antibodies and related products"*[575] describes The necessary controls to be performed on the drug before marketing. In particular, regarding the control of impurities and contaminants, an excerpt is given: "Monoclonal antibodies commonly exhibit several sources of heterogeneity (e.g., C-terminal lysine processing, N-terminal pyroglutamate, deamidation, oxidation, isomerization, fragmentation, disulfide bond mismatch, N-linked oligosaccharides, glycation), leading to a complex purity/impurity profile comprising several molecular entities or variants. Multimers and aggregates should also be properly characterized using a combination of methods.

The formation of aggregates, sub-visible and visible particles in the drug product is important and should be studied and monitored closely during batch release and stability studies.

---

[569] Muller PY, Brennan FR.
Safety assessment and dose selection for first-in-human clinical trials with immunomodulatory monoclonal antibodies. Clin Pharmacol Ther. 2009 Mar;85(3):247-58. doi: 10.1038/clpt.2008.273.
https://pubmed.ncbi.nlm.nih.gov/19177065/

[570] European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). Guideline on strategies to identify and mitigate risks for first in human clinical trials with investigational medicinal products. Doc. Ref. EMEA/CHMP/SWP/28367/07. *EMA website*
https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-strategies-identify-mitigate-risks-first-human-early-clinical-trials-investigational_en.pdf

[571] European Medicines Agency. ICH topic M 3 (R2): non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. Note for guidance on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals (CPMP/ICH/286/95).
https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorization_en.pdf

[572] European Medicines Agency. ICH topic S 6: preclinical safety evaluation of biotechnology-derived pharmaceuticals. Note for guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals (CPMP/ICH/302/95).
https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s6r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals-step-5_en.pdf

[573] Lappin G, Garner RC.
The utility of microdosing over the past 5 years.
Expert Opin Drug Metab Toxicol. 2008 Dec;4(12):1499-506. doi: 10.1517/17425250802531767
https://pubmed.ncbi.nlm.nih.gov/19040326/

[574] European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). Position paper on non-clinical safety studies to support clinical trials with a single microdose. CPMP/SWP/2599/02
https://www.ema.europa.eu/en/documents/scientific-guideline/concept-paper-development-chmp-guideline-non-clinical-requirements-support-early-phase-i-clinical_en.pdf

[575] Guideline on development, production, characterization and specification for monoclonal antibodies and related products
EMA/CHMP/BWP/532517/2008 Committee for medicinal products for human use (CHMP)
https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-development-production-characterisation-specification-monoclonal-antibodies-related_en.pdf

Potential process-related impurities (e.g., HCP, host cell DNA, cell culture residues, downstream processing residues) should be identified and evaluated qualitatively and/or quantitatively, as appropriate.
Contaminants, which include all accidentally introduced materials not intended to be part of the manufacturing process (e.g., microbial species, endotoxins) should be strictly avoided and/or adequately controlled. Where non-endotoxic pro-inflammatory contaminants, such as peptidoglycan, are suspected, the use of additional tests, such as monocyte activation test, should be considered.
Considering that glycosylation may impact the pharmacokinetics of the product and may modulate its immunogenic properties,[576] appropriate acceptance criteria should be considered for this attribute.
Accordingly, the tests and acceptance limits for relevant glycosylation structures should be carefully considered (e.g., relative amounts of G0, G1, and/or G2 Fc fragments, levels of galactosylation, fucosylation, and sialylation) taking into account the expected and potential impact of this attribute on biological activity in the context of the clinical situation (e.g., the presence of functional effector functions not required for the intended mechanism of action, Fab glycosylation).

To these factors influencing variability in clinical response, it should be added that soluble receptors can bind to the monoclonal antibody resulting in altered pharmacokinetics due to changes in clearance or volume. Binding to soluble receptors may increase inter-subject variability in pharmacokinetic parameters due to differences in circulating receptor levels among individuals.
Binding capacity to plasma proteins (albumin, α-glycoprotein acid) should be studied when considered relevant. Other specific binding proteins may affect the pharmacokinetics of various proteins, as exemplified by growth hormone (GH) binding to GH-binding proteins and insulin-like growth factor (IGF-1) binding in plasma to transporter proteins. Binding proteins can also cause difficulties in quantifying the drug substance in blood or plasma.[577]

Prediction of capacity to cause CRS.
The cytokine storm was observed after intravenous administration of mAb, in the case discussed TGN1412, and the serum cytokines found *in vivo* could be synthesized and released from circulating leukocytes.

---

[576] Liu L.
Antibody glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins.
J Pharm Sci. 2015 Jun;104(6):1866-1884. doi: 10.1002/jps.24444.
https://pubmed.ncbi.nlm.nih.gov/25872915/

Bumbaca D, Boswell CA, Fielder PJ, Khawli LA.
Physiochemical and biochemical factors influencing the pharmacokinetics of antibody therapeutics.
AAPS J. 2012 Sep;14(3):554-8. doi: 10.1208/s12248-012-9369-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3385840/

Zhou Q, Qiu H.
The Mechanistic Impact of N-Glycosylation on Stability, Pharmacokinetics, and Immunogenicity of Therapeutic Proteins.
J Pharm Sci. 2019 Apr;108(4):1366-1377. doi: 10.1016/j.xphs.2018.11.029.
https://pubmed.ncbi.nlm.nih.gov/30471292/

Ryman JT, Meibohm B.
Pharmacokinetics of Monoclonal Antibodies.
CPT Pharmacometrics Syst Pharmacol. 2017 Sep;6(9):576-588. doi: 10.1002/psp4.12224.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5613179/

Datta-Mannan A.
Mechanisms Influencing the Pharmacokinetics and Disposition of Monoclonal Antibodies and Peptides.
Drug Metab Dispos. 2019 Oct;47(10):1100-1110. doi: 10.1124/dmd.119.086488.
https://dmd.aspetjournals.org/content/47/10/1100

[577] Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins
EMA Doc. Ref. CHMP/EWP/89249/2004
https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-investigation-pharmacokinetics-therapeutic-proteins_en.pdf

Therefore, *in vitro* assays have been established that rely on incubating TGN1412 with human whole blood or cell populations such as peripheral blood mononuclear cells.[578] To identify and validate preclinical screens relevant to CRS, it would be useful for the scientific community to have access to TGN1412 and related CD28-specific mAbs and immunostimulatory antibodies and cytokines.
Preclinical predictive screening tests should meet four key criteria for SRC.
First, they should be performed on a range of human cell types (preferably derived from the target population) that respond to potential mechanisms for CRS, such as blood and tissue cells, but especially endothelial cells.
Second, they should allow for validated and technically feasible readings.
Third, to determine their predictive power and limitations, they should consider a range of biological products and controls-TGN1412 is a necessary test reagent.
Finally, they should have predictive abilities not only for CRS but also for immune and tissue cell activation, Toll-like receptor activation, capillary leakage, disseminated intravascular coagulation, cardiovascular shock, and systemic inflammatory response syndrome.
In addition to improved *in vitro* tissue screening, other essential approaches to consider when evaluating the safety of biological products include testing for molecules in the local circulation (e.g., the nose or skin) in humans and in combinations of humans and animals in *vivo* and *in vitro* models.
One approach that needs more consideration is the use of microdosing studies[579] , with careful pharmacokinetic and pharmacodynamic evaluation in preliminary human studies.
Provided that previous animal data are available regarding target distribution and efficacy, this approach could include whole body as well as microscopic imaging to allow assessment of molecule distribution[580] and customized assays to determine any biological or clinical effects of the molecule.

Monoclonal antibodies in controlling the cytokine storm caused by COVID-19

Patients with coronavirus disease have huge numbers of inflammatory immune cells, such as T lymphocytes and macrophages, which are stimulated and infiltrated into respiratory tissues in response to chemokines and cytokines generated and released by the infected cells.
In mild COVID-19 infection, SARS-CoV-2 is eliminated by these cells, whereas in severe infection, major cytokines, such as interleukin-6 (IL-6), IL-1β, IL-17, IFN-γ and TNF-α, are produced by inflammatory cells in an uncontrolled response leading to the so-called cytokine storm.[581]

---

[578] Stebbings R, et al
"Cytokine storm" in the phase I trial of monoclonal antibody TGN1412: better understanding the causes to improve preclinical testing of immunotherapeutics.
J Immunol. 2007 Sep 1;179(5):3325-31. doi: 10.4049/jimmunol.179.5.3325.
https://doi.org/10.4049/jimmunol.179.5.3325

Findlay L, Eastwood D, Stebbings R, Sharp G, Mistry Y, Ball C, Hood J, Thorpe R, Poole S.
Improved in vitro methods to predict the in vivo toxicity in humans of therapeutic monoclonal antibodies including TGN1412. J Immunol Methods. 2010 Jan 31;352(1-2):1-12. doi: 10.1016/j.jim.2009.10.013.
https://pubmed.ncbi.nlm.nih.gov/19895813/

[579] Lappin G, Garner RC.
The utility of microdosing over the past 5 years.
Expert Opin Drug Metab Toxicol. 2008 Dec;4(12):1499-506. doi: 10.1517/17425250802531767.
https://pubmed.ncbi.nlm.nih.gov/19040326/

[580] Willmann JK, van Bruggen N, Dinkelborg LM, Gambhir SS.
Molecular imaging in drug development.
Nat Rev Drug Discov. 2008 Jul;7(7):591-607. doi: 10.1038/nrd2290.
https://pubmed.ncbi.nlm.nih.gov/18591980/

Bullen A.
Microscopic imaging techniques for drug discovery.
Nat Rev Drug Discov. 2008 Jan;7(1):54-67. doi: 10.1038/nrd2446.
https://pubmed.ncbi.nlm.nih.gov/18079755/
[581] Tabll AA, Shahein YE, Omran MM, Elnakib MM, Ragheb AA, Amer KE.

Available monoclonal antibodies or specific antagonists that can help control cytokine production, during COVID-19 infection, are listed in the Table below.

Cytokine		Available mAb/antagonist		Adverse effects of using the antibody
Name	Action/results	Name	Action/results	
IL-6	Destroys the alveoli membrane/hemorrhage in lungs then fibrosis	1-Tocilizumab (mAb) 2-Sarilumab (mAb) 3-Clazakizumab (mAb)	Binds then block the action of IL-6. No direct action on the virus/Inhibits the destructive effect of the virus.	1-Increase in hepatic enzymes 2-Skin allergy 3-Infection by opportunistic fungi
IL-1β	Produced by macrophages, induces fever/respiratory fibrosis	Canakinumab (mAb)	Blocks IL-1β	Not identified
IL-17	Produced by lymphoid cells, activates T-helper 17 cells, activates secretion of proinflammatory mediators and infiltration of neutrophils/lung tissue damage	No available	–	Not identified
IFN-γ	Produced by CD^+, CD^+8, NK cells/lung tissue damage	Emapalumab (mAb)	Blocks IFN-γ/decrease the acute respiratory distress syndrome caused by the SARS-CoV-2	Not identified
TNF-α	One of the first cytokines produced during viral infection, induces differentiation od dendritic cells	XPro1595 (soluble protein used in Alzheimer's disease)	Inhibits the TNF-α binding to its receptor	Not identified

https://content.iospress.com/articles/human-antibodies/hab200441

The following is an excerpt from what is described on the AIFA website on the use of monoclonal antibodies for COVID-19:

The European Commission, on the advice of the European Medicines Agency (EMA), has authorized the following medicines containing monoclonal antibodies, alone or in combination, against the SARS-CoV-2 virus spike protein:

- **casirivimab-imdevimab** combination called **Ronapreve** (from the pharmaceutical company Regeneron/Roche) for the treatment and prevention of COVID-19;
- **regdanvimab** called **Regkirona** (from the pharmaceutical company Celltrion Healthcare Hungary Kft) for the treatment of COVID-19;
- **sotrovimab** called **Xevudy** (from the GSK company) for the treatment of COVID-19;

A review of monoclonal antibodies in COVID-19: Role in immunotherapy, vaccine development and viral detection.
Hum Antibodies. 2021;29(3):179-191. doi: 10.3233/HAB-200441.
https://content.iospress.com/articles/human-antibodies/hab200441

A. Esmaeilzadeh A, Elahi R.
Immunobiology and immunotherapy of COVID-19: A clinically updated overview.
J Cell Physiol. 2021 Apr;236(4):2519-2543. doi: 10.1002/jcp.30076.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7675260/

Tanaka T, Narazaki M, Kishimoto T.
Immunotherapeutic implications of IL-6 blockade for cytokine storm.
Immunotherapy. 2016 Jul;8(8):959-70. doi: 10.2217/imt-2016-0020.
https://pubmed.ncbi.nlm.nih.gov/27381687/

- **tixagevimab-cilgavimab** combination called **Evusheld** (from the pharmaceutical company AstraZeneca) for pre-exposure prophylaxis of COVID-19.

Italy implemented the European authorizations with Determinations No. 155 and No. 156 of November 25, 2021, published in Official Gazette No. 282 of November 26, 2021, for Ronapreve and Regkirona, respectively, No. 169 of December 23, 2021 (published in Official Gazette No. 305 of December 24 2021) for Xevudy and No. 53 of April 13, 2022 (Official Gazette No. 88 of April 14, 2022) for Evusheld , classifying the medicines for the purpose of reimbursement by the National Health Service in "non-negotiated C [C(nn)]" with the following supply regime: *medicine subject to a restrictive prescription, to be renewed from time to time, saleable to the public on prescription by hospital centers identified by the regions (RNRL).* These medicines are subject to additional monitoring.
This allows rapid identification of new safety information. Healthcare providers are asked to report any suspected adverse reactions. Records for all drugs being monitored are available within the lists posted on the "Updated List of Registries and Web-based Therapeutic Plans" page.

List of monoclonal antibodies against SARS-Cov-2 and for COVID-19 therapy
[582]https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/treatments-covid-19/covid-19-treatments-authorise

Tixagevimab and cilgavimab neutralize SARS-CoV-2 spike (original Wuhan-1 sequence) Evusheld Tixagevimab/Cilgavimab https://www.ema.europa.eu/en/medicines/human/EPAR/evusheld

Anti IL-1
Kineret Anakinra https://www.ema.europa.eu/en/medicines/human/EPAR/kineret
https://www.giornaledicardiologia.it/archivio/3666/articoli/36514/

Regdanvimab neutralizes SARS-CoV-2 spike (original Wuhan-1 sequence) Regkirona
Regdanvimab https://www.ema.europa.eu/en/medicines/human/EPAR/regkirona

Tocilizumab binds to the receptor for interleukin-6
RoActemra https://www.ema.europa.eu/en/medicines/human/EPAR/roactemra

[582] List of critical medicines for COVID-19 public health emergency (PHE) under Regulation (EU) 2022/123 EMA/285556/2022 https://www.ema.europa.eu/en/documents/other/list-critical-medicines-covid-19-public-health-emergency-phe-under-regulation-eu-2022/123_en.pdf

Anti-SARS-CoV-2 Monoclonal Antibodies
https://www.covid19treatmentguidelines.nih.gov/therapies/anti-sars-cov-2-antibody-products/anti-sars-cov-2-monoclonal-antibodies/

Kreuzberger N, et al
SARS-CoV-2-neutralizing monoclonal antibodies for treatment of COVID-19.
Cochrane Database Syst Rev. 2021 Sep 2;9(9):CD013825. doi: 10.1002/14651858.CD013825.pub2.
https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013825.pub2/full

Anti-SARS-CoV-2 Monoclonal Antibodies
https://www.idsociety.org/covid-19-real-time-learning-network/therapeutics-and-interventions/monoclonal-antibodies/

COVID-19 Manufacturing for Monoclonal Antibodies
https://healthpolicy.duke.edu/sites/default/files/2020-06/Issue%20Brief%20-%20COVID-19%20Manufacturing%20of%20Monoclonal%20Antibodies.pdf

Monoclonal Antibodies for the Coronavirus (Updated May 5th)
Science 28 Apr 2020
https://www.science.org/content/blog-post/monoclonal-antibodies-coronavirus-updated-may-5th

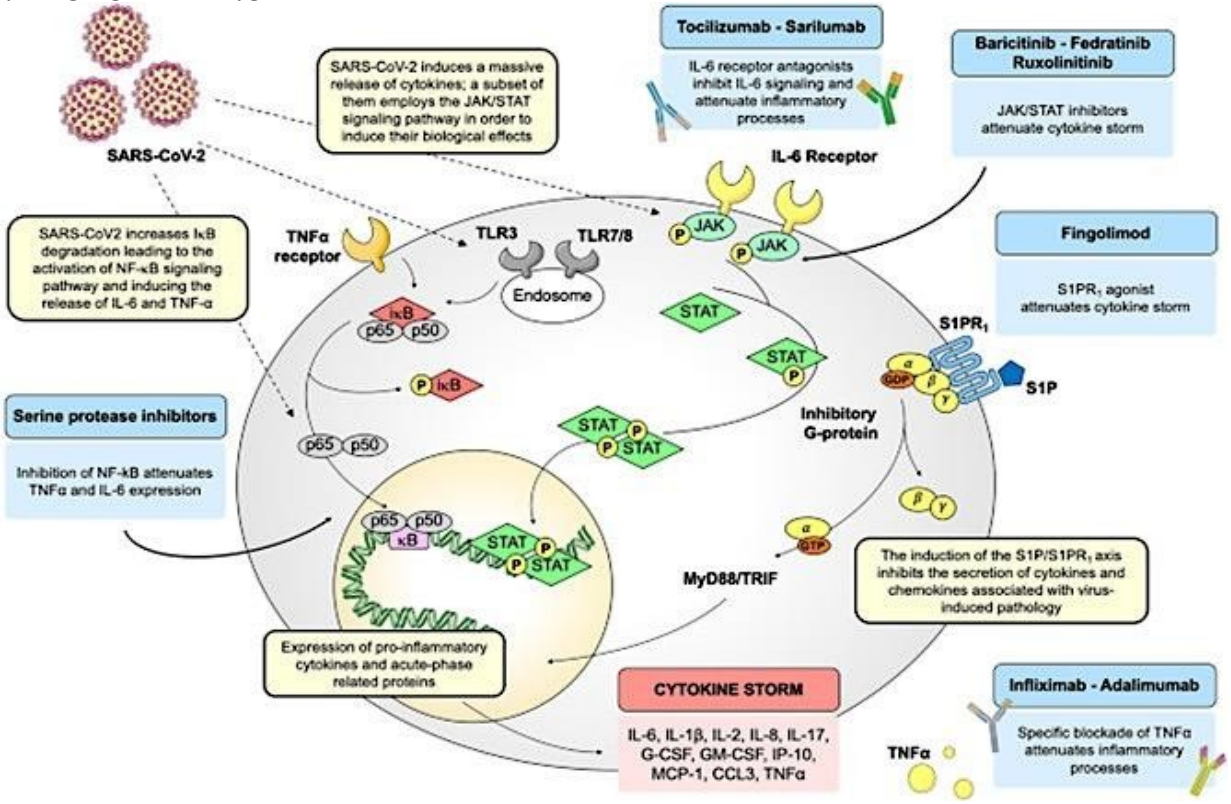
COVID-19 Monoclonal Antibodies: What They Are, How They Work, Side Effects
https://www.my-personaltrainer.it/benessere/anticorpi-monoclonali-covid-19.html

Casirivimab and imdevimab neutralize SARS-CoV-2 spike (original Wuhan-1 sequence) Ronapreve
https://www.ema.europa.eu/en/medicines/human/EPAR/ronapreve

Sotrovimab neutralizes SARS-CoV-2 spike (original Wuhan-1 sequence) Xevudy
https://www.ema.europa.eu/en/medicines/human/EPAR/xevudy

Risks associated with the use of monoclonal antibodies against SARS-Cov-2

As with other monoclonal antibodies, infusion-related reactions are potential adverse treatment reactions. [583] Infusion-related reactions are characterized by hot flashes, fever/chills, back or abdominal pain, nausea/vomiting, itching or rash and usually occur 30-60 minutes after the start of the infusion.
Monoclonal antibody therapy is not indicated in severe cases requiring hospitalization.[584]
It has been found that monoclonal antibodies may be associated with worse outcomes for patients requiring high-flow oxygen or mechanical ventilation.



[583] Brobst B, Borger J.
Benefits And Risks Of Administering Monoclonal Antibody Therapy For Coronavirus (COVID-19).
2022 Apr 28. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK574507/.

[584] An EUA for bamlanivimab and etesevimab for COVID-19.
Med Lett Drugs Ther. 2021 Apr 05;63(1621):49-50
https://pubmed.ncbi.nlm.nih.gov/33830966/

An EUA for sotrovimab for treatment of COVID-19.
Med Lett Drugs Ther. 2021 Jun 28;63(1627):97-xx98.
https://pubmed.ncbi.nlm.nih.gov/34181630/

An EUA for casirivimab and imdevimab for COVID-19.
Med Lett Drugs Ther. 2020 Dec 28;62(1614):201-202.
https://pubmed.ncbi.nlm.nih.gov/33451174/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8662023/
Schematic representation of SARS-CoV-2-driven signaling pathways and potential drug targets. Images reproduced from Catanzaro et al. 61 This article is licensed under a creative commons 4.0 international attribution license. http://creativecommons.org/licenses/by/4.0/. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-6, interleukin-6; JAK, Janus kinase; NF-κB, nuclear factor-κB; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor-α

Monoclonal antibody-dependent disease potentiation

As discussed earlier, monoclonal antibodies used in the treatment of SARS- Cov-2 infection can directly interfere with viral pathogenesis in multiple ways.[585]

Binding of a neutralizing antibody to the virion can prevent binding and/or fusion of target cells. In addition, binding of the antibody opsonizes infected virions or cells for phagocytic uptake.

If viral proteins are intercalated into target cell membranes during viral egress, monoclonal antibodies can facilitate target cell death through complement fixation and membrane attack complex (MAC) activation or antibody-dependent cytotoxicity.

These mechanisms can cause apoptosis or necrosis of the infected cell, and in some cases opsonization of a virion can facilitate viral pathogenesis through antibody-dependent enhancement. [586]

As detailed above, ADE can occur through two distinct mechanisms: pathogen-specific antibodies can increase infection through viral uptake and replication in immune cells expressing the Fcγ receptor (FcγR), or they can induce increased immune activation as a result of Fc-mediated effector functions or immune complex formation.

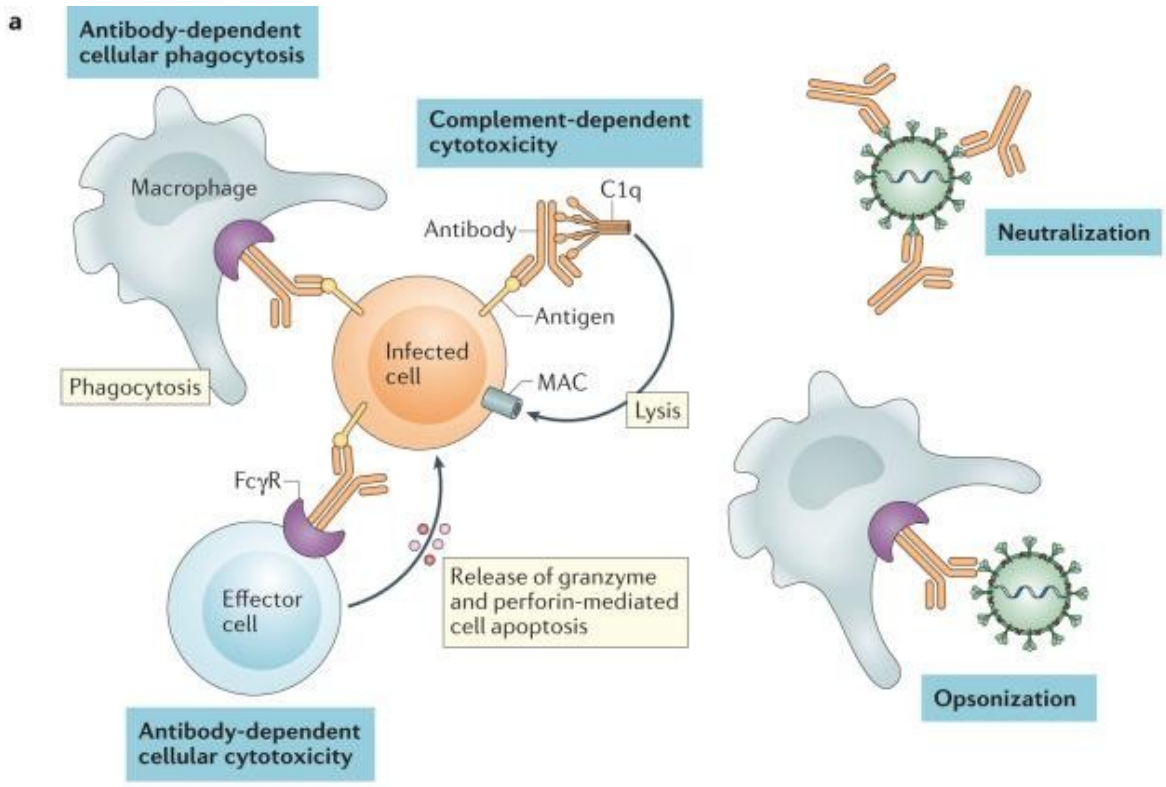
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8054133/
Mechanism of action of monoclonal antibodies for viral infection and antibody-dependent enhancement.
a | Monoclonal antibodies can directly interfere with viral pathogenesis in multiple ways. First, binding of a neutralizing antibody to the virion can prevent binding and/or fusion of target cells. In addition, binding of the antibody opsonizes infected virions or cells for phagocytic uptake. If viral proteins are intercalated into target cell membranes during viral egress, monoclonal antibodies can facilitate target cell death through complement fixation and membrane attack complex (MAC) activation or antibody-dependent cytotoxicity. These mechanisms can cause apoptosis or necrosis of the infected cell. b | In some cases, opsonization of a virion can facilitate viral pathogenesis in a process called antibody-dependent enhancement (ADE). ADE can occur through two distinct mechanisms. First, pathogen-specific antibodies could enhance infection t h r o u g h viral uptake and replication in immune cells expressing the Fcγ receptor (FcγR). Second, ADE may be mediated through increased immune activation by Fc-mediated effector functions or by the formation of immune complexes.

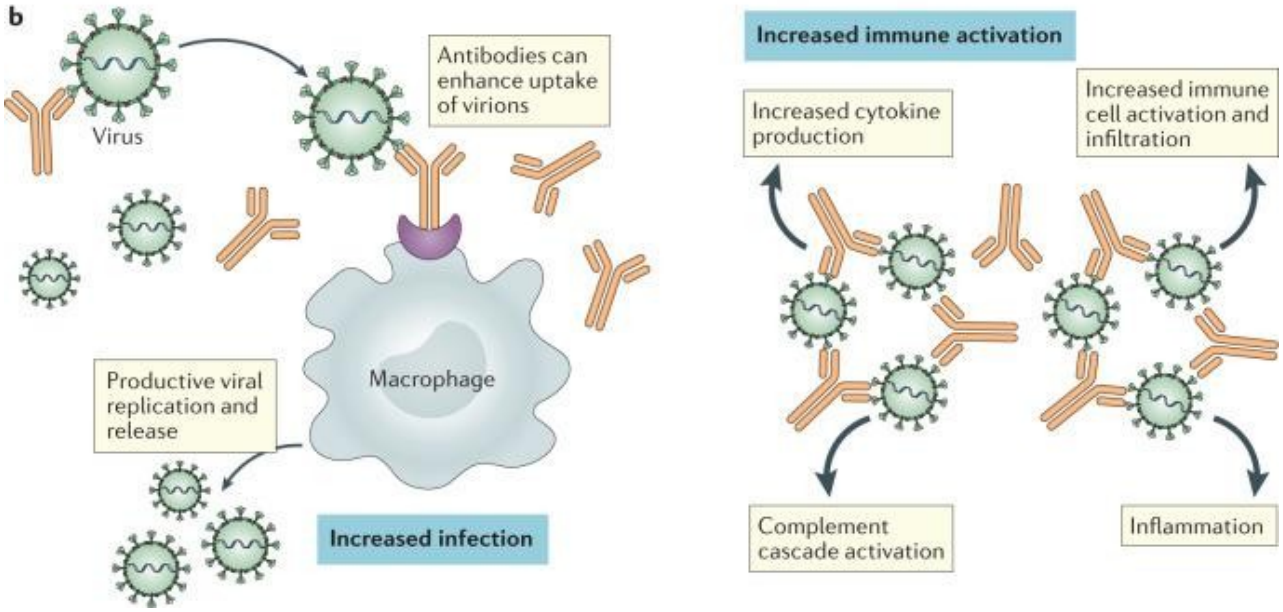
[585] Taylor PC, Adams AC, Hufford MM, de la Torre I, Winthrop K, Gottlieb RL.
Neutralizing monoclonal antibodies for treatment of COVID-19.
Nat Rev Immunol. 2021 Jun;21(6):382-393. doi: 10.1038/s41577-021-00542-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8054133/

Quiros-Roldan E, Amadasi S, Zanella I, Degli Antoni M, Storti S, Tiecco G, Castelli F.
Monoclonal Antibodies against SARS-CoV-2: Current Scenario and Future Perspectives.
Pharmaceuticals (Basel). 2021 Dec 6;14(12):1272. doi: 10.3390/ph14121272.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8707981/

Kaplon H, Chenoweth A, Crescioli S, Reichert JM.
Antibodies to watch in 2022.
MAbs. 2022 Jan-Dec;14(1):2014296. doi: 10.1080/19420862.2021.2014296.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8765076/

[586] Goncalvez AP, Engle RE, St Claire M, Purcell RH, Lai CJ.
Monoclonal antibody-mediated enhancement of dengue virus infection in vitro and in vivo and strategies for prevention.
Proc Natl Acad Sci U S A. 2007 May 29;104(22):9422-7. doi: 10.1073/pnas.0703498104.


a
Antibody-dependent cellular phagocytosis
Macrophage
Phagocytosis
Complement-dependent cytotoxicity
C1q
Antibody
Antigen
Infected cell
MAC
Lysis
FcγR
Effector cell
Release of granzyme and perforin-mediated cell apoptosis
Antibody-dependent cellular cytotoxicity
Neutralization
Opsonization


b
Virus
Antibodies can enhance uptake of virions
Macrophage
Productive viral replication and release
Increased infection
Increased immune activation
Increased cytokine production
Increased immune cell activation and infiltration
Complement cascade activation
Inflammation

Safety of Tocilizumab

Among the monoclonal antibodies initially used against SARS-Cov-2 and currently marketed, tocilizumab (TCZ) has been associated with cases of elevated liver function enzyme tests (LFTs)[587] , gastrointestinal perforation[588] , diverticulitis , neutropenia, hypertension, allergic reactions, skin rash (toxic erythema[589]) and infusion-related reactions. [590]

In the study by Pettit et al. 64 patients were included in each group, 17 infections in the TCZ group (23%) and 6 (8%) infections in the control group occurred >48 hours after admission (P = .013).

Most infections were bacterial, and pneumonia was the most common manifestation. Late-onset infections were significantly more common among those receiving TCZ.

Among patients who received TCZ, increases in LFTs were observed in 51%, neutropenia in 1.4%, and hypertension in 8%. Combining TCZ-related infections and toxicities, 61% of patients had a possible post-TCZ complication, and the mortality rate among those who received TCZ was higher than the control (39% vs. 23%, P = .03). [591]

In the review of pharmacovigilance data conducted by Jakaran et al,[592] a total of 1005 adverse drug events reported by 513 people were examined.

Analyzing the data according to the number of adverse reactions reported, most were reported by the 18-64 age group (46.26%) and more than half by males.

Reports came more from Europe and the Americas than from Asia, Africa and Oceania.

Only 12% of the adverse reactions were reported from clinical trials; the rest were reported spontaneously.

Eighty percent of these adverse reactions were serious and 20% were fatal.

The distribution of adverse reactions is shown in the following diagram:

[587] Serviddio G, Villani R, Stallone G, Scioscia G, Foschino-Barbaro MP, Lacedonia D.
Tocilizumab and liver injury in patients with COVID-19.
Therap Adv Gastroenterol. 2020 Oct 7;13:1756284820959183. doi: 10.1177/1756284820959183
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7545299/

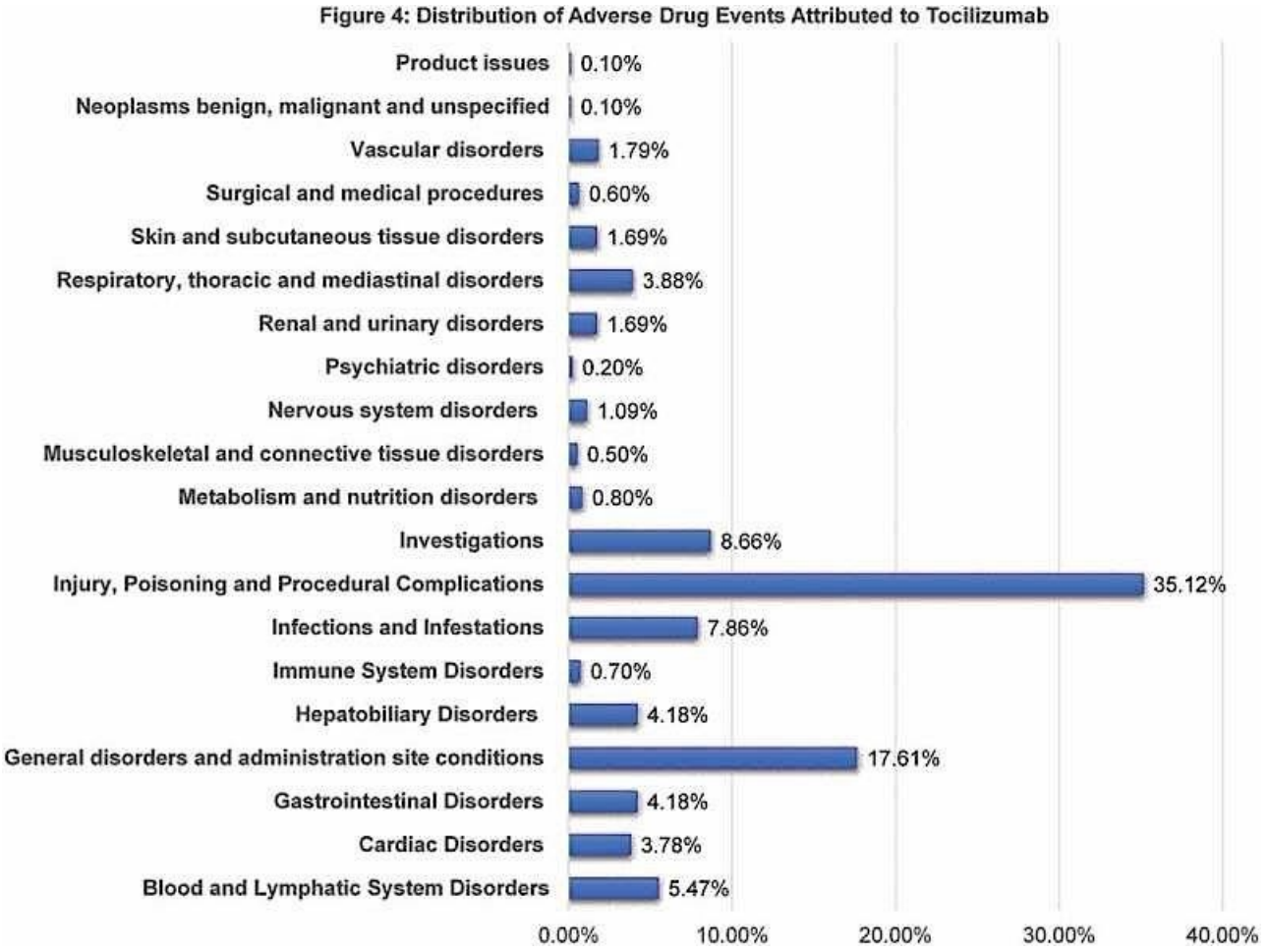
[588] Bruce-Hickman D, Sajeed SM, Pang YH, Seow CS, Chen W, Gulati Kansal M.
Bowel ulceration following tocilizumab administration in a COVID-19 patient.
BMJ Open Gastroenterol. 2020 Aug;7(1):e000484. doi: 10.1136/bmjgast-2020-000484.
https://pubmed.ncbi.nlm.nih.gov/32816957/

[589] Sernicola A, Carnicelli G, Di Fraia M, Chello C, Furlan C, Muharremi R, Paolino G, Grieco T.
'Toxic erythema' and eosinophilia associated with tocilizumab therapy in a COVID-19 patient.
J Eur Acad Dermatol Venereol. 2020 Aug;34(8):e368-e370. doi: 10.1111/jdv.16620.
https://pubmed.ncbi.nlm.nih.gov/32386438/

[590] Boretti A, Banik B.
Modulation of Covid-19 cytokine storm by tocilizumab.
J Med Virol. 2022 Mar;94(3):823-828. doi: 10.1002/jmv.27380.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8662023/

[591] Pettit NN, Nguyen CT, Mutlu GM, Wu D, Kimmig L, Pitrak D, Pursell K.
Late onset infectious complications and safety of tocilizumab in the management of COVID-19.
J Med Virol. 2021 Mar;93(3):1459-1464. doi: 10.1002/jmv.26429.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7436682/

[592][592] Charan J, Dutta S, Kaur R, Bhardwaj P, Sharma P, Ambwani S, Jahan I, Abubakar AR, Islam S, Hardcastle TC, Rahman NAA, Lugova H, Haque M.
Tocilizumab in COVID-19: a study of adverse drug events reported in the WHO database.
Expert Opin Drug Saf. 2021 Sep;20(9):1125-1136. doi: 10.1080/14740338.2021.1946513.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8290369/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8290369/

Regarding monoclonal antibodies against the SARS-Cov-2 spike, numerous studies are reported in the literature demonstrating the rapid formation of new monoclonal antibody-resistant variants as another mechanism of the damage caused by these drugs. This topic will be discussed further in the section on natural and vaccine variants.

Further aspects of monoclonal antibody safety are explored in the following popular article

Prof. Francesco Cappello Cure or procured misfortune? Synthetic monoclonal antibodies. [593]

[593] https://www.francescocappello.com/2022/01/14/cura-o-procurata-sventura-anticorpi-sintetici-monoclonali/

THE GLYCOBIOLOGY OF VIRAL INFECTIONS

Deepening

GLYCOBIOLOGY

Varki A, Cummings RD, Esko JD, et al, editors. Essentials of Glycobiology [Internet]. 4th edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2022.
https://www.ncbi.nlm.nih.gov/books/NBK579918/ doi: 10.1101/9781621824213

Video
N linked glycosylation
Lecture 3 Protein glycosylation in ER

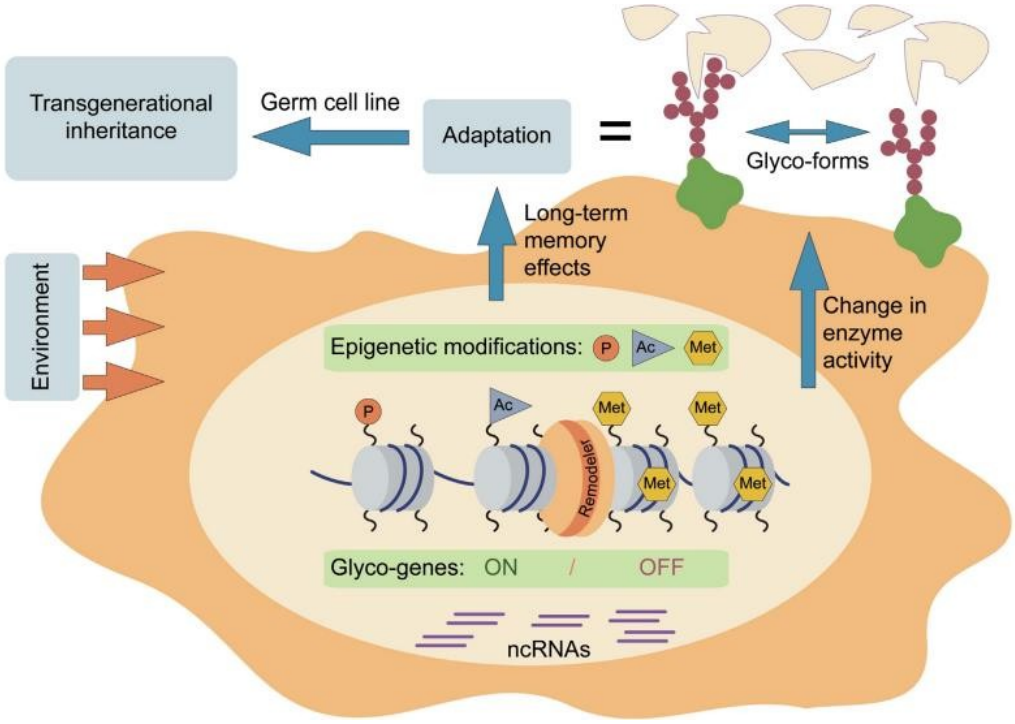
Glycans are sugar molecules consisting of modular arrangements of monosaccharide units often attached to proteins and lipids. Glycans vary in size from mono- and oligosaccharides a few nanometers long, to polysaccharide chains of thousands of subunits and several micrometers long.
The monosaccharide constituents of glycans are linked together by glycosidic bonds from many of the hydroxylated carbon atoms that form each monosaccharide ring, and thus the structures of glycans can be extensively branched.
Glycans decorate most extracellular proteins and many lipids (the combined molecules are called glycoconjugates),[594] are mainly found on the outer surface of the cell membrane and are also secreted into the extracellular spaces.
Unlike proteins and RNAs, glycans are not constructed from a mold. Their synthesis is an assembly process of sequential addition, removal, and modification of sugar subunits. The shape and diversity of glycan structures are protein-driven, but they are also intrinsically dependent on diet and physiological conditions.[595]

Therefore, in addition to being defined by an individual's genetic background, the glycome (defined as the set of all glycans in an analyzed tissue or organism) is shaped by environmentally induced changes in gene expression.
Hereditary (genetic) and acquired (epigenetic-environmental) factors that modulate glycosylation affect numerous molecular processes, including interactions with specific receptors or the half-life of numerous membrane proteins.
Both quantitative and qualitative changes in the repertoire of glycan structures have been found in many complex diseases and cancer.

[594] Marth JD.
A unified vision of the building blocks of life.
Nat Cell Biol. 2008 Sep;10(9):1015-6. doi: 10.1038/ncb0908-1015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892900/

[595] Zoldoš V, Novokmet M, Bečeheli I, Lauc G.
Genomics and epigenomics of the human glycome.
Glycoconj J. 2013 Jan;30(1):41-50. doi: 10.1007/s10719-012-9397-y.
https://pubmed.ncbi.nlm.nih.gov/22648057/

Kunej T.
Rise of Systems Glycobiology and Personalized Glycomedicine: Why and How to Integrate Glycomics with Multiomics Science?
OMICS. 2019 Dec;23(12):615-622. doi: 10.1089/omi.2019.0149.
https://pubmed.ncbi.nlm.nih.gov/31651212/



https://pubmed.ncbi.nlm.nih.gov/23999089/
Schematic representation of adaptation to changes in the environment through epigenetic regulation of glycoforms. Epigenetic mechanisms are mediators between the environment and glycoforms, which are reflected in glycan end structures on membrane glycoproteins that interact with microbes. Glycoform expression is epigenetically regulated by DNA methylation and chromatin modifications, which include covalent modifications of histone tails (phosphorylation, acetylation, methylation, etc.) and ATP-dependent remodeling complexes; small noncoding RNAs also play a regulatory role. The transcriptional state of the glycoform can change in response to external and intrinsic signals in order to achieve an appropriate functional change in protein glycosylation, which can become adaptive through long-term epigenetic effects and even move to the next generation

Because the structures of mature glycans are not encoded in the genome, their evolutionary history can only be discovered indirectly. For example, the evolution of glycan-modifying proteins may in turn reveal changes in glycans.
The gain and loss of sugar transporters, transferases and glycosidases, have a direct impact on the range of glycans that an organism can synthesize and degrade or use in the diet. [596]

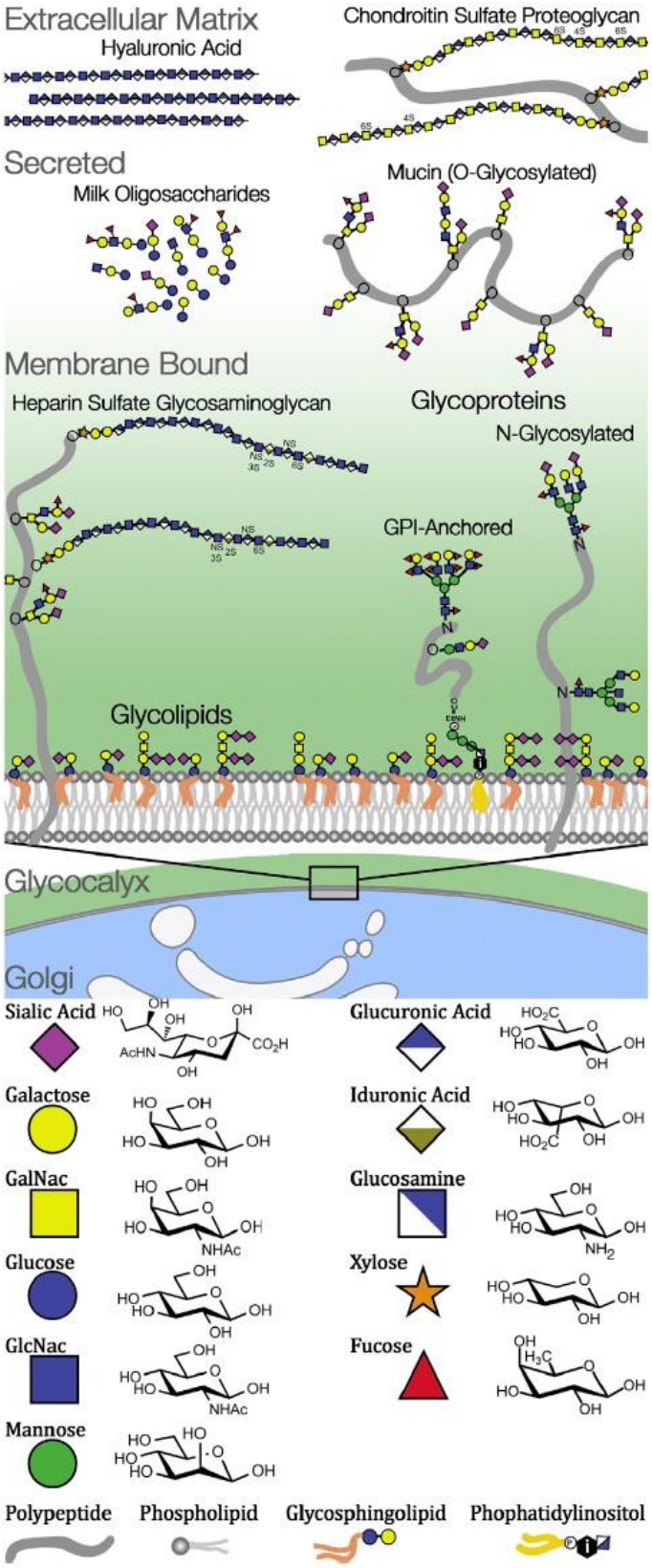
[596] Schjoldager KT, Narimatsu Y, Joshi HJ, Clausen H.
Global view of human protein glycosylation pathways and functions.
Nat Rev Mol Cell Biol. 2020 Dec;21(12):729-749. doi: 10.1038/s41580-020-00294-x.
https://pubmed.ncbi.nlm.nih.gov/33087899/

Reily C, Stewart TJ, Renfrow MB, Novak J.
Glycosylation in health and disease.
Nat Rev Nephrol. 2019 Jun;15(6):346-366. doi: 10.1038/s41581-019-0129-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6590709/

Schnaar RL.
Glycans and glycan-binding proteins in immune regulation: A concise introduction to glycobiology for the allergist.
J Allergy Clin Immunol. 2015 Mar;135(3):609-15. doi: 10.1016/j.jaci.2014.10.057.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4355172/

Bennun SV, Hizal DB, Heffner K, Can O, Zhang H, Betenbaugh MJ.
Systems Glycobiology: Integrating Glycogenomics, Glycoproteomics, Glycomics, and Other 'Omics Data Sets to Characterize Cellular Glycosylation Processes.
J Mol Biol. 2016 Aug 14;428(16):3337-3352. doi: 10.1016/j.jmb.2016.07.005.
https://pubmed.ncbi.nlm.nih.gov/27423401/

Rabinovich GA, van Kooyk Y, Cobb BA.
Glycobiology of immune responses.
Ann N Y Acad Sci. 2012 Apr;1253:1-15. doi: 10.1111/j.1749-6632.2012.06492.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3884643/



Glycans are sugars composed of building blocks of monosaccharides. These basic units can be linked into chains and branched structures. Mammals use only about a dozen basic units of monosaccharides, but these are combined into many different types of glycan structures. Glycans can be linked with different bonds to proteins (N- and O-glycans, glycosaminoglycans, proteoglycans) and lipids (glycolipids, GPI-anchored proteins). Glycans can also be secreted as free oligo- or polysaccharides (hyaluronic acid, milk oligosaccharides). In eukaryotic cells, N-glycans are attached to glycoproteins in the endoplasmic reticulum and extensively remodeled in the Golgi before moving outside the cell. In prokaryotes, N- and O-glycans are built up gradually in the cytoplasm but not extensively pruned or remodeled. Bacteria and archaea use hundreds of different monosaccharides, but only rarely are these linked to the more complex structures typical of glycans in eukaryotes

Glycans and glycoconjugates are classified in several ways. N-linked and O-linked glycans are classified according to their chemical binding to a protein.

Glycosaminoglycans can be recognized by their monosaccharide composition. Other glycoconjugates, such as GPI-anchored proteins and glycolipids, can be recognized by the bonds and combinations of their constituent molecules. Some monosaccharides, glycans and glycoconjugates are expressed only in particular phylogenetic lineages (mammalian glycosaminoglycans), others exist in most cellular life (N-glycans). There are many known rare glycans, monosaccharides, linkages and modifications, and it is likely that many more remain to be discovered.

https://www.nature.com/articles/s41581-019-0129-4

Glycans can be covalently attached to proteins and lipids to form glycoconjugates; glycans in these compounds are classified according to their binding to lipid, glycan, or protein parts. Glycoproteins consist of glycans and chains of glycans bound to the nitrogen and oxygen atoms of amino acid residues and are therefore referred to as N-glycans and O-glycans, respectively. N-glycans consist of N-acetylglucosamine (GlcNAc) attached by a β1-glycosidic bond to the nitrogen atom of the amine group of Asn (N) at the Asn-X-Ser/Thr glycosylation consensus motif (where X denotes any amino acid except Pro). These branched and highly heterogeneous N-glycan structures consist of a central glycan containing two GlcNAc residues and three mannose (Man) residues. Perhaps the most diverse form of protein glycosylation is O-glycosylation, in which glycans attach to the oxygen atom of the hydroxyl groups of residues Ser

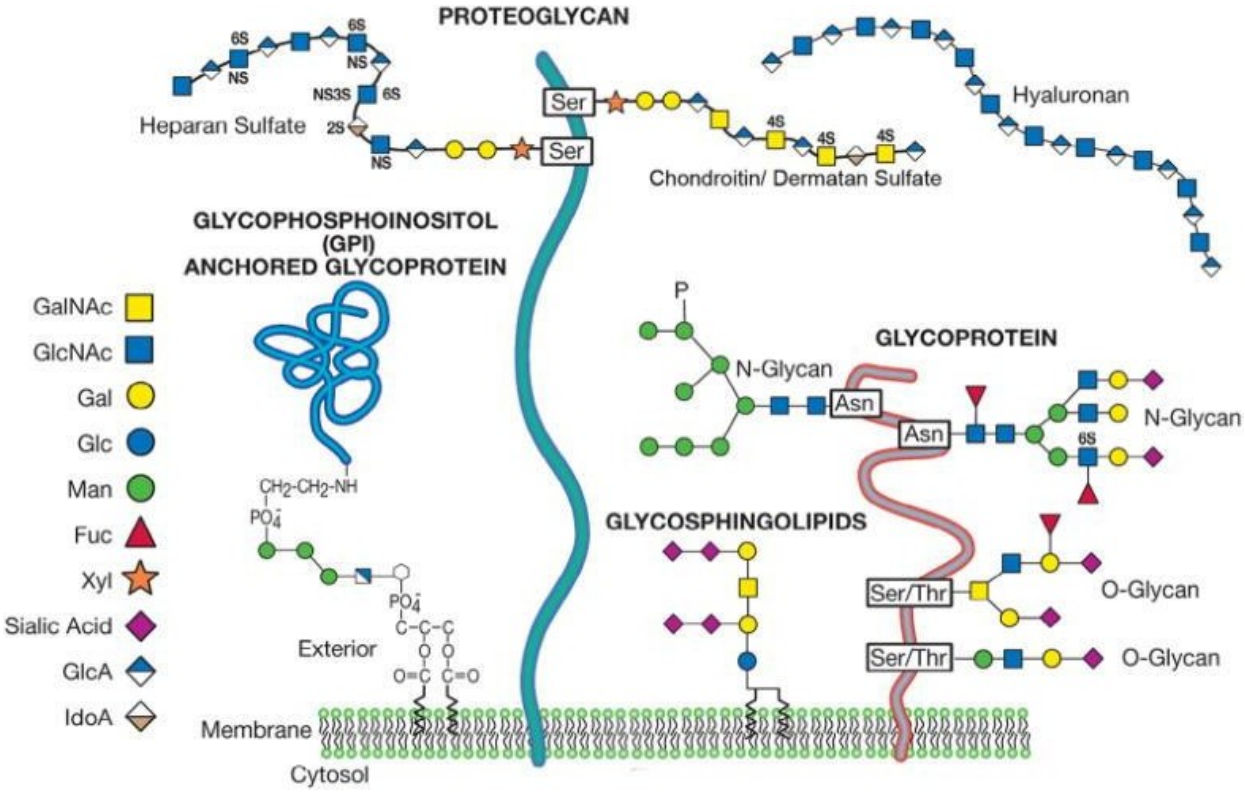
(S) or Thr (T). O-glycans can be further subclassified based on the initial sugar attached to the protein and the additional sugar structures added to the initial glycan. For example, mucin-type O-glycosylation indicates that the initial glycan is N-acetylgalactosamine (GalNAc); mucin-type glycans can be further classified on the basis of the glycans attached to the initial GalNAc. Other types of O-glycans, such as O-linked fucose (Fuc) and O-linked man, are often found in specific proteins or protein domains, such as epidermal growth factor (EGF) repeats, thrombospondin type I (TSR) repeats, or dystroglycan . N-glycans and O-glycans are often coated with negatively charged sialic acid. O-GlcNAc is a unique type of O-glycosylation synthesized by O-GlcNAc transferase; it occurs in the cytosol and nucleus. (continued)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4762723/

Proteoglycans represent an important class of glycoproteins that are defined by long chains of glycosaminoglycans (GAGs) attached to proteins through a tetrasaccharide core consisting of glucuronic acid (GlcA)-galactose (Gal)-xylose (Xyl); this carbohydrate core

Varki A, Cummings RD, Esko JD, et al, editors.
Essentials of Glycobiology. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009.
https://www.ncbi.nlm.nih.gov/books/NBK1908/
https://ncfg.hms.harvard.edu/files/ncfg/files/cummings_overview_of_glycans.pdf

is attached to the hydroxyl group of Ser on Ser-Gly-X-Gly amino acid motifs. GAG proteoglycans can be further classified according to the number, composition and degree of sulfation of their repeating disaccharide units; common GAGs include heparan sulfate, chondroitin sulfate and dermatan sulfate. Glycoproteins anchored to glycosylphosphatidylinositol (GPI) represent another important class of glycoconjugates. These glycoproteins are bound to the carboxyl terminus through a phosphoethanolamine bond attached to a trimannosyl-nonacetylated glucosamine core (Man3-GlcN); the GlcN residue is bound to phosphatidylinositol, which is incorporated into the cell membrane. Glycosphingolipids are a class of glycoconjugates in which glycans, such as Gal or glucose (Glc), are attached to cell membrane lipids. Another important class of glycans are GAGs that are not attached to protein cores, such as hyaluronan, which is synthesized on the plasma membrane by sequential addition of GlcA and GlcNAc. IdoA, hyduronic acid.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5556687/

Nitrogen-bound glycans (N-glycans)

N-glycans are oligosaccharides, each covalently linked to a protein by the nitrogen (N) atom of an asparagine. The potential N-linked glycosylation sites are called "sequons" and have a consensus N-X-S/T amino acid sequence.

N-linked glycans are found in all three domains of life, but they are synthesized differently in eukaryotes than in eubacteria or archaea.[597]

In eukaryotes, N-linked glycosylation regulates protein folding. Changes in N-glycan composition track the process of protein folding in the endoplasmic reticulum. After folding, glycoproteins mature in the Golgi apparatus, where attached N-glycans are restructured into their mature forms.[598]

In the Golgi, N-glycans are first trimmed to an oligomannose base and then constructed into complex multiramified forms.[599] The Golgi is a multi-compartmental vesicular system, and the presence or absence of enzyme

[597] Schwarz F, Aebi M.
Mechanisms and principles of N-linked protein glycosylation.
Curr Opin Struct Biol. 2011 Oct;21(5):576-82. doi: 10.1016/j.sbi.2011.08.005.
https://pubmed.ncbi.nlm.nih.gov/21978957/

[598] Stanley P, Schachter H, Taniguchi N.
N-Glycans. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME, editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 8.
https://www.ncbi.nlm.nih.gov/books/NBK1917/

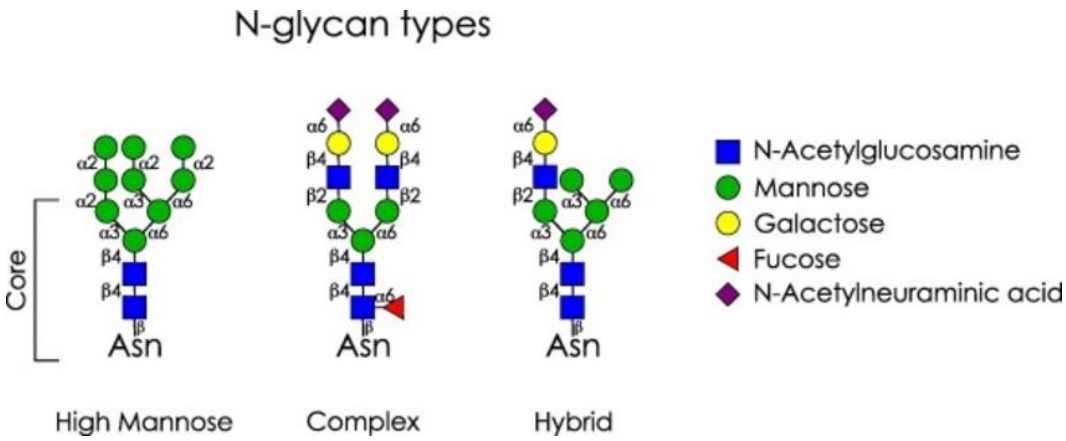
[599] Hang I, Lin CW, Grant OC, Fleurkens S, Villiger TK, Soos M, Morbidelli M, Woods RJ, Gauss R, Aebi M.

glycan modifiers and nucleotide sugar donors in a given Golgi compartment regulates N-glycan composition.

N-glycan structures vary widely in different organisms. Mammalian N-glycans are most often terminated by sialic acids. Plant N-glycans are never terminated in sialic acids and contain modifications not observed in mammalian N-glycans.

Fungi produce N-glycans rich in mannose residues, while invertebrates produce hybrid N-glycans with fewer branches than vertebrates and with some of the same modifications as plants.[589]

Invertebrates such as gastropods also use different monosaccharides.[600] In general, multicellular organisms produce a greater abundance and diversity of N-glycans than bacteria and archaea. The diversity of multicellular N-glycans may help define cell identity and modulate cell-cell signaling during development.[601]



https://doi.org/10.1016/j.ejpb.2016.01.005

Types of N-glycans. The three different types (High Mannose, Complex, and Hybrid) share a common central structure that includes the first two N-acetylglucosamine residues and the first three mannose residues.

O-linked glycans (O-glycans)

O-glycans are chains of oligosaccharides conjugated to a protein by the oxygen atom of a serine or threonine, and they contain fewer branches and fewer mannose residues than N-glycans.

O-glycans are abundant in animal secretions such as mucus on epithelia, and mucin glycoproteins can be 80% O-glycans en masse and form protective hydrated gels. Most O-glycans are attached to a protein by a GalNac monosaccharide.

Analysis of site-specific N-glycan remodeling in the endoplasmic reticulum and the Golgi.
Glycobiology. 2015 Dec;25(12):1335-49. doi: 10.1093/glycob/cwv058.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4634314/

Higel F, Seidl A, Sörgel F, Friess W. N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins. Eur J Pharm Biopharm. 2016 Mar;100:94-100. doi: 10.1016/j.ejpb.2016.01.005. Epub 2016 Jan 13. PMID: 26775146. https://doi.org/10.1016/j.ejpb.2016.01.005.

[600] Stepan H, Staudacher E.
Optimization of monosaccharide determination using anthranilic acid and 1-phenyl-3-methyl-5-pyrazolone for gastropod analysis.
Anal Biochem. 2011 Nov 1;418(1):24-9. doi: 10.1016/j.ab.2011.07.005.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3169793/

[601] Lowe JB, Marth JD.
A genetic approach to Mammalian glycan function.
Annu Rev Biochem. 2003;72:643-91. doi: 10.1146/annurev.biochem.72.121801.161809.
https://pubmed.ncbi.nlm.nih.gov/12676797/

Esmail S, Manolson MF.
Advances in understanding N-glycosylation structure, function, and regulation in health and disease.
Eur J Cell Biol. 2021 Sep-Nov;100(7-8):151186. doi: 10.1016/j.ejcb.2021.151186.
https://doi.org/10.1016/j.ejcb.2021.151186

Recently, it was discovered that O-linked GlcNac directly interacts with cellular metabolism and phosphorylation[602] and that O-GlcNac glycans can act as direct sensors of physiological state. [603]
Glycosaminoglycans are also attached to proteins by the oxygen atom of a serine, but they are not typically classified as oxygen-bound glycans.

Glycosaminoglycans and proteoglycans

Glycosaminoglycans (GAGs) are among the largest molecules synthesized by animals.[604] Their synthesis begins with an O-linked xylose, which is then elongated by two galactose molecules.
Many thousands of N-acetylglucosamine and glucuronic acid disaccharides are added to this initial trisaccharide to form giant chains.
Importantly, GAGs undergo further modifications: glucuronic acid can be epimerized into hyduronic acid, and N-acetylglucosamines can be N-deacetylated and N-sulfated, N-acetylated, or O-sulfated.
The six mammalian glycosaminoglycans, chondroitin sulfate (**CS)**, dermatan sulfate (**DS)**, heparin (**HP)**, heparan sulfate (**HS**)[605] , hyaluronan (**HA**)[606] and keratan sulfate (**KS**)[607] , are linear polysaccharides consisting of disaccharide repeats.
These post-translational modifications generate complex chemical patterns along each GAG chain with important signaling functions. [608]

---

[602] Zhao L, Feng Z, Yang X, Liu J.
The regulatory roles of O-GlcNAcylation in mitochondrial homeostasis and metabolic syndrome.
Free Radic Res. 2016 Oct;50(10):1080-1088. doi: 10.1080/10715762.2016.1239017.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5466075/

[603] Butkinaree C, Park K, Hart GW.
O-linked beta-N-acetylglucosamine (O-GlcNAc): Extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress.
Biochim Biophys Acta. 2010 Feb;1800(2):96-106. doi: 10.1016/j.bbagen.2009.07.018.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2815129/

[604] Casale J, Crane JS.
Biochemistry, Glycosaminoglycans. [Updated 2022 Mar 27]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK544295/.

[605] Gallagher J.
Fell-Muir Lecture: Heparan sulphate and the art of cell regulation: a polymer chain conducts the protein orchestra.
Int J Exp Pathol. 2015 Aug;96(4):203-31. doi: 10.1111/iep.12135.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4561558/

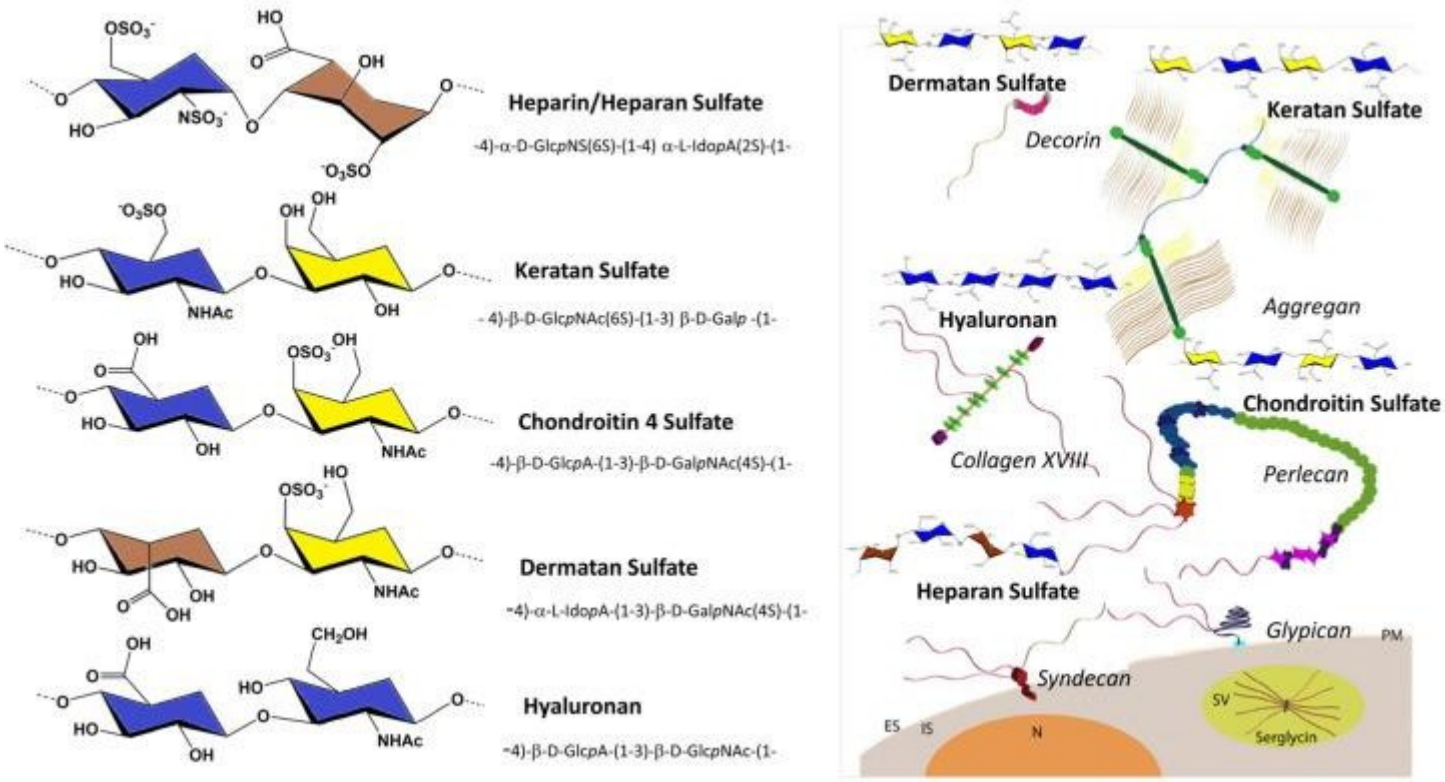
Li JP, Kusche-Gullberg M.
Heparan Sulfate: Biosynthesis, Structure, and Function.
Int Rev Cell Mol Biol. 2016;325:215-73. doi: 10.1016/bs.ircmb.2016.02.009.
https://pubmed.ncbi.nlm.nih.gov/27241222/

[606] Garantziotis S, Savani RC.
Hyaluronan biology: A complex balancing act of structure, function, location and context.
Matrix Biol. 2019 May;78-79:1-10. doi: 10.1016/j.matbio.2019.02.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6774756/

[607] Caterson B, Melrose J.
Keratan sulfate, a complex glycosaminoglycan with unique functional capability.
Glycobiology. 2018 Apr 1;28(4):182-206. doi: 10.1093/glycob/cwy003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5993099/

Pomin VH.
Keratan sulfate: an up-to-date review.
Int J Biol Macromol. 2015 Jan;72:282-9. doi: 10.1016/j.ijbiomac.2014.08.029.
https://pubmed.ncbi.nlm.nih.gov/25179279/

[608] Vallet SD, Clerc O, Ricard-Blum S.
Glycosaminoglycan-Protein Interactions: The First Draft of the Glycosaminoglycan Interactome.
J Histochem Cytochem. 2021 Feb;69(2):93-104. doi: 10.1369/0022155420946403.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7841700/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8615939/
Representation of the chemical constitution of the five families of GAGs and six categories of proteoglycans (aggrecan; decorin, perlecan and collagen; glypican; syndecan and serglycan). ES, extracellular; È, intracellular; N, nucleus; SV, secretory vesicle.

GAGs play numerous biological roles, ranging from embryonic development[609] , extracellular matrix (ECM) assembly, to regulation of cell signaling[610] in various physiological and pathological contexts[611] such as angiogenesis[612] , cancer[613] ,

[609] Kramer KL.
Specific sides to multifaceted glycosaminoglycans are observed in embryonic development.
Semin Cell Dev Biol. 2010 Aug;21(6):631-7. doi: 10.1016/j.semcdb.2010.06.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2923045/

Poulain FE, Yost HJ.
Heparan sulfate proteoglycans: a sugar code for vertebrate development?
Development. 2015 Oct 15;142(20):3456-67. doi: 10.1242/dev.098178.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4631762/

[610] Nikitovic D, Pérez S.
Preface for the Special Issue on the Exploration of the Multifaceted Roles of Glycosaminoglycans: GAGs.
Biomolecules. 2021 Nov 4;11(11):1630. doi: 10.3390/biom11111630.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8615939/

[611] Soares da Costa D, Reis RL, Pashkuleva I.
Sulfation of Glycosaminoglycans and Its Implications in Human Health and Disorders.
Annu Rev Biomed Eng. 2017 Jun 21;19:1-26. doi: 10.1146/annurev-bioeng-071516-044610.
https://pubmed.ncbi.nlm.nih.gov/28226217/

[612] Chiodelli P, Bugatti A, Urbinati C, Rusnati M.
Heparin/Heparan sulfate proteoglycans glycomic interactome in angiogenesis: biological implications and therapeutic use.
Molecules. 2015 Apr 10;20(4):6342-88. doi: 10.3390/molecules20046342.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6272510/

Spinelli FM, Vitale DL, Demarchi G, Cristina C, Alaniz L.
The immunological effect of hyaluronan in tumor angiogenesis.
Clin Transl Immunology. 2015 Dec 4;4(12):e52. doi: 10.1038/cti.2015.35.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4685440/

[613] Morla S.

inflammation[614] , neural development[615] , neurodegenerative diseases[616] and host-pathogen interactions.[617] These roles are mediated by GAG interactions with a variety of proteins, including soluble proteins (growth factors, morphogens, and chemokines[618]), ECM proteins and bioactive fragments, membrane receptors such as integrins[619] and lipoproteins[620] .

Glycosaminoglycans and Glycosaminoglycan Mimetics in Cancer and Inflammation.
Int J Mol Sci. 2019 Apr 22;20(8):1963. doi: 10.3390/ijms20081963.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6514582/

Vitale D, Kumar Katakam S, Greve B, Jang B, Oh ES, Alaniz L, Götte M.
Proteoglycans and glycosaminoglycans as regulators of cancer stem cell function and therapeutic resistance.
FEBS J. 2019 Aug;286(15):2870-2882. doi: 10.1111/febs.14967.
https://febs.onlinelibrary.wiley.com/doi/epdf/10.1111/febs.14967

[614] Johnson P, Arif AA, Lee-Sayer SSM, Dong Y.
Hyaluronan and its interactions with immune cells in the healthy and inflamed lung.
Front Immunol. 2018 Nov 29;9:2787. doi: 10.3389/fimmu.2018.02787.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6281886/

[615] Saied-Santiago K, Bülow HE.
Diverse roles for glycosaminoglycans in neural patterning.
Dev Dyn. 2018 Jan;247(1):54-74. doi: 10.1002/dvdy.24555.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5866094/

[616] Quittot N, Sebastiao M, Bourgault S.
Modulation of amyloid assembly by glycosaminoglycans: from mechanism to biological significance.
Biochem Cell Biol. 2017 Jun;95(3):329-337. doi: 10.1139/bcb-2016-0236.
https://pubmed.ncbi.nlm.nih.gov/28177755/

Nishitsuji K, Uchimura K.
Sulfated glycosaminoglycans in protein aggregation diseases.
Glycoconj J. 2017 Aug;34(4):453-466. doi: 10.1007/s10719-017-9769-4.
https://pubmed.ncbi.nlm.nih.gov/28401373/
[617] Aquino RS, Park PW.
Glycosaminoglycans and infection.
Front Biosci (Landmark Ed). 2016 Jun 1;21(6):1260-77. doi: 10.2741/4455.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975577/

[618] Monneau Y, Arenzana-Seisdedos F, Lortat-Jacob H.
The sweet spot: how GAGs help chemokines guide migrating cells.
J Leukoc Biol. 2016 Jun;99(6):935-53. doi: 10.1189/jlb.3MR0915-440R.
https://pubmed.ncbi.nlm.nih.gov/26701132/

Proudfoot AEI, Johnson Z, Bonvin P, Handel TM.
Glycosaminoglycan Interactions with Chemokines Add Complexity to a Complex System.
Pharmaceuticals (Basel). 2017 Aug 9;10(3):70. doi: 10.3390/ph10030070. PMID: 28792472; PMCID: PMC5620614.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5620614/

[619] Vorup-Jensen T, Chi L, Gjelstrup LC, Jensen UB, Jewett CA, Xie C, Shimaoka M, Linhardt RJ, Springer TA.
Binding between the integrin alphaXbeta2 (CD11c/CD18) and heparin.
J Biol Chem. 2007 Oct 19;282(42):30869-77. doi: 10.1074/jbc.M706114200.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4142650/

Faye C, Moreau C, Chautard E, Jetne R, Fukai N, Ruggiero F, Humphries MJ, Olsen BR, Ricard-Blum S.
Molecular interplay between endostatin, integrins, and heparan sulfate.
J Biol Chem. 2009 Aug 14;284(33):22029-22040. doi: 10.1074/jbc.M109.002840.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2755927/

[620] Ma Z, Mao C, Jia Y, Fu Y, Kong W.
Extracellular matrix dynamics in vascular remodeling.
Am J Physiol Cell Physiol. 2020 Sep 1;319(3):C481-C499. doi: 10.1152/ajpcell.00147.2020.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7509265/

Olsson U, Ostergren-Lundén G, Moses J.
Glycosaminoglycan-lipoprotein interaction.
Glycoconj J. 2001 Oct;18(10):789-97. doi: 10.1023/a:1021155518464.
https://pubmed.ncbi.nlm.nih.gov/12441668/

GAGs also bind to numerous pathogens[621] , including viruses[622] , bacteria[623] , parasites[624] and fungi. [625] The interaction of HS with a number of viruses contributes to their binding to host cells or their increased concentration on the cell surface, while HS-proteoglycans may act as entry receptors for some viruses. [626]

[621] Aquino RS, Park PW.
Glycosaminoglycans and infection.
Front Biosci (Landmark Ed). 2016 Jun 1;21(6):1260-77. doi: 10.2741/4455.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975577/

Aquino RS, Lee ES, Park PW.
Diverse functions of glycosaminoglycans in infectious diseases.
Prog Mol Biol Transl Sci. 2010;93:373-94. doi: 10.1016/S1877-1173(10)93016-0.
https://pubmed.ncbi.nlm.nih.gov/20807653/

Kamhi E, Joo EJ, Dordick JS, Linhardt RJ.
Glycosaminoglycans in infectious disease.
Biol Rev Camb Philos Soc. 2013 Nov;88(4):928-43. doi: 10.1111/brv.12034.
https://pubmed.ncbi.nlm.nih.gov/23551941/

Jinno A, Park PW.
Role of glycosaminoglycans in infectious disease.
Methods Mol Biol. 2015;1229:567-85. doi: 10.1007/978-1-4939-1714-3_45.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4624626/

[622] Ströh LJ, Stehle T.
Glycan Engagement by Viruses: Receptor Switches and Specificity.
Annu Rev Virol. 2014 Nov;1(1):285-306. doi: 10.1146/annurev-virology-031413-085417.
https://pubmed.ncbi.nlm.nih.gov/26958723/

Dietrich MH, Harprecht C, Stehle T.
The bulky and the sweet: How neutralizing antibodies and glycan receptors compete for virus binding.
Protein Sci. 2017 Dec;26(12):2342-2354. doi: 10.1002/pro.3319.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5699497/

[623] García B, Merayo-Lloves J, Martin C, Alcalde I, Quirós LM, Vazquez F.
Surface Proteoglycans as Mediators in Bacterial Pathogens Infections.
Front Microbiol. 2016 Feb 24;7:220. doi: 10.3389/fmicb.2016.00220.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4764700/

[624] Merida-de-Barros DA, Chaves SP, Belmiro CLR, Wanderley JLM.
Leishmaniasis and glycosaminoglycans: a future therapeutic strategy?
Parasit Vectors. 2018 Oct 3;11(1):536. doi: 10.1186/s13071-018-2953-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6171297/

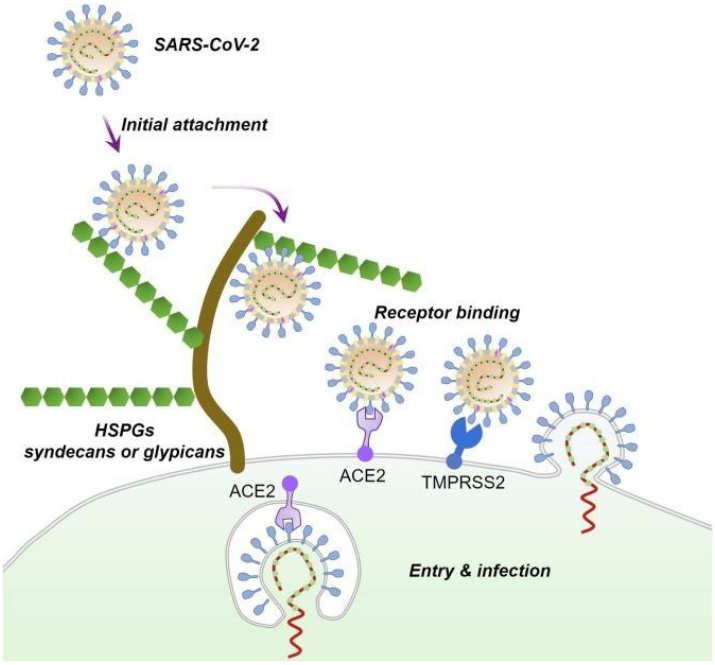
Fatoux-Ardore M, Peysselon F, Weiss A, Bastien P, Pratlong F, Ricard-Blum S.
Large-scale investigation of Leishmania interaction networks with host extracellular matrix by surface plasmon resonance imaging.
Infect Immun. 2014 Feb;82(2):594-606. doi: 10.1128/IAI.01146-13. Epub 2013 Nov 25. Erratum in: Infect Immun. 2014 Apr;82(4):1741.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3911396/

[625] Aquino RS, Park PW.
Glycosaminoglycans and infection.
Front Biosci (Landmark Ed). 2016 Jun 1;21(6):1260-77. doi: 10.2741/4455.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4975577/

Jinno A, Park PW.
Role of glycosaminoglycans in infectious disease.
Methods Mol Biol. 2015;1229:567-85. doi: 10.1007/978-1-4939-1714-3_45.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4624626/

[626] Cagno V, Tseligka ED, Jones ST, Tapparel C.
Heparan Sulfate Proteoglycans and Viral Attachment: True Receptors or Adaptation Bias?
Viruses. 2019 Jul 1;11(7):596. doi: 10.3390/v11070596.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6669472/

It has recently been reported that HP binds to both the monomeric and trimeric spike glycoprotein of SARS-CoV-2. The interaction between HP and the S1 SARS-CoV-2 spike protein is mediated by the receptor binding domain of the protein. [627]



[627] Möller S, Theiß J, Deinert TIL, Golat K, Heinze J, Niemeyer D, Wyrwa R, Schnabelrauch M, Bogner E. High-Sulfated Glycosaminoglycans Prevent Coronavirus Replication.
Viruses. 2022 Feb 17;14(2):413. doi: 10.3390/v14020413.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8877876/

Mycroft-West CJ, et al.
Heparin Inhibits Cellular Invasion by SARS-CoV-2: Structural Dependence of the Interaction of the Spike S1 Receptor-Binding Domain with Heparin.
Thromb Haemost. 2020 Dec;120(12):1700-1715. doi: 10.1055/s-0040-1721319.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7869224/

Clausen TM, et al
SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2.
Cell. 2020 Nov 12;183(4):1043-1057.e15. doi: 10.1016/j.cell.2020.09.033.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7489987/

West, Dunhao Su, et al.
The 2019 coronavirus (SARS-CoV-2) surface protein (Spike) S1 Receptor Binding Domain undergoes conformational change upon heparin binding Courtney Mycroft-
bioRxiv 2020.02.29.971093; doi: https://doi.org/10.1101/2020.02.29.971093
https://www.biorxiv.org/content/10.1101/2020.02.29.971093v2.full.pdf

Kalra RS, Kandimalla R.
Engaging the spikes: heparan sulfate facilitates SARS-CoV-2 spike protein binding to ACE2 and potentiates viral infection.
Signal Transduct Target Ther. 2021 Jan 29;6(1):39. doi: 10.1038/s41392-021-00470-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7844799/

Paiardi G, Richter S, Oreste P, Urbinati C, Rusnati M, Wade RC.
The binding of heparin to spike glycoprotein inhibits SARS-CoV-2 infection by three mechanisms.
J Biol Chem. 2022 Feb;298(2):101507. doi: 10.1016/j.jbc.2021.101507.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8683219/

Kim SY, Jin W, Sood A, Montgomery DW, Grant OC, Fuster MM, Fu L, Dordick JS, Woods RJ, Zhang F, Linhardt RJ.
Characterization of heparin and severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) spike glycoprotein binding interactions.
Antiviral Res. 2020 Sep;181:104873. doi: 10.1016/j.antiviral.2020.104873.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7347485/

Schuurs ZP, et al
Evidence of a putative glycosaminoglycan binding site on the glycosylated SARS-CoV-2 spike protein N-terminal domain.
Comput Struct Biotechnol J. 2021;19:2806-2818. doi: 10.1016/j.csbj.2021.05.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8093007/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7816574/
One possible mechanism for SARS-CoV-2 entry and infection. In the early stage of the infection process, SARS-CoV-2 may first interact with HSPGs on the surface of susceptible cells using the S protein protruding from the virus particle. This initial attack may promote subsequent binding of the virus to the high-affinity ACE2 entry receptor. The transmembrane protease serine 2 (TMPRSS2) on the host cell surface and other host cell proteases can aid in virus entry by cleaving the S protein at the S1/S2 and/or S2' sites.

Glycoaminoglycan-specific protein-protein interactions[628] and with microbial proteins are being intensively studied as important targets for pharmacological interventions. [629]

As will be discussed in more detail later, heparin binding accelerates the aggregation of pathological amyloid proteins present in the brain, and Idrees et al[630] demonstrated by molecular docking studies that SARS-CoV-2 S1 RBD binds to a number of heparin-binding proteins prone to aggregation including Aβ, α-synuclein, tau, prions, and TDP-43 RRM.
These interactions suggest that the heparin binding site on S1 protein could promote the binding of amyloid proteins to the viral surface and thus could initiate the aggregation of these proteins and consequently lead to neurodegeneration in the brain.

Glycosaminoglycans and gut microbiota

Another very important function of glycosaminoglycans is related to their presence in the human colon in free forms and as part of proteoglycans.
Their utilization is critical for the colonization and proliferation of gut bacteria and also for host health. Therefore, it is essential to determine the members of the gut bacteria that degrade GAGs and their enzymatic machinery for GAG depolymerization.[631]

---

[628] Almer J, Gesslbauer B, Kungl AJ.
Therapeutic strategies to target microbial protein-glycosaminoglycan interactions.
Biochem Soc Trans. 2018 Dec 17;46(6):1505-1515. doi: 10.1042/BST20170485.
https://pubmed.ncbi.nlm.nih.gov/30381333/

Vallet SD, Berthollier C, Ricard-Blum S.
The glycosaminoglycan interactome 2.0.
Am J Physiol Cell Physiol. 2022 Jun 1;322(6):C1271-C1278. doi: 10.1152/ajpcell.00095.2022.
https://pubmed.ncbi.nlm.nih.gov/35544698/

Shi D, Sheng A, Chi L.
Glycosaminoglycan-Protein Interactions and Their Roles in Human Disease.
Front Mol Biosci. 2021 Mar 9;8:639666. doi: 10.3389/fmolb.2021.639666.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7985165/

Raman R, Sasisekharan V, Sasisekharan R.
Structural insights into biological roles of protein-glycosaminoglycan interactions.
Chem Biol. 2005 Mar;12(3):267-77. doi: 10.1016/j.chembiol.2004.11.020.
https://pubmed.ncbi.nlm.nih.gov/15797210/

Kogut MM, Marcisz M, Samsonov SA.
Modeling glycosaminoglycan-protein complexes.
Curr Opin Struct Biol. 2022 Apr;73:102332. doi: 10.1016/j.sbi.2022.102332.
https://pubmed.ncbi.nlm.nih.gov/35152187/

[629] Almer J, Gesslbauer B, Kungl AJ.
Therapeutic strategies to target microbial protein-glycosaminoglycan interactions.
Biochem Soc Trans. 2018 Dec 17;46(6):1505-1515. doi: 10.1042/BST20170485.
https://pubmed.ncbi.nlm.nih.gov/30381333/

[630] Idrees D, Kumar V.
SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration.
Biochem Biophys Res Commun. 2021 May 21;554:94-98. doi: 10.1016/j.bbrc.2021.03.100.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7988450/

[631] Rawat PS, Seyed Hameed AS, Meng X, Liu W.
Utilization of glycosaminoglycans by the human gut microbiota: participating bacteria and their enzymatic machineries.
Gut Microbes. 2022 Jan-Dec;14(1):2068367. doi: 10.1080/19490976.2022.2068367.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9067506/

Koropatkin NM, Cameron EA, Martens EC.

The human gut microbiota (HGM) is now considered one of the most metabolically active "organs" of the human host[632] and contributes to human health in numerous ways.[633]
HGM collectively catabolizes indigestible fiber and several host glycans to produce short-chain fatty acids (SCFAs).[634]
In particular, colonic glycans, including mucosal carbohydrates, glycosaminoglycans (GAGs) and glycosphingolipid, provide a continuous source of nutrition for the HGM.[635]

How glycan metabolism shapes the human gut microbiota.
Nat Rev Microbiol. 2012 Apr 11;10(5):323-35. doi: 10.1038/nrmicro2746.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4005082/

Tuncil YE, Xiao Y, Porter NT, Reuhs BL, Martens EC, Hamaker BR.
Reciprocal Prioritization to Dietary Glycans by Gut Bacteria in a Competitive Environment Promotes Stable Coexistence.
mBio. 2017 Oct 10;8(5):e01068-17. doi: 10.1128/mBio.01068-17.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5635687/

Eilam O, Zarecki R, Oberhardt M, Ursell LK, Kupiec M, Knight R, Gophna U, Ruppin E.
Glycan degradation (GlyDeR) analysis predicts mammalian gut microbiota abundance and host diet-specific adaptations.
mBio. 2014 Aug 12;5(4):e01526-14. doi: 10.1128/mBio.01526-14.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4145686/

Ouwerkerk JP, de Vos WM, Belzer C.
Glycobiome: bacteria and mucus at the epithelial interface.
Best Pract Res Clin Gastroenterol. 2013 Feb;27(1):25-38. doi: 10.1016/j.bpg.2013.03.001.
https://pubmed.ncbi.nlm.nih.gov/23768550/

[632] Baquero F, Nombela C.
The microbiome as a human organ.
Clin Microbiol Infect. 2012 Jul;18 Suppl 4:2-4. doi: 10.1111/j.1469-0691.2012.03916.x.
https://doi.org/10.1111/j.1469-0691.2012.03916.x

Yadav M, Verma MK, Chauhan NS.
A review of metabolic potential of human gut microbiome in human nutrition.
Arch Microbiol. 2018 Mar;200(2):203-217. doi: 10.1007/s00203-017-1459-x
https://pubmed.ncbi.nlm.nih.gov/29188341/

[633] Louis P, Hold GL, Flint HJ.
The gut microbiota, bacterial metabolites and colorectal cancer.
Nat Rev Microbiol. 2014 Oct;12(10):661-72. doi: 10.1038/nrmicro3344.
https://pubmed.ncbi.nlm.nih.gov/25198138/

[634] Cameron EA, Sperandio V.
Frenemies: Signaling and Nutritional Integration in Pathogen-Microbiota-Host Interactions.
Cell Host Microbe. 2015 Sep 9;18(3):275-84. doi: 10.1016/j.chom.2015.08.007.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4567707/

Cockburn DW, Koropatkin NM.
Polysaccharide Degradation by the Intestinal Microbiota and Its Influence on Human Health and Disease.
J Mol Biol. 2016 Aug 14;428(16):3230-3252. doi: 10.1016/j.jmb.2016.06.021.
https://pubmed.ncbi.nlm.nih.gov/27393306/

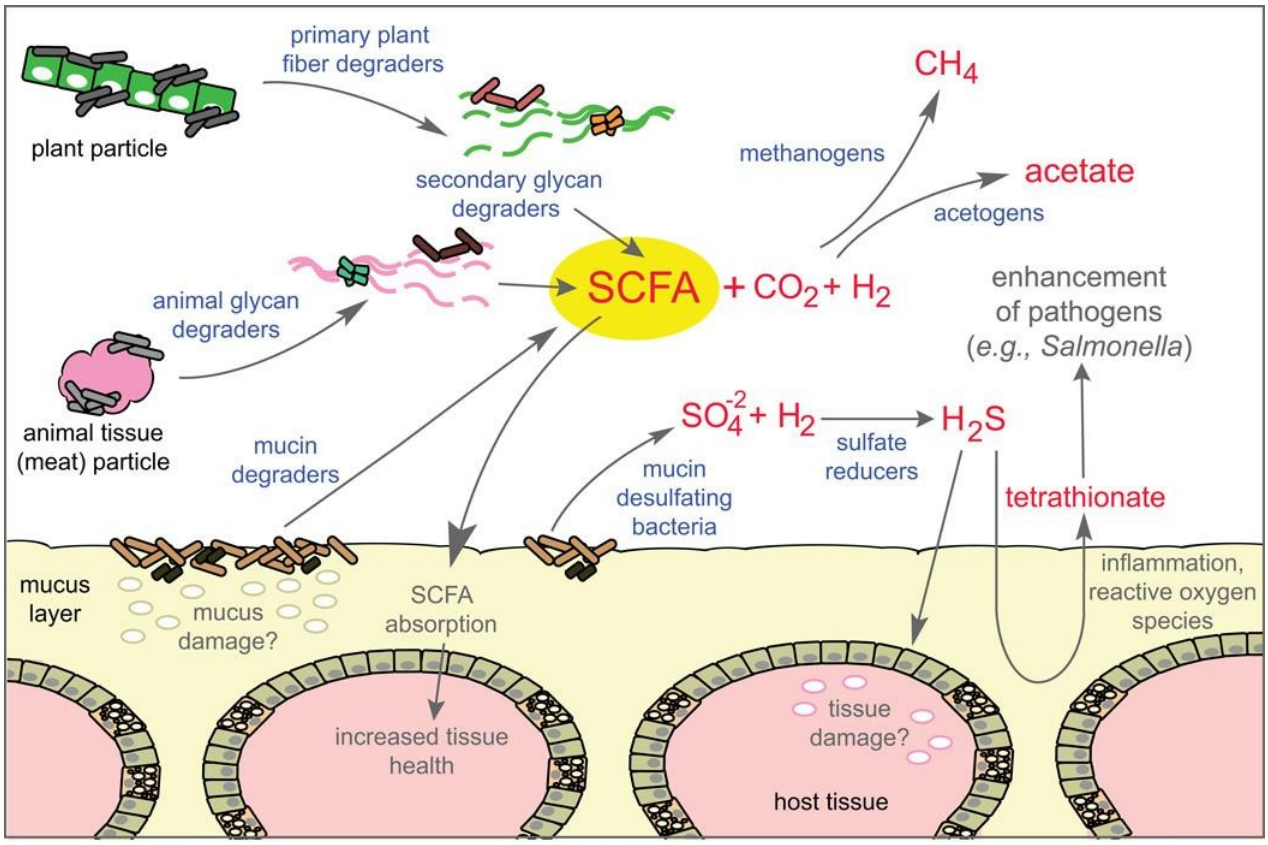
Liu F, Zhang N, Li Z, Wang X, Shi H, Xue C, Li RW, Tang Q.
Chondroitin sulfate disaccharides modified the structure and function of the murine gut microbiome under healthy and stressed conditions.
Sci Rep. 2017 Jul 28;7(1):6783. doi: 10.1038/s41598-017-05860-6.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5533764/

Pan L, Ai X, Fu T, Ren L, Shang Q, Li G, Yu G.
In vitro fermentation of hyaluronan by human gut microbiota: Changes in microbiota community and potential degradation mechanism.
Carbohydr Polym. 2021 Oct 1;269:118313. doi: 10.1016/j.carbpol.2021.118313.
https://pubmed.ncbi.nlm.nih.gov/34294327/

Coker JK, Moyne O, Rodionov DA, Zengler K.
Carbohydrates great and small, from dietary fiber to sialic acids: How glycans influence the gut microbiome and affect human health.
Gut Microbes. 2021 Jan-Dec;13(1):1-18. doi: 10.1080/19490976.2020.1869502.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7899658/

[635] Hooper LV, Midtvedt T, Gordon JI.
How host-microbial interactions shape the nutrient environment of the mammalian intestine.
Annu Rev Nutr. 2002;22:283-307. doi: 10.1146/annurev.nutr.22.011602.092259.

In contrast, carbohydrates in the diet can vary in types and amounts depending on diet and meal timing.[636]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4005082/
Microhabitat glycans and food chains in the gut
An illustration of the ways in which different gut microorganisms are thought to interact during the processing of various glycan substrates. Digest derived from plant cell wall or meat particles will be rich in source-specific glycans, such as cellulose, hemicellulose and pectin (plant) or glycosaminoglycans and cellular glycoproteins (meat). These types of nutrients are likely to enter the distal intestine as particulate forms that will be attacked by primary glycan degraders (e.g., Roseburia, Eubacterium, Clostridium, Ruminococcus, and Bifidobacterium species) that are able to bind directly to these insoluble particles and digest their glycans. After the initial degradation of glycan-containing particles, more soluble glycan fragments can be digested by other bacteria, which contribute to the released pool of SCFA fermentation products derived from primary and secondary glycan degraders. A similar food chain of primary and secondary degraders in the mucus layer has been proposed; whereby some primary species are able to directly degrade high molecular weight mucin glycoproteins and others are optimized to target the resulting oligosaccharide products. Fermentation of bacterial glycans is enhanced by removal of downstream H_2 consumers, which convert this gas to methane, acetate or hydrogen sulfide depending on the types of microorganisms present. The latter pathway also requires free sulfate, which can be derived from many food products, but also from the degradation of animal proteins, sulfated glycans abundant in animal tissues (e.g., chondroitin sulfate) or in mucus. The resulting H_2S is toxic to host cells, but is readily

---

https://pubmed.ncbi.nlm.nih.gov/12055347/

Pudlo NA, Urs K, Kumar SS, German JB, Mills DA, Martens EC.
Symbiotic Human Gut Bacteria with Variable Metabolic Priorities for Host Mucosal Glycans.
mBio. 2015 Nov 10;6(6):e01282-15. doi: 10.1128/mBio.01282-15.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4659458/

Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC.
The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria.
Proc Natl Acad Sci U S A. 2008 Sep 30;105(39):15064-9. doi: 10.1073/pnas.0803124105.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2567493/

[636] Vogt SL, Peña-Díaz J, Finlay BB.
Chemical communication in the gut: Effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens.
Anaerobe. 2015 Aug;34:106-15. doi: 10.1016/j.anaerobe.2015.05.002. epub 2015 May 7. PMID: 25958185.
https://pubmed.ncbi.nlm.nih.gov/25958185/.

Li Z, Quan G, Jiang X, Yang Y, Ding X, Zhang D, Wang X, Hardwidge PR, Ren W, Zhu G.
Effects of metabolites derived from gut microbiota and hosts on pathogens.
Front Cell Infect Microbiol. 2018 Sep 14;8:314. doi: 10.3389/fcimb.2018.00314.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6152485/

metabolized and detoxified by colonic tissue to form thiosulfate. In the context of mucosal inflammation, thiosulfate can be converted to tetrathionate by reactive oxygen species, an event that has recently been linked to the metabolic enhancement of the intestinal pathogen *Salmonella enterica* subspecies *typhimurium*.

Since these degradation products are also bioactive, their disordered use could be harmful.[637] In addition, the total content of individual GAGs has been shown to be altered in inflammatory bowel disorders (IBD).[638] GAG-PGs are essential for the development of intestinal tissue during embryogenesis,[639] and various HSPGs and CSPGs are involved in the modulation of cell shape, cell motility, contact inhibition, and intestinal morphogenesis.[640]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4005082/
Utilization of glycan along the length of the intestine and its potential health effects
A schematic of the color-coded human ileum and colon to reflect potential gradients of glycans (schematized according to the color bar below). The solubility and digestibility of dietary glycans passing through the lumen are variable, so each glycan is likely to be digested at a different rate. The thickness of the intestinal mucus also follows a longitudinal gradient along the intestine, but may be reciprocal to that of glycan digestibility, with the greatest thickness present in the sigmoid colon and rectum where mostly insoluble/indigestible glycans are likely to be present. The insets on the left and right show patterns of luminal and mucosal niches in the ileum and distal (sigmoid) colon. In the ileum, the mucus layer is relatively thin, the transit time of the contents is faster, and the bacteria are likely to target the more soluble and rapidly digestible glycans, such as inulin and several side chains of oligosaccharides, such as α-arabinans and β-galactans , which are commonly attached to the pectin backbone (ramnogalacturonan). In contrast, the distal colon has a much thicker mucus layer, the transit time is slower, and the residual glycans that feed bacterial growth are likely to be less soluble and thus take longer to degrade. Note the presence of inner and outer mucus layers, with bacterial colonization largely present only in the outer layer. One possible reason for the increased mucus thickness in the distal intestine could be to protect the epithelium from more prolonged exposure to more bacteria, which have more time to proliferate given the slower transit rate. It is widely accepted that higher fiber intake in the diet is beneficial for colon health. In light of this idea, it is interesting to note that the incidence of colon cancer in several developed countries in North America, Europe, and Asia shows a decrease in abundance in the distal colon and increases in more proximal regions in recent decades. One explanation offered for this phenomenon relates to changing dietary habits in these societies, particularly reduced fiber intake and increased consumption of animal fat and protein. This trend could alter the microbiota or its metabolism in the more distal regions, leading to carcinogenesis through several possible mechanisms (reduced transit time, increased production of toxic metabolites, or decreased production of protective metabolites such as butyrate).

---

[637] Desai MS, et al
A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility.
Cell. 2016 Nov 17;167(5):1339-1353.e21. doi: 10.1016/j.cell.2016.10.043.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5131798/

Tao Liao, Yan-Ping Chen, Li-Li Tan, Chang-Qing Li, Qi Wang, Shui-Qing Huang, Xin-An Huang, Qin Xu, Qing-Ping Zeng
Chondroitin sulfate flourishes gut sulfatase-secreting bacteria to damage mucus layers, leak bacterial debris, and trigger inflammatory lesions in mice bioRxiv 145714; doi: https://doi.org/10.1101/145714
https://www.biorxiv.org/content/10.1101/145714v1.full.pdf

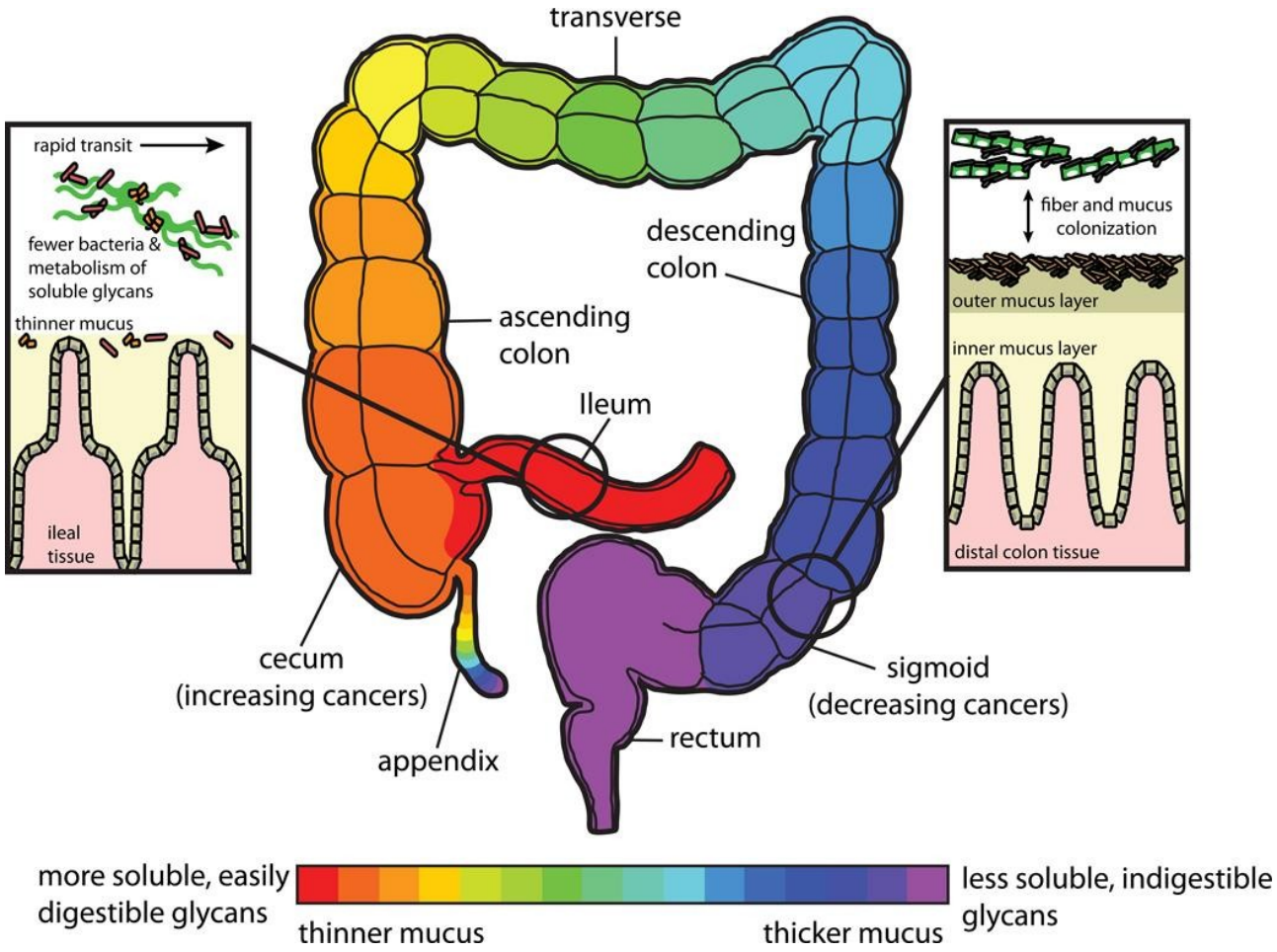
[638] Murch SH, MacDonald TT, Walker-Smith JA, Levin M, Lionetti P, Klein NJ.
Disruption of sulphated glycosaminoglycans in intestinal inflammation.
Lancet. 1993 Mar 20;341(8847):711-4. doi: 10.1016/0140-6736(93)90485-y.
https://pubmed.ncbi.nlm.nih.gov/8095623/

[639] Newgreen DF, Hartley L.
Extracellular matrix and adhesive molecules in the early development of the gut and its innervation in normal and spotting lethal rat embryos.
Acta Anat (Basel). 1995;154(4):243-60. doi: 10.1159/000147776.
https://pubmed.ncbi.nlm.nih.gov/8773711/

[640] Simon-Assmann P, Bouziges F, Vigny M, Kedinger M.
Origin and deposition of basement membrane heparan sulfate proteoglycan in the developing intestine.
J Cell Biol. 1989 Oct;109(4 Pt 1):1837-48. doi: 10.1083/jcb.109.4.1837.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2115786/

Oshiro M, Ono K, Suzuki Y, Ota H, Katsuyama T, Mori N.
Immunohistochemical localization of heparan sulfate proteoglycan in human gastrointestinal tract.
Histochem Cell Biol. 2001 May;115(5):373-80. doi: 10.1007/s004180100271.
https://pubmed.ncbi.nlm.nih.gov/11449885/

Tang F, Lord MS, Stallcup WB, Whitelock JM.
Cell surface chondroitin sulphate proteoglycan 4 (CSPG4) binds to the basement membrane heparan sulphate proteoglycan, perlecan, and is involved in cell adhesion.
J Biochem. 2018 May 1;163(5):399-412. doi: 10.1093/jb/mvy008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5905647/



Dysregulation in the production and binding of these proteoglycans to the basement membrane has been shown to be oncogenic in the colon.[641] Mice deficient in the enzyme required for the initiation of HS sulfation showed altered colonic histology and an increased rate of apoptosis of the colonic epithelium.[642] Another important function of GAG-PGs is the maintenance of intestinal barrier function.[643] Membrane homeostasis is achieved by modulating epithelial regeneration

[641] Tang F, Lord MS, Stallcup WB, Whitelock JM.
Cell surface chondroitin sulphate proteoglycan 4 (CSPG4) binds to the basement membrane heparan sulphate proteoglycan, perlecan, and is involved in cell adhesion.
J Biochem. 2018 May 1;163(5):399-412. doi: 10.1093/jb/mvy008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5905647/

[642] Jao TM, Li YL, Lin SW, Tzeng ST, Yu IS, Yen SJ, Tsai MH, Yang YC.
Alteration of colonic epithelial cell differentiation in mice deficient for glucosaminyl N-deacetylase/N-sulfotransferase 4.
Oncotarget. 2016 Dec 20;7(51):84938-84950. doi: 10.18632/oncotarget.12915.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5356710/

[643] Bode L, Salvestrini C, Park PW, Li JP, Esko JD, Yamaguchi Y, Murch S, Freeze HH.
Heparan sulfate and syndecan-1 are essential in maintaining murine and human intestinal epithelial barrier function.
J Clin Invest. 2008 Jan;118(1):229-38. doi: 10.1172/JCI32335.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2117765/

Dias AM, Pereira MS, Padrão NA, Alves I, Marcos-Pinto R, Lago P, Pinho SS.
Glycans as critical regulators of gut immunity in homeostasis and disease.
Cell Immunol. 2018 Nov;333:9-18. doi: 10.1016/j.cellimm.2018.07.007.
https://pubmed.ncbi.nlm.nih.gov/30049413/

Brazil JC, Parkos CA.
Finding the sweet spot: glycosylation mediated regulation of intestinal inflammation.
Mucosal Immunol. 2022 Feb;15(2):211-222. doi: 10.1038/s41385-021-00466-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8591159/

through HSPGs[644] and HA[645] and loss of barrier function is directly associated with enteropathy with protein loss, colitis and IBD.[646]

Proteoglycans

All GAGs, except HA (hyaluronan), are found covalently bound to specific proteins as proteoglycans. The spatial and temporal expression patterns of proteoglycans are mainly dictated by the central protein.
A proteoglycan consists of a central protein and one or more covalently attached GAG chains of unbranched polysaccharides composed of repeated disaccharide units. In most proteoglycans, GAGs constitute more than 50 percent of the total molecular mass and mediate biological functions.
GAG biosynthesis is initiated by the formation of a covalent bond between the reducing end of a xylosyl residue (Xyl) and the hydroxyl part of some serine residues within a Ser- Gly dipeptide sequence often repeated two or more times in the central protein.
This is followed by formation of the -GlcA-Gal-Xyl tetrasaccharide binding domain (where GlcA is glucuronic acid and Gal is galactose), polymerization of a characteristic disaccharide unit, and modification of the newly synthesized polysaccharide chain, catalyzed at each step by specific enzymes.

GAGs are defined by the nature (composition and chemical bonding) of the repeating disaccharide unit, which includes a hexosamine [e.g., N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNac)] and uronic acid [e.g., GlcA, hyduronic acid (IdoA)].
The characteristic disaccharide unit of **HS/heparin** is GlcAβ1→ 4GlcNAcα1→4, of **CS** is GlcAβ1→3GalNAcβ1→4, of **dermatan sulfate** (DS) is IdoAβ1→3GalNAcβ1→4, of **keratan sulfate** (KS) is Galβ1→4GalNAcβ1→3, and of **hyaluronan** (HA) is GlcAβ1→3GlcNAcβ1→4.
Except for HA, GAGs are modified in the Golgi complex by different sulfation and epimerization reactions. Because the polymerization and modification reactions do not complete, the biosynthetic process generates an exceptionally diverse range of GAG structures. [647]

---

[644] Fröhling M, et al
Syndecan-4 Modulates Epithelial Gut Barrier Function and Epithelial Regeneration in Experimental Colitis.
Inflamm Bowel Dis. 2018 Nov 29;24(12):2579-2589. doi: 10.1093/ibd/izy248.
https://pubmed.ncbi.nlm.nih.gov/30053064/

[645] Zheng L, Riehl TE, Stenson WF.
Regulation of colonic epithelial repair in mice by Toll-like receptors and hyaluronic acid.
Gastroenterology. 2009 Dec;137(6):2041-51. doi: 10.1053/j.gastro.2009.08.055.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2789856/

[646] Suzuki K, et al
Pivotal Role of Carbohydrate Sulfotransferase 15 in Fibrosis and Mucosal Healing in Mouse Colitis.
PLoS One. 2016 Jul 13;11(7):e0158967. doi: 10.1371/journal.pone.0158967.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4943596/

[647] Bartlett AH, Park PW.
Proteoglycans in host-pathogen interactions: molecular mechanisms and therapeutic implications.
Expert Rev Mol Med. 2010 Feb 1;12:e5. doi: 10.1017/S1462399409001367.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4634875/

Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M.
Functions of cell surface heparan sulfate proteoglycans.
Annu Rev Biochem. 1999;68:729-77. doi: 10.1146/annurev.biochem.68.1.729.
https://pubmed.ncbi.nlm.nih.gov/10872465/

Park PW, Reizes O, Bernfield M.
Cell surface heparan sulfate proteoglycans: selective regulators of ligand-receptor encounters.
J Biol Chem. 2000 Sep 29;275(39):29923-6. doi: 10.1074/jbc.R000008200.
https://doi.org/10.1074/jbc.R000008200

Kreuger J, Spillmann D, Li JP, Lindahl U.
Interactions between heparan sulfate and proteins: the concept of specificity.
J Cell Biol. 2006 Jul 31;174(3):323-7. doi: 10.1083/jcb.200604035.

Proteoglycans, particularly those harboring structurally diverse HS chains, bind to and regulate a multitude of biological molecules through their GAG chains. The list includes growth factors, cytokines, chemokines, proteinases, antimicrobial factors, ECM (extracellular matrix) components, and many more [648].

Although cell surface proteoglycans can act as primary receptors for some ligands, in most cases cell surface proteoglycans function as coreceptors that capture ligands and facilitate the encounter between ligands and their respective signaling receptors.

This also applies to the interaction of proteoglycans with microbial pathogens, where most HS-binding microbial pathogens use HSPGs on the cell surface as coreceptors to facilitate their interaction with secondary internalization receptors.

Proteoglycans can also regulate protein-protein interactions by affecting the stability, conformation, and oligomerization state of the ligand or receptor, and some microbial adhesins and secreted virulence factors subvert these mechanisms to enhance pathogenic activities.

In addition, proteoglycans can function as soluble molecules because they can be released from the cell surface or ECM by proteolytic cleavage.

Once solubilized, proteoglycans exhibit similar or distinct functions from their immobilized counterparts, and some bacterial pathogens are known to exploit soluble proteoglycans to inhibit host defense mechanisms.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4634875/

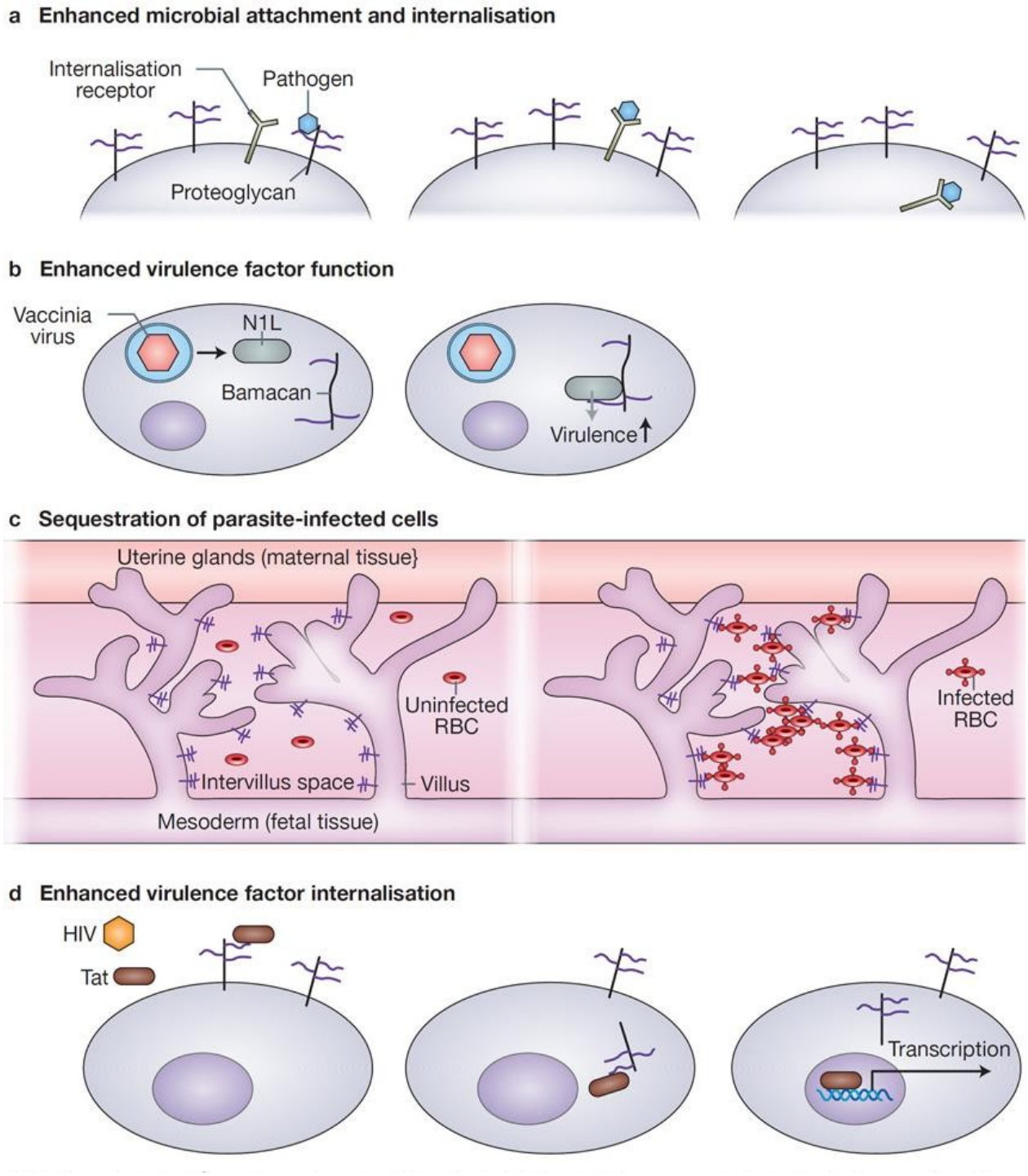
Mechanisms of proteoglycans in microbial attachment, entry and spread

(a) Increased microbial attachment and internalization. Pathogens use proteoglycans as coreceptors to increase the concentration of the pathogen on the cell surface, facilitating binding to specific secondary receptors. This binding often results in internalization of the pathogen. **(b)** Enhanced virulence factor function. The vaccination virus produces the virulence factor N1L after internalization. N1L binds to CSPG bamacan, resulting in enhanced viral growth in vitro and neurovirulence in vivo. **(c)** Sequestration of parasite-infected cells. Placental tissue expresses CS-A (purple), which binds Plasmodium falciparum-infected red blood cells, leading to their sequestration and clinical manifestations including anemia. **(d)** Increased internalization of virulence factor. HIV Tat binds to cell surface HSPGs and is then internalized where it can activate transcription. Abbreviations: CS-A, chondroitin sulfate A; CSPG, chondroitin sulfate proteoglycan; HIV, human immunodeficiency virus; HSPG, heparan sulfate proteoglycan; red blood cells, red blood cells; Tat, transactivator of transcription.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2064228/

[648] Sarkar A, Desai UR.
A Simple Method for Discovering Druggable, Specific Glycosaminoglycan-Protein Systems. Elucidation of Key Principles from Heparin/Heparan Sulfate-Binding Proteins.
PLoS One. 2015 Oct 21;10(10):e0141127. doi: 10.1371/journal.pone.0141127.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4619353/

Weiss RJ, Esko JD, Tor Y.
Targeting heparin and heparan sulfate protein interactions.
Org Biomol Chem. 2017 Jul 21;15(27):5656-5668. doi: 10.1039/c7ob01058c.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5567684/



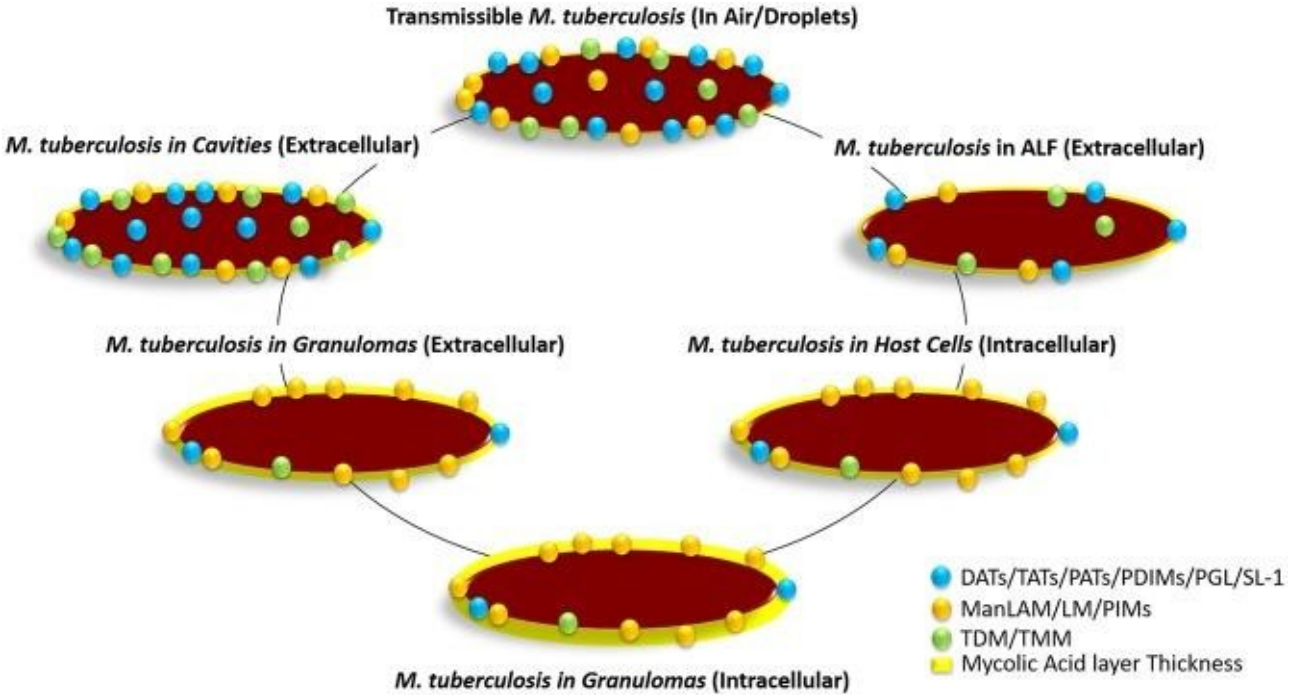
Glycoconjugated glycolipids

Glycolipids are oligosaccharides conjugated to lipid molecules. Gangliosides are sialic acid containing glycosphingolpids, forms associated with lipid rafts on the outer cell membrane. Some cell types may have outer membrane leaflets that consist almost entirely of glycolipids rather than phospholipids.[649]

[649] Schnaar RL, Suzuki A, Stanley P.
Glycosphingolipids. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME, editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 10. https://www.ncbi.nlm.nih.gov/books/NBK1909/

The spatial orientation of glycans is important because their binding is often multivalent, involving multiple chemical contacts in close proximity. The clustering of glycans on the cell surface by glycolipids can therefore have important functional effects.

An important example of a pathogen that uses glycolipids to protect itself from the host is *Mycobacterium tuberculosis*, whose cell envelope has evolved over time to make the bacterium transmissible and adaptable to the human host.
In this context, the cell envelope of *M. tuberculosis* consists of a barrier filled with lipids, some of them unique, that give the pathogen a shield against the different host environments that the mycobacterium will encounter in the different stages of infection. This lipid barrier is composed mainly of glycolipids that can be characterized by three different subsets: glycolipids containing trehalose, mannose, and 6-deoxypyranose. [650]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6930167/
Hypothetical distribution of glycolipids on the surface of the cell envelope of the bacterium M. tuberculosis at different stages of infection. It is believed that the transmissible bacterium contains a large number of glycolipids (DAT, TAT, PAT, SL-1, TMM, TDM, PDIMS, PIM, etc.) on the cell envelope, which drives t h e hydrophobicity of the surface, which in turn promotes its transmission through the air. After infection and after reaching the alveolar space,
M. tuberculosis comes into contact with alveolar lining fluid (ALF; hypophase of the lung mucosa). The ALF contains a series of homeostatic hydrolases that significantly alter the surface of the cell envelope of M. tuberculosis by removing glycolipids and lipoglycans, and thus somewhat altering the hydrophobicity of the cell surface and determining how the bacterium will interact with alveolar host cells. After phagocytosis by MAs, the bacterium has been shown to hyperproduce PIM, as well as switch to metabolic networks such as beta-oxidation, glyoxylate shunt, and reverse methylcitrate cycle. This metabolic switch within host cells allows the bacterium to break down captured and host long-chain fatty acids and cholesterol to generate acetyl-CoA and propionyl-CoA, increasing its mycolic acid production at the expense of glycolipid production. This metabolic state of the bacterium is believed to be maintained in the latency stages when the bacterium remains intracellular within granulomas. After reactivation, the metabolic state of the bacterium changes again by increasing glycolipid production at the expense of mycolic acid production, restoring the surface hydrophobicity of the bacterium while remaining extracellular within the granulomas. This hydrophobicity of the bacterial surface is further accentuated when M. tuberculosis escapes the destroyed granulomas by becoming extracellular within the cavities, where host-driven hydrolytic tissue destruction facilitates the escape of M. tuberculosis

[650] Garcia-Vilanova A, Chan J, Torrelles JB.
Underestimated Manipulative Roles of Mycobacterium tuberculosis Cell Envelope Glycolipids During Infection.
Front Immunol. 2019 Dec 18;10:2909. doi: 10.3389/fimmu.2019.02909.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6930167/

Lowary TL.
Twenty Years of Mycobacterial Glycans: Furanosides and Beyond.
Acc Chem Res. 2016 Jul 19;49(7):1379-88. doi: 10.1021/acs.accounts.6b00164.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4955529/

into the bloodstream and the airways become transmissible again, closing the loop. The relative number of molecules and size are not accurately represented reflecting published experimental data.

Glycoconjugates anchored with GPI (glycosylphosphatidylinositol)
GPI-anchored glycoproteins are complex molecules that combine a lipid, a glycan and a protein. The lipid is a phosphoinositol tail linked to a short oligosaccharide glycan, which in turn is linked to a polypeptide by a phosphoethanolamine bond.
The polypeptide components of most GPI-anchored proteins are further glycosylated and may contain N- and O-glycans and even glycosaminoglycans called glypicans.
Interestingly, GPI-anchored proteins can transfer information from one cell to another, can be released from the surface of one cell and incorporated into the membranes of other cells.[651]

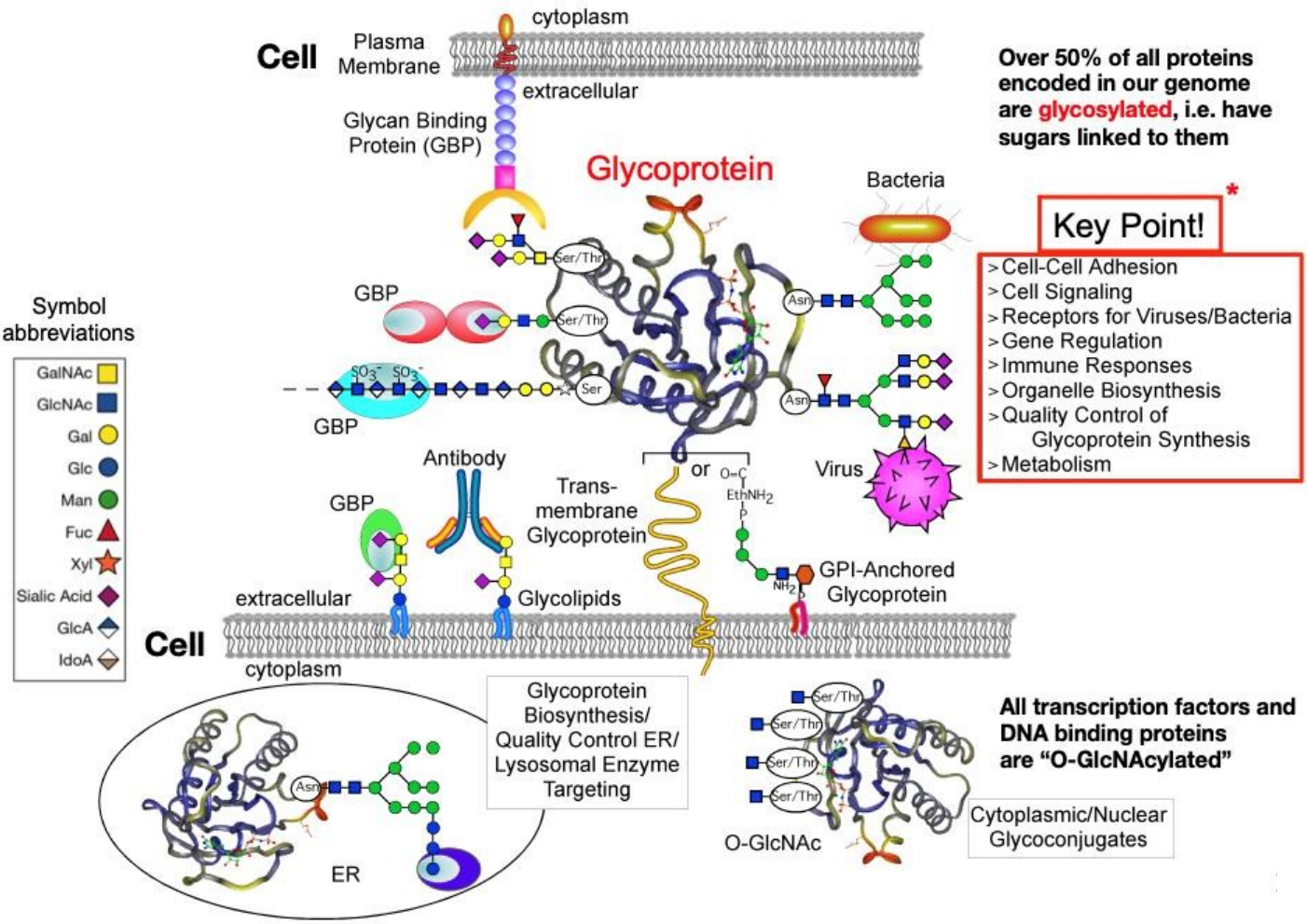
Rare glycans and glycoconjugates
There are many other classes of glycans that are less abundant than the classes described above. Some have only a few known examples; others are expressed only in particular phylogenetic lineages.
It is likely that a huge unexplored diversity of glycans exists in bacteria and archaea. Because they lack an ER-Golgi compartment, these taxa have evolved different ways of assembling glycans than eukaryotes.
It is already known that the capsids and glycoconjugates of bacteria and archaea contain a wide diversity of complex glycans, and the study of these is in its infancy.[652] Recent years have seen the discovery of many additional classes of glycans including: O-xylose, O-fucose, C-mannose and S-linked glycans on cysteine residues.[653]

[651] Ferguson MAJ, Kinoshita T, Hart GW.
Glycosylphosphatidylinositol Anchors. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME, editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 11.
https://www.ncbi.nlm.nih.gov/books/NBK1966/

[652] Schäffer C, Graninger M, Messner P.
Prokaryotic glycosylation. Proteomics. 2001 Feb;1(2):248-61. doi: 10.1002/1615-9861(200102)1:2<248::AID-PROT248>3.0.CO;2-K.
https://pubmed.ncbi.nlm.nih.gov/11680871/

[653] Freeze HH, Haltiwanger RS.
Other Classes of ER/Golgi-derived Glycans. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME, editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 12.
https://www.ncbi.nlm.nih.gov/books/NBK1947/

Defaus S, Gupta P, Andreu D, Gutiérrez-Gallego R.
Mammalian protein glycosylation--structure versus function.
Analyst. 2014 Jun 21;139(12):2944-67. doi: 10.1039/c3an02245e.
https://pubmed.ncbi.nlm.nih.gov/24779027/



https://ncfg.hms.harvard.edu/files/ncfg/files/cummings_overview_of_glycans.pdf

Glycosylation and the immune system

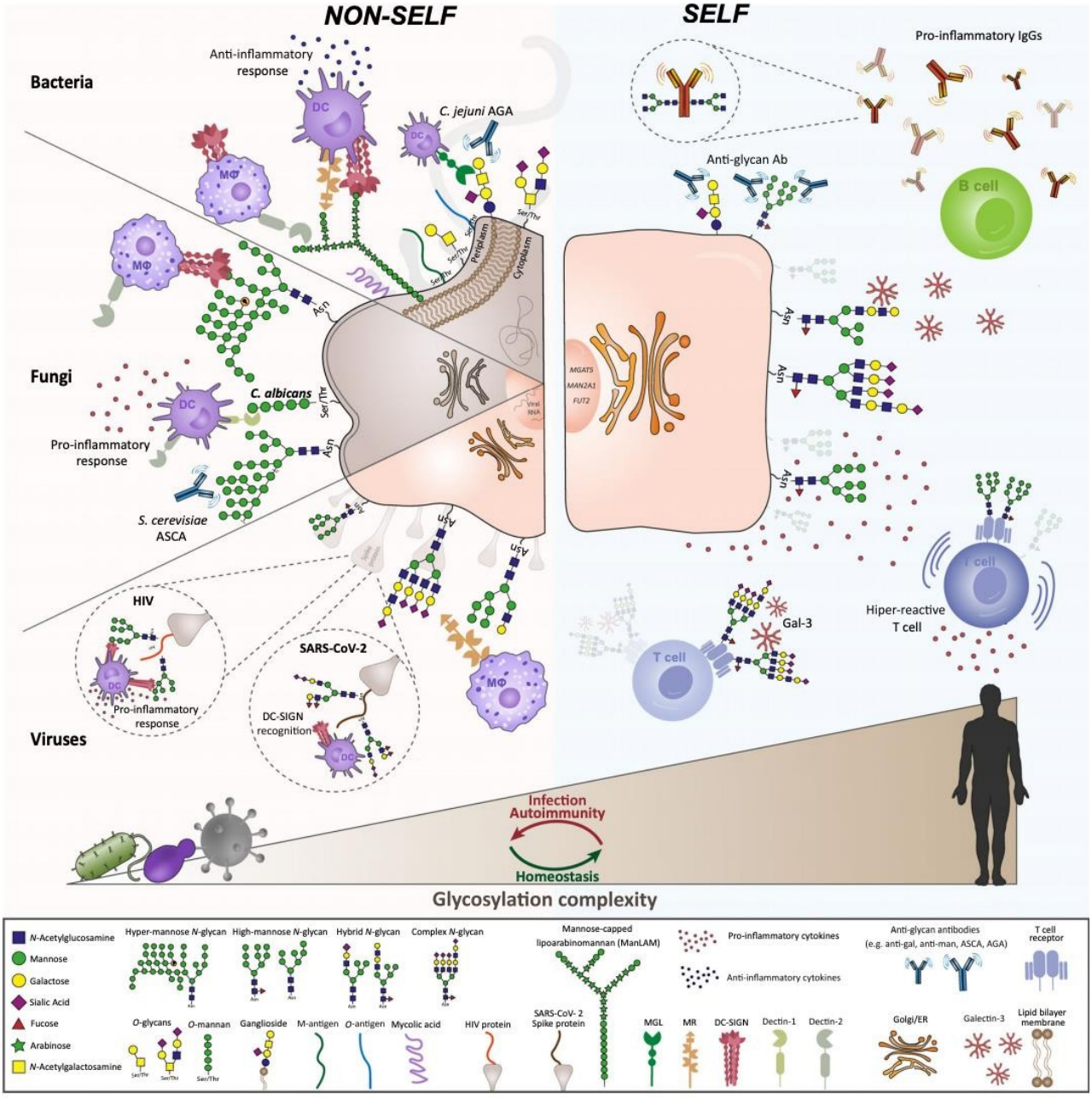
Chronic inflammation is characterized by numerous systemic physiological and biochemical changes, most of which are mediated by abundantly secreted proinflammatory cytokines.
They are the key molecules responsible for activating the proinflammatory potential of innate and adaptive immunity, which often leads to tissue destruction[654] . In addition, chronic inflammation is characterized by marked changes in glycosylation[655] .
N-glycans are found on the surface of key entities involved in the inflammatory response, including endothelial adhesion molecules, immune cells of innate and adaptive immunity, secreted immunoglobulins, and acute phase proteins (APPs).

[654] Feghali CA, Wright TM.
Cytokines in acute and chronic inflammation.
Front Biosci. 1997 Jan 1;2:d12-26. doi: 10.2741/a171.
https://pubmed.ncbi.nlm.nih.gov/9159205/

[655] Gudelj I, Lauc G, Pezer M.
Immunoglobulin G glycosylation in aging and diseases.
Cell Immunol. 2018 Nov;333:65-79. doi: 10.1016/j.cellimm.2018.07.009.
https://doi.org/10.1016/j.cellimm.2018.07.009

Groux-Degroote S, Cavdarli S, Uchimura K, Allain F, Delannoy P.
Glycosylation changes in inflammatory diseases.
Adv Protein Chem Struct Biol. 2020;119:111-156. doi: 10.1016/bs.apcsb.2019.08.008.
https://pubmed.ncbi.nlm.nih.gov/31997767/

Their N-glycan composition has been shown to be modulated by abundantly secreted proinflammatory cytokines that regulate the expression of glycotransferases (GTs) and influence the availability of the substrate required for N-glycan biosynthesis. Overall, the changes in N-glycosylation observed in chronic inflammation are diverse but highly dependent on the particular subset of immune cells. Affected features of N-glycan structure include changes in the number of branches, changes in the composition of N-glycan structure, and diversification of saccharide bonds resulting in different ligand epitopes. As a result, altered N-glycosylation can significantly affect leukocyte trafficking, trigger a shift toward more proinflammatory effector functions of leukocytes and initiate proinflammatory transformation of secreted immunoglobulins and APPs, ultimately leading to the development of various inflammatory diseases.[656]



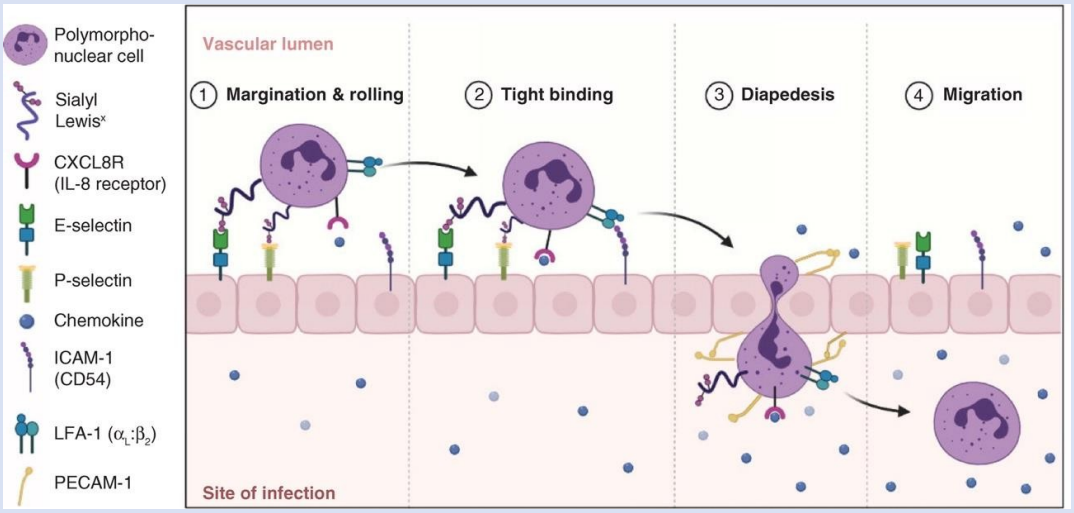
https://febs.onlinelibrary.wiley.com/doi/pdf/10.1002/1873-3468.14347

[656] Radovani B, Gudelj I.
N-Glycosylation and Inflammation; the Not-So-Sweet Relation.
Front Immunol. 2022 Jun 27;13:893365. doi: 10.3389/fimmu.2022.893365
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9272703/

Glycans as central molecules in discriminating self/non-self identity. The complexity of glycosylation is an evolutionary process in which pathogens show a distinct pattern of glycosylation compared with higher organisms such as mammals, including humans. Different glycan patterns among microorganisms and humans lay the foundation for molecular sensing and self/non-self discrimination. Both innate and adaptive immune cells are equipped with a variety of glycan-recognizing receptors (C-type lectins, galectins, and siglecs) that are able to specifically sense and recognize different glycan structures, instructing pro- or anti-inflammatory responses associated with infection, inflammation, and autoimmunity. Antibodies against specific glycan structures on the surface of microorganisms (antiglycan antibodies) are also involved in glycan-dependent recognition processes, representing important players in host immune responses and consequently in infection and inflammation. In addition, glycans can also modulate the activity and function of immune cells, such as T cells, as well as the effector functions of antibodies.

To successfully pass through the endothelium, immune cells undergo a complex process involving ligand-dependent binding followed by surface lamination, adhesion, and finally transendothelial migration[657] .
Each step in this cascade depends on the interaction between endothelial adhesion molecules and their counter-receptors expressed on the surface of leukocytes.
Key players in the leukocyte transmigration process are selectins, integrins, intercellular and vascular adhesion molecules (ICAM and VCAM), platelet endothelial cell adhesion molecules (PECAM), and junctional adhesion molecules (JAM)[658] .

Leukocyte transmigration



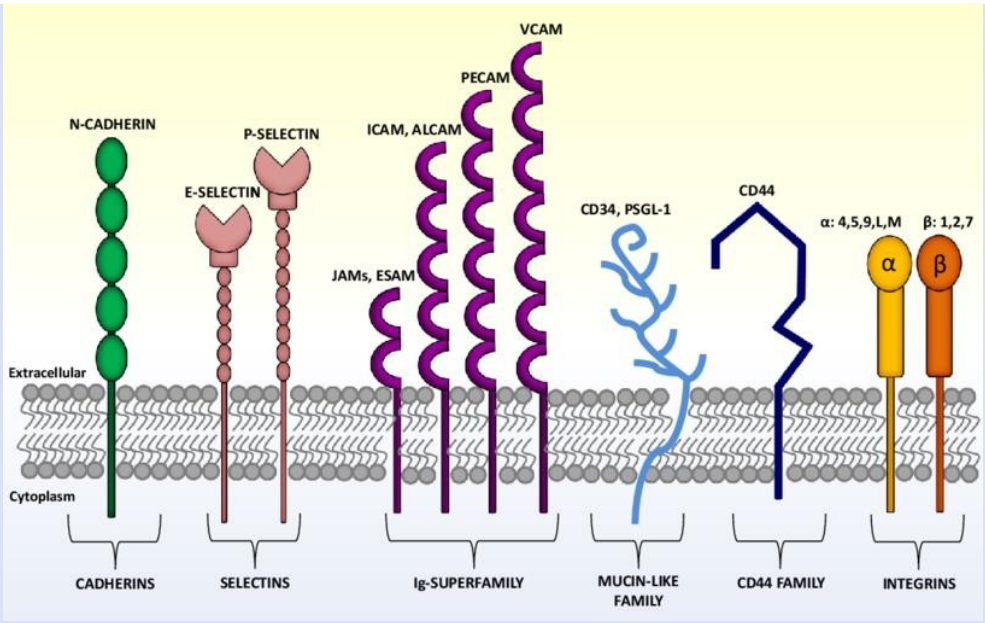
https://www.futuremedicine.com/doi/10.2217/ijh-2021-0017

Adhesion molecules

[657] Kelly M, Hwang JM, Kubes P.
Modulating leukocyte recruitment in inflammation.
J Allergy Clin Immunol. 2007 Jul;120(1):3-10. doi: 10.1016/j.jaci.2007.05.017
https://pubmed.ncbi.nlm.nih.gov/17559914/

Yano K, Gale D, Massberg S, Cheruvu PK, Monahan-Earley R, Morgan ES, Haig D, von Andrian UH, Dvorak AM, Aird WC.
Phenotypic heterogeneity is an evolutionarily conserved feature of the endothelium.
Blood. 2007 Jan 15;109(2):613-5. doi: 10.1182/blood-2006-05-026401.
https://doi.org/10.1182/blood-2006-05-026401

[658] Ley K, Laudanna C, Cybulsky MI, Nourshargh S.
Getting to the site of inflammation: the updated leukocyte adhesion cascade.
Nat Rev Immunol. 2007 Sep;7(9):678-89. doi: 10.1038/nri2156. PMID: 17717539.
https://pubmed.ncbi.nlm.nih.gov/17717539/



https://pubmed.ncbi.nlm.nih.gov/26495446/

Intercellular adhesion molecules
Adhesion molecules.
Stable adhesion

video

Cell Adhesion Molecules/ CAMs/ Integrins/ Adhesion molecules/ cadherins/selectins.
Adhesion in Leukocyte Extravasation

Most endothelial adhesion molecules are strongly N-glycosylated[659] , which is crucial for successful leukocyte trafficking as defined by the "zipper code" hypothesis[660] .
In circulation, leukocytes encounter various proteins and sugars expressed on endothelial surfaces. Efficient leukocyte adhesion is achieved only when a specific combination of an adhesion molecule protein and N-glycan is expressed[651] .
Adhesion molecules are not normally expressed in resting cells, while their expression is upregulated in inflammation, via the cytokine-induced signaling pathway, such as NF-κB[661] .

[659] Scott DW, Patel RP.
Endothelial heterogeneity and adhesion molecules N-glycosylation: implications in leukocyte trafficking in inflammation.
Glycobiology. 2013 Jun;23(6):622-33. doi: 10.1093/glycob/cwt014.
https://pubmed.ncbi.nlm.nih.gov/23445551/

[660] Renkonen J, Tynninen O, Häyry P, Paavonen T, Renkonen R.
Glycosylation might provide endothelial zipper codes for organ-specific leukocyte traffic into inflammatory sites. Am J Pathol. 2002 Aug;161(2):543-50. doi: 10.1016/S0002-9440(10)64210-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1850742/

[661] Gotsch U, Jäger U, Dominis M, Vestweber D.
Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-alpha in vivo.
Cell Adhes Commun. 1994 Apr;2(1):7-14. doi: 10.3109/15419069409014198.
https://pubmed.ncbi.nlm.nih.gov/7526954/

Rahman A, Kefer J, Bando M, Niles WD, Malik AB.
E-selectin expression in human endothelial cells by TNF-alpha-induced oxidant generation and NF-kappaB activation.
Am J Physiol. 1998 Sep;275(3):L533-44. doi: 10.1152/ajplung.1998.275.3.L533.
https://pubmed.ncbi.nlm.nih.gov/9728048/

Hubbard AK, Rothlein R.
Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades.
Free Radic Biol Med. 2000 May 1;28(9):1379-86. doi: 10.1016/s0891-5849(00)00223-9.

In addition, the N-glycan cycle involved in leukocyte trafficking is tightly controlled by inflammation[662] . Because inflammation-dependent modulation of adhesion molecule expression and N-glycan biosynthesis is critical for the innate immune response, dysregulation of this axis may be crucial for the transition from an innate immune response to inflammatory disease.

Innate immune response

In any infection or tissue injury, inflammation is triggered when innate immune cells recognize molecular patterns foreign to a tissue, called pathogen-associated molecular patterns (PAMPs), and initiate a cascade of inflammatory responses.

These innate immune cells include tissue-derived macrophages, natural killer cells (NK cells) and dendritic cells (DCs), as well as circulating leukocytes such as monocytes and neutrophils[663] . To communicate with other immune cells and exert their immunomodulatory functions, they often rely on N-glycans expressed on their surface and counter-receptors expressed by their binding partners.

Neutrophils are polymorphonuclear leukocytes long known to be key players in the recognition and elimination of pathogens in acute inflammation, but their role in chronic inflammatory and autoimmune diseases, such as psoriasis, RA, and SLE, has also been described[664] .

N-glycosylation has been shown to contribute to important neutrophil effector functions, such as extravasation, phagocytosis, degranulation and neutrophil extracellular trap (NET) formation. [665]

https://pubmed.ncbi.nlm.nih.gov/10924857/

[662] Stolfa G, Mondal N, Zhu Y, Yu X, Buffone A Jr, Neelamegham S.
Using CRISPR-Cas9 to quantify the contributions of O-glycans, N-glycans and Glycosphingolipids to human leukocyte-endothelium adhesion.
Sci Rep. 2016 Jul 26;6:30392. doi: 10.1038/srep30392.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4960646/

Dewald JH, Colomb F, Bobowski-Gerard M, Groux-Degroote S, Delannoy P.
Role of Cytokine-Induced Glycosylation Changes in Regulating Cell Interactions and Cell Signaling in Inflammatory Diseases and Cancer.
Cells. 2016 Nov 29;5(4):43. doi: 10.3390/cells5040043.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5187527/

Mitoma J, et al
Critical functions of N-glycans in L-selectin-mediated lymphocyte homing and
recruitment. Nat Immunol. 2007 Apr;8(4):409-18. doi: 10.1038/ni1442.
https://pubmed.ncbi.nlm.nih.gov/17334369/

[663] Akira S, Uematsu S, Takeuchi O.
Pathogen recognition and innate immunity.
Cell. 2006 Feb 24;124(4):783-801. doi: 10.1016/j.cell.2006.02.015. ì
https://doi.org/10.1016/j.cell.2006.02.015

Beutler B.
Innate immunity: an overview.
Mol Immunol. 2004 Feb;40(12):845-59. doi: 10.1016/j.molimm.2003.10.005.
https://pubmed.ncbi.nlm.nih.gov/14698223/

[664] Caielli S, Banchereau J, Pascual V.
Neutrophils come of age in chronic inflammation.
Curr Opin Immunol. 2012 Dec;24(6):671-7. doi: 10.1016/j.coi.2012.09.008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3684162/

Whittaker Hawkins RF, et al
ICAM1+ neutrophils promote chronic inflammation via ASPRV1 in B cell-dependent autoimmune encephalomyelitis.
JCI Insight. 2017 Dec 7;2(23):e96882. doi: 10.1172/jci.insight.96882.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5752297/

[665] Kelm M, Lehoux S, Azcutia V, Cummings RD, Nusrat A, Parkos CA, Brazil JC.
Regulation of neutrophil function by selective targeting of glycan epitopes expressed on the integrin CD11b/CD18.
FASEB J. 2020 Feb;34(2):2326-2343. doi: 10.1096/fj.201902542R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7018557/

Filep JG.
Targeting Neutrophils for Promoting the Resolution of Inflammation.

Monocytes and tissue macrophages are part of the mononuclear phagocyte system, which plays a central role in inflammation through antigen presentation, phagocytosis, and cytokine-mediated immune modulation[666] . These mononuclear leukocytes are considered hallmarks of the transition from acute to chronic inflammation, as their accumulation is the result of cytokine-induced neutrophil apoptosis and increased production of monocyte chemotactic protein (MCP-1).
In the past decade, N-glycosylation has gained much attention as a tool by which inflammation orchestrates the immune response of monocytes and macrophages.
There are three main steps involved in the accumulation of macrophages in the inflamed environment: recruitment of monocytes from the circulation, differentiation into macrophages, and activation of macrophages at the site of inflammation[657] . All three steps are under the direct influence of altered N-glycosylation. [667]

DCs (**dendritic cells**) are antigen-presenting cells with the ability to take up antigens in the periphery and expose them to lymphocytes, thus bridging the gap between the innate and adaptive immune response [668]. A specific subset of monocyte-derived DCs (Mo-DCs) plays a key role in inflammation [669]. The surface of Mo-DCs is covered with glycoproteins decorated predominantly with sialylated glycans[670] . DC sialylation is regulated during both differentiation and maturation, and has been found to significantly affect DC functions such as antigen uptake, phagocytosis, and T-cell initiation[671] .

NK (natural killer) **cells** are known for their role in cell-mediated cytotoxicity and secretion of proinflammatory cytokines[672] , critical for both promotion of inflammation and immune regulation[673] .

Front Immunol. 2022 Mar 16;13:866747. doi: 10.3389/fimmu.2022.866747.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8966391/

[666] Hume DA, Irvine KM, Pridans C.
The Mononuclear Phagocyte System: The Relationship between Monocytes and Macrophages.
Trends Immunol. 2019 Feb;40(2):98-112. doi: 10.1016/j.en.2018.11.007.
https://pubmed.ncbi.nlm.nih.gov/30579704/

[667] Regal-McDonald K, Patel RP.
Selective Recruitment of Monocyte Subsets by Endothelial N-Glycans.
Am J Pathol. 2020 May;190(5):947-957. doi: 10.1016/j.ajpath.2020.01.006.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7221295/

[668] Iwasaki A, Medzhitov R.
Control of adaptive immunity by the innate immune system.
Nat Immunol. 2015 Apr;16(4):343-53. doi: 10.1038/ni.3123.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4507498/

[669] Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA.
Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. Immunity. 1999 Dec;11(6):753-61. doi: 10.1016/s1074-7613(00)80149-1.
https://doi.org/10.1016/S1074-7613(00)80149-1

[670] Schauer R.
Sialic acids as regulators of molecular and cellular interactions.
Curr Opin Struct Biol. 2009 Oct;19(5):507-14. doi: 10.1016/j.sbi.2009.06.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7127376/

[671] Carrascal, Mylène & Silva, Zélia & Crespo, Hélio & Cabral, Maria & Videira, Paula..
Sialylation and dendritic cells: Bridging innate and adaptive immune responses.
Carbohydrate Chemistry. (2011) 37. 94-116. 10.1039/9781849732765-00094.
https://www.researchgate.net/publication/285963460_Sialylation_and_dendritic_cells_Bridging_innate_and_adaptive_immune_responses[6]
[72] Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S.
Functions of natural killer cells.
Nat Immunol. 2008 May;9(5):503-10. doi: 10.1038/ni1582.
https://pubmed.ncbi.nlm.nih.gov/18425107/

[673] Hush B, Bryceson YT.
Natural killer cells in inflammation and autoimmunity.

The effector functions of NK cells are regulated by a series of activating and inhibitory receptors expressed on their surface, with glycosylation playing a crucial role in receptor-ligand recognition. FcγRIIIa (CD16a) is the most abundantly expressed activating receptor on circulating NK cells[674] , and its role in antibody-dependent cell-mediated cytotoxicity (ADCC) is well established[675] .
In particular, a huge increase in the binding affinity of proinflammatory afucosylated IgG was observed when N-glycan oligomannoses were present on FcγRIIIa[676] .
In addition, higher levels of N-glycan complexes sialylated on FcγRIIIa have been shown to correlate with
A lower affinity for antibody binding[677] .
In their recent review, Rosenstock and Kaufmann describe an important contribution of sialic acids to NK cell functions, both through the expression of sialic acid-binding receptors and through the presence of sialic acids on their surface[678] .

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9272703/
Overview of altered N-glycosylation pathways in innate immune cells during chronic inflammation. The main contributors to alterations in N-glycosylation are proinflammatory cytokines (e.g., TNFα, IL-2, IFN-α, IFN-γ) that are released in excess during inflammation. Here, the affected structural elements of N-glycans on the surface of innate leukocytes (neutrophils, macrophages, NK cells and DCs) are shown together with associated glycosyltransferases and glycosidases. In neutrophils, the increase of Lex motif on integrin MAC-1 leads to dysregulated neutrophil migration, while the binding of Lex-decorated MAC-1 to DC-SIGN further triggers DC activation. While neutrophil granules (e.g., HNE) secreted by neutrophils carry truncated N-glycans, the presence of sialylated complex N-glycans and/or the sLex motif on Siglec contrast entities contributes to neutrophil inflammatory potential in a context-dependent manner. Proinflammatory cytokines enhance monocyte transport and direct their differentiation into M1 proinflammatory macrophages while contributing to the absence of sialylated N-glycans, Gal-3 cleavage, and increased expression of Siglec-1. While surface-bound Siglec-1 is involved in the autoimmune response in rheumatoid arthritis (RA), soluble Siglec-1 is a marker in interferonopathy. In addition, the Gal-1/IFN-β feedback loop involved in the cessation of inflammation appears to be dysregulated in chronic inflammation. Similar to macrophages, mature DCs also lack terminal sialic acids, plausibly due to inflammation-mediated decrease in sialyltransferase and/or increase in neuraminidase activity. Regarding NK cells, t h e presence of N-glycan oligomannoses on FcγRIIIa significantly increases ADCC, while cytokine-induced increase in sialylation abrogates Siglec-9-dependent NK cell inhibition by cis-binding. BACE1, beta-site APP cleavage enzyme-1; Gal, galectin; hAGP-1; hepatic α1 acid glycoprotein; HNE, human neutrophil elastase; IFN, interferon; IL, interleukin; ICAM-1, intercellular adhesion molecule-1; MAC-1, macrophage antigen-1; Man, Mannosidase; MMP-12, metalloproteinase of

---

Cytokine Growth Factor Rev. 2018 Aug;42:37-46. doi: 10.1016/j.cytogfr.2018.08.001.
https://pubmed.ncbi.nlm.nih.gov/30122459/

[674] Lanier LL, Le AM, Civin CI, Loken MR, Phillips JH.
The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. J Immunol. 1986 Jun 15;136(12):4480-6.
https://pubmed.ncbi.nlm.nih.gov/3086432/

[675] Battella S, Cox MC, Santoni A, Palmieri G.
Natural killer (NK) cells and anti-tumor therapeutic mAb: unexplored interactions.
J Leukoc Biol. 2016 Jan;99(1):87-96. doi: 10.1189/jlb.5VMR0415-141R.
https://pubmed.ncbi.nlm.nih.gov/26136506/

[676] Subedi GP, Barb AW.
CD16a with oligomannose-type N-glycans is the only "low-affinity" Fc γ receptor that binds the IgG crystallizable fragment with high affinity in vitro. J Biol Chem. 2018 Oct 26;293(43):16842-16850. doi: 10.1074/jbc.RA118.004998.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6204906/

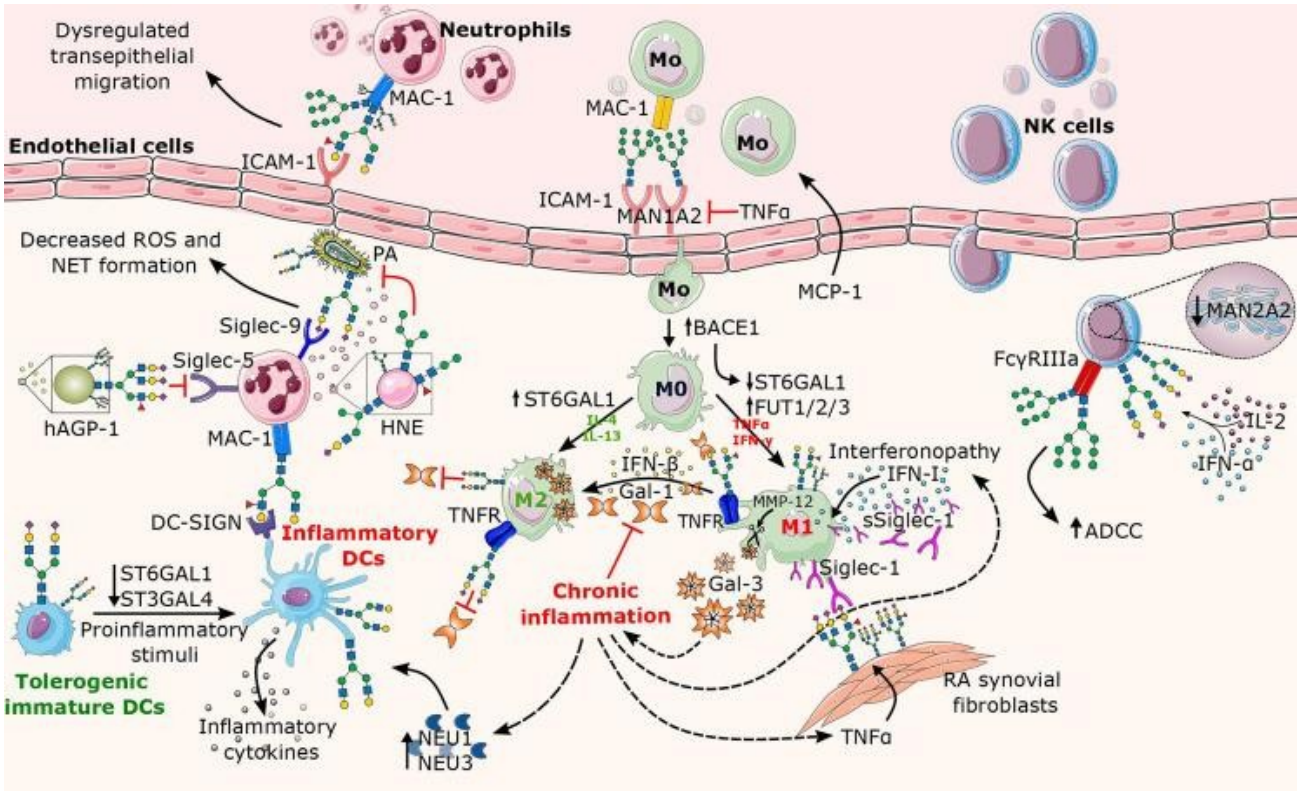
Patel KR, Roberts JT, Subedi GP, Barb AW.
Restricted processing of CD16a/Fc γ receptor IIIa N-glycans from primary human NK cells impacts structure and function.
J Biol Chem. 2018 Mar 9;293(10):3477-3489. doi: 10.1074/jbc.RA117.001207.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5846152/

Patel KR, Rodriguez Benavente MC, Lorenz WW, Mace EM, Barb AW.
Fc γ receptor IIIa/CD16a processing correlates with the expression of glycan-related genes in human natural killer cells.
J Biol Chem. 2021 Jan-Jun;296:100183. doi: 10.1074/jbc.RA120.015516.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7948478/

[677] Hayes JM, Frostell A, Karlsson R, Müller S, Martín SM, Pauers M, Reuss F, Cosgrave EF, Anneren C, Davey GP, Rudd PM.
Identification of Fc Gamma Receptor Glycoforms That Produce Differential Binding Kinetics for Rituximab.
Mol Cell Proteomics. 2017 Oct;16(10):1770-1788. doi: 10.1074/mcp.M117.066944.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5629263/

[678] Rosenstock P, Kaufmann T.
Sialic Acids and Their Influence on Human NK Cell Function.
Cells. 2021 Jan 29;10(2):263. doi: 10.3390/cells10020263.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7911748/

12 matrix; MCP-1, monocyte chemoattractant protein-1; NEU, neuraminidase; NAGP-1, neutrophil α1 acid glycoprotein; PA, Pseudomonas aeruginosa.



Adaptive response

In adaptive immunity, glycans are essential for signal transduction and cell-cell interactions. N-glycans have been shown to regulate important steps in lymphocyte biology, such as T- and B-cell activity, cell differentiation and proliferation, and the function of secreted antibodies in chronic inflammation. [679]

T-cell function in inflammation is highly pleiotropic and depends on intra- and intercellular communication, often mediated by N-glycans and their corresponding binding partners.

In this regard, alterations in the N-glycome of T lymphocytes can significantly affect their activation, differentiation, survival, and cytokine production, often leading to autoimmunity, chronic inflammation, or cancer[680] .

[679] Wright RD, Cooper D.
Glycobiology of leukocyte trafficking in inflammation.
Glycobiology. 2014 Dec;24(12):1242-51. doi: 10.1093/glycob/cwu101.
https://pubmed.ncbi.nlm.nih.gov/25258391/

Maverakis E, Kim K, Shimoda M, Gershwin ME, Patel F, Wilken R, Raychaudhuri S, Ruhaak LR, Lebrilla CB.
Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: a critical review.
J Autoimmun. 2015 Feb;57:1-13. doi: 10.1016/j.jaut.2014.12.002. ì
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4340844/

[680] Chien MW, Fu SH, Hsu CY, Liu YW, Sytwu HK.
The Modulatory Roles of N-glycans in T-Cell-Mediated Autoimmune Diseases.
Int J Mol Sci. 2018 Mar 8;19(3):780. doi: 10.3390/ijms19030780.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5877641/

Coder BD, Wang H, Ruan L, Su DM.
Thymic involution perturbs negative selection leading to autoreactive T cells that induce chronic inflammation. J Immunol. 2015 Jun 15;194(12):5825-37. doi: 10.4049/jimmunol.1500082.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4458423/

Under homeostatic conditions, galectins are the main immune regulators of T cells, with Gal-1, Gal- 3, and Gal-9 consistently showing immunosuppressive effects. [681]

Lectine

As discussed above, host cells, as well as virtually all bacteria, parasites, fungi and viruses, carry glycan structures on the cell surface, and it has been proposed that these molecules may serve to display information critical to host-microbe communication.

This information is, at least in part, decoded by proteins or lectins that bind glycans[682] , and act as receptors that could be secreted or expressed on the cell surface of immune cells. Lectin families, including C-type lectins, siglecs, and galectins, contain one or more carbohydrate recognition domains (CRDs) responsible for sugar recognition.

The specificity of glycan binding and cell type-specific expression of these receptors are well documented. While galectins are secreted, most C-type lectins and all known siglecs are membrane-bound proteins[673] .

---

[681] Sundblad V, Morosi LG, Geffner JR, Rabinovich GA.
Galectin-1: A Jack-of-All-Trades in the Resolution of Acute and Chronic Inflammation.
J Immunol. 2017 Dec 1;199(11):3721-3730. doi: 10.4049/jimmunol.1701172.
https://www.jimmunol.org/content/199/11/3721.long

Cerliani JP, Blidner AG, Toscano MA, Croci DO, Rabinovich GA.
Translating the 'Sugar Code' into Immune and Vascular Signaling Programs.
Trends Biochem Sci. 2017 Apr;42(4):255-273. doi: 10.1016/j.tibs.2016.11.003.
https://pubmed.ncbi.nlm.nih.gov/27986367/

Ilarregui JM, Croci DO, Bianco GA, Toscano MA, Salatino M, Vermeulen ME, Geffner JR, Rabinovich GA.
Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10.
Nat Immunol. 2009 Sep;10(9):981-91. doi: 10.1038/ni.1772.
https://pubmed.ncbi.nlm.nih.gov/19668220/

Thiemann S, Baum LG.
Galectins and Immune Responses-Just How Do Those Things They Do?
Annu Rev Immunol. 2016 May 20;34:243-64. doi: 10.1146/annurev-immunol-041015-055402.
https://pubmed.ncbi.nlm.nih.gov/26907217/

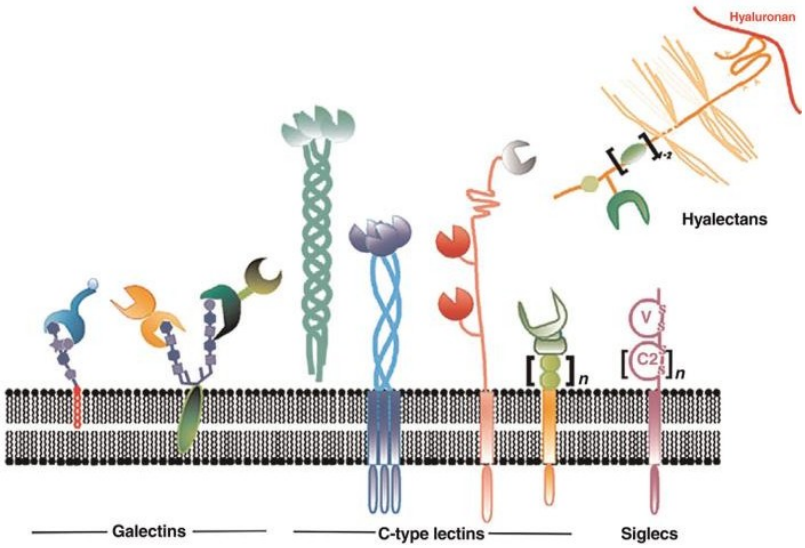
Grigorian A, Torossian S, Demetriou M.
T-cell growth, cell surface organization, and the galectin-glycoprotein lattice.
Immunol Rev. 2009 Jul;230(1):232-46. doi: 10.1111/j.1600-065X.2009.00796.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3059806/

Colomb F, Giron LB, Trbojevic-Akmacic I, Lauc G, Abdel-Mohsen M.
Breaking the Glyco-Code of HIV Persistence and Immunopathogenesis.
Curr HIV/AIDS Rep. 2019 Apr;16(2):151-168. doi: 10.1007/s11904-019-00433-w.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6441623/

Rabinovich GA, Toscano MA.
Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation.
Nat Rev Immunol. 2009 May;9(5):338-52. doi: 10.1038/nri2536.
https://pubmed.ncbi.nlm.nih.gov/19365409/

[682] van Kooyk Y, Rabinovich GA.
Protein-glycan interactions in the control of innate and adaptive immune responses.
Nat Immunol. 2008 Jun;9(6):593-601. doi: 10.1038/ni.f.203.
https://pubmed.ncbi.nlm.nih.gov/18490910/

Ledeen RW, Kopitz J, Abad-Rodríguez J, Gabius HJ.
Glycan Chains of Gangliosides: Functional Ligands for Tissue Lectins (Siglecs/Galectins).
Prog Mol Biol Transl Sci. 2018;156:289-324. doi: 10.1016/bs.pmbts.2017.12.004.
https://pubmed.ncbi.nlm.nih.gov/29747818/



https://pubmed.ncbi.nlm.nih.gov/29747818/
Illustration of the strategic ways in which carbohydrate recognition domains (CRDs) in animal and human lectins are positioned to achieve optimal ligand selection (e.g., to separate self from nonself glycan profiles in innate immunity) and topological complementarity. From left to right, CRDs are displayed in the three subtypes within the galectin family (chimeric, prototype, and tandem-repeat arrangements that bind to a ganglioside or an N-glycan of branched complex type without or with terminal α2,3 -sialylation), presentation of CRDs (C-type or fibrinogen-like domain) in serum and collectins or ficolins of surfactants attached to their collagen stems and noncovalent binding of lectin sites in C-type transmembrane lectins by an α-helical coiled-coil are administered stems (e.g. asialoglycoprotein and Kupffer cell receptors, C-type scavenger receptor lectin, CD23, DC-SIGN or DC-SIGNR). Similar to tandem-repeat galectins, the C-type lectin family also has a branch of members with this design, namely immulectins1, -2 and -3. Next, tandem repeat display in the macrophage-specific mannose receptor (which is also found on dendritic cells, hepatic endothelial cells, renal mesangial cells, retinal pigment epithelial cells and tracheal smooth muscle cells) and the related C-type lectin Endo180 with eight domains as well as in the cation-independent P-type lectin with 15 domains is presented. The ability to bind sugar is limited to a few domains as illustrated. The occurrence of lectin activity for the pituitary hormone-bearing glycoprotein GalNAc-4-SO4 in the cysteine-rich domain, a member of the β-fold family with a domain (QxW)3 in the N-terminal section of the macrophage mannose receptor (amino acids 8-128), which is connected via a fibronectin -type-II-containing repeat to the tandem repeat section, is also included in the schematic design for these lectins with more than one CRD type per protein chain. Moving further to the right-hand side, the association of a distal CRD in selectins (linked to an epidermal growth factor (EGF)-like domain and two to nine complement-binding consensus repeats) or in the siglec subfamily of type I lectins using 1 -16 immunoglobulin C2-set-like units as equivalent spacers to allow the CRD (V-set) to reach contact ligands and modulate the ability to serve in cis- or trans-interactions on the cell surface. Alterations dependent on the strength of the topological arrangement of the two distal domains in selectins represent selectin capture bonds, a canonical immunoreceptor tyrosine-based inhibitory motif (ITIM) along with a putative tyrosine-based motif is often present in the intracellular portion of siglecs. C2-Set domains linked to fibronectin-type-III repeats establish the extracellular section of type I L1 lectins and neural cell adhesion molecule (NCAM). In the matrix, modular proteoglycans (hyalectans/lectans: aggrecan, brevican, neurocan, and versican) interact (i) with hyaluronan (and also link protein) via the link-protein type modules of the G1 N-terminal domain (and an Ig-like module), (ii) with receptors that bind to glycosaminoglycan chains in the central region, and (iii) with carbohydrates or proteins (fibulins-1 and -2 and tenascin-R) via lectin C-like domain flanked by consensus repeat-like EGF and complement module binding. (For details, see Gabius H-J. Animal and human lectins. In: Gabius H-J, ed.The Sugar Code. Fundamentals of Glycosciences. Weinheim, Germany: Wiley-VCH; 2009: 317-328.)

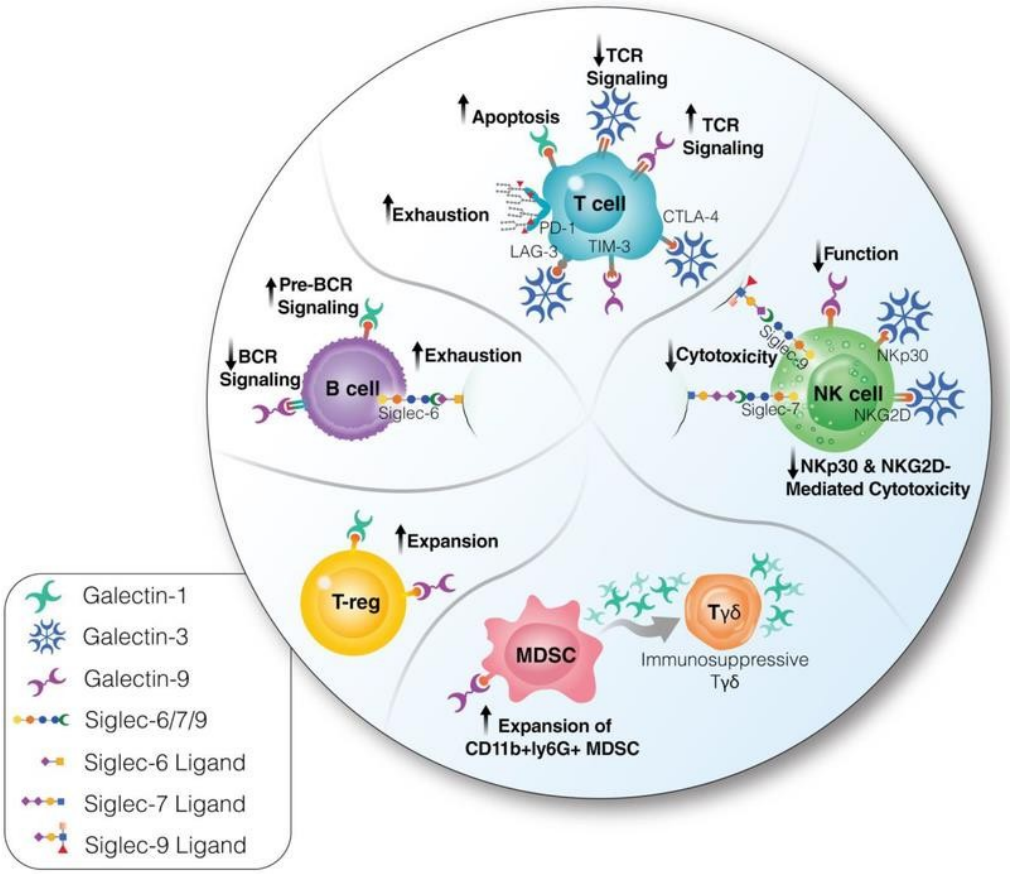
Although galectins are synthesized and stored in the cytoplasmic compartment, they are passively released from dying cells or actively secreted by activated inflammatory cells following tissue damage caused by pathogens. Because of similarities with other DAMPs or alarmins, including cytosolic localization, release following tissue damage, and nonclassical externalization, it has been hypothesized that galectins act as potential DAMPs*[683] .

[683] Rabinovich GA, Toscano MA.
Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation.
Nat Rev Immunol. 2009 May;9(5):338-52. doi: 10.1038/nri2536.
https://pubmed.ncbi.nlm.nih.gov/19365409/

Dam TK, Brewer CF.
Lectins as pattern recognition molecules: the effects of epitope density in innate immunity.
Glycobiology. 2010 Mar;20(3):270-9. doi: 10.1093/glycob/cwp186.
https://pubmed.ncbi.nlm.nih.gov/19939826/

Roh JS, Sohn DH.
Damage-Associated Molecular Patterns in Inflammatory Diseases.

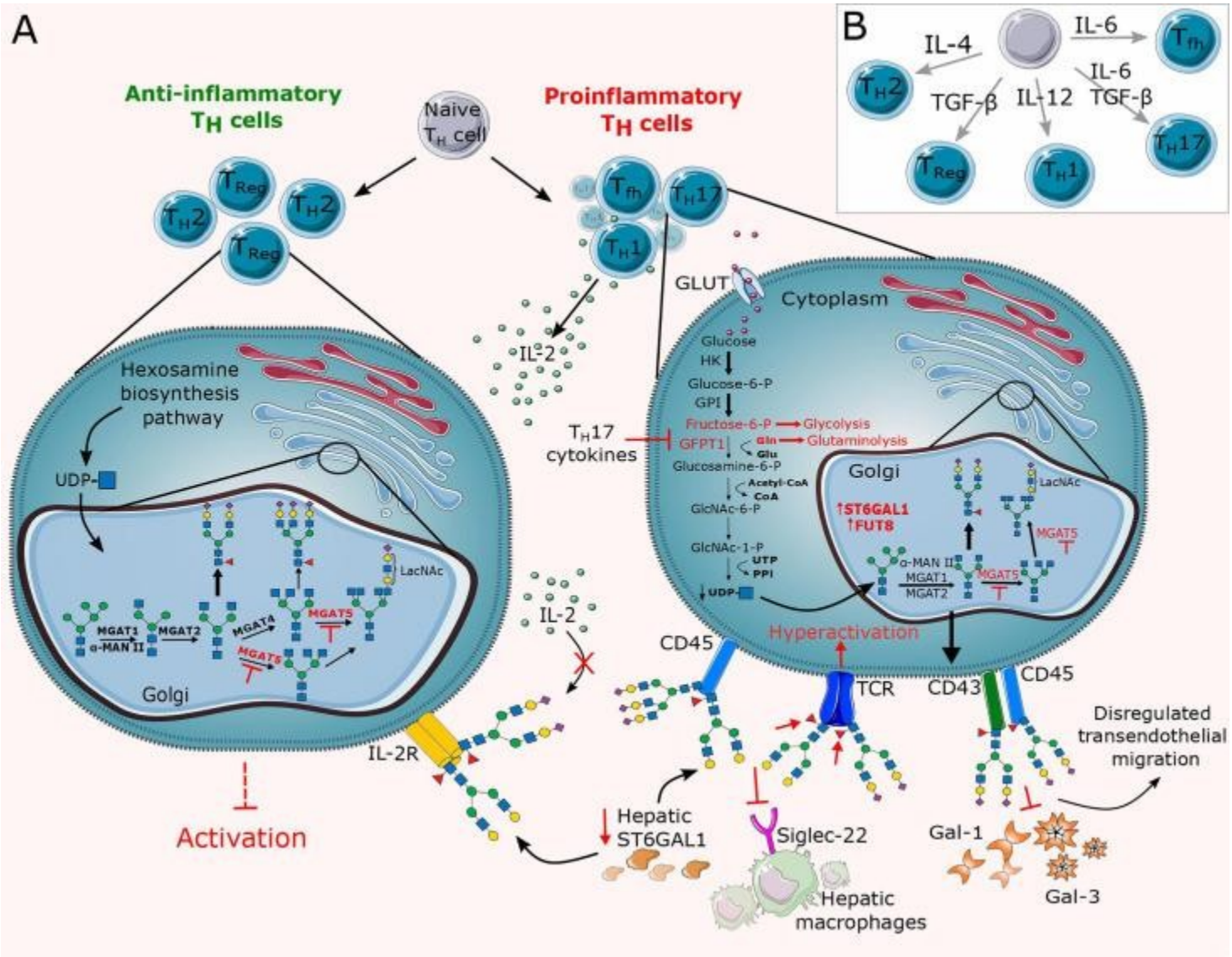
** Damage-associated molecular patterns (DAMPs) are endogenous dangerous molecules that are released from damaged or dying cells and activate the innate immune system by interacting with pattern recognition receptors (PRRs). Although DAMPs contribute to host defense, they promote pathological inflammatory responses.*



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6441623/
Cell-surface glycan-lectin interactions mediate signals that define various cellular processes and immunological functions central t o HIV infection. The specific structure of a glycan allows it to bind to specific lectins, leading to activation of downstream signaling pathways. These pathways are critical for a variety of cellular processes and immunological functions. T cells. Galectin-1 induces apoptosis of T lymphocytes. Galectin-9 induces TCR signaling, while galectin-3 reduces it. Galectin-3 alters T-cell function through interaction with LAG3 and other negative immune checkpoints. Finally, fucosylation of PD-1 affects its function. NK cells. Siglecs-7 and -9 inhibit NK activity. Galectin-9 alters NK function/cytotoxicity and cytokine production through a Tim-3-independent mechanism. Galectin-3 antagonizes NK cell-mediated antitumor immunity by decreasing the affinity of MHC I-related chain A (MICA) for the NKG2D receptor or by acting as an inhibitory ligand of the NKp30 receptor. B cells. Siglec-6 induces B-cell depletion. Galectin-1 is a pre-B cell receptor ligand that induces receptor clustering, leading to efficient B cell differentiation. Galectin-9 suppresses BCR signaling. T-reg. Galectin-1 and -9 can expand T-reg. myeloid-derived suppressor cells (MDSCs). Galectin-9/Tim3 interaction drives expansion of CD11b+ly6G+ MDSCs. Granulocytic MDSCs induce γδ-T cells to produce galectin-1, thus transforming them into immunosuppressive cells. These glycan-lectin interactions represent potential new targets for improving immune function during HIV infection to cure HIV or prevent HIV-associated immune dysfunction and subsequent development of immune dysfunction-associated diseases.

Immune Netw. 2018 Aug 13;18(4):e27. doi: 10.4110/in.2018.18.e27.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6117512/

Sato S, St-Pierre C, Bhaumik P, Nieminen J.
Galectins in innate immunity: dual functions of host soluble beta-galactoside-binding lectins as damage-associated molecular patterns (DAMPs) and as receptors for pathogen-associated molecular patterns (PAMPs).
Immunol Rev. 2009 Jul;230(1):172-87. doi: 10.1111/j.1600-065X.2009.00790.x.
https://pubmed.ncbi.nlm.nih.gov/19594636/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9272703/
Overview of altered N-glycosylation pathways regarding T lymphocytes during chronic inflammation. (A) The differentiation of lymphocytes and thus their surface N-glycosome is under the direct influence of cytokines and stimulation by antigen-presenting cells (APCs). Cytokines control the differentiation in favor of proinflammatory T lymphocytes (Th1, Th17, Tfh), thus altering their N-glycome by dysregulating the expression of glycosyltransferases such as MGAT5, ST6GAL1, and FUT8 and abrogating the availability of substrate for the hexosamine biosynthesis pathway (HBP). The resulting N-glycan changes significantly reduce the binding affinity of inhibitory galectins and Siglecs. (B) Schematic representation of relevant cytokines responsible for T-cell differentiation. GLUT, glucose transporter; TCR, T cell receptor; Tfh, follicular helper T cell; Th, T helper cell; Treg, regulatory T cell.

B lymphocytes are the central immune effector cells in the humoral branch of adaptive immunity. [684] In addition to their function as precursors of antibody-secreting plasma cells, B lymphocytes are involved in the suppression of T lymphocytes and the secretion of relevant cytokines that control adaptive immunity[685] . N-glycosylation has an enormous impact on the proliferation, differentiation, and effector functions of B cells, but research on this topic lags far behind that of T cells.

[684] Forthal DN.
Functions of Antibodies.
Microbiol Spectr. 2014 Aug 15;2(4):1-17
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159104/

[685] Lund FE.
Cytokine-producing B lymphocytes-key regulators of immunity.
Curr Opin Immunol. 2008 Jun;20(3):332-8. doi: 10.1016/j.coi.2008.03.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2474694/

In B cells, among the best understood roles for lectin-glycan interactions are those of sialoglycans and Siglecs in BCR signaling.
Sialic acids are often referred to as "self-signaling" inhibitors because of their high local concentration on the surface of B lymphocytes. Therefore, it is not surprising that Siglecs are considered the main immunomodulators of B lymphocytes.[686]

The final but no less important role of B lymphocytes is the secretion of immunoglobulins (Igs), the main executive glycoproteins of the humoral adaptive immune response.
All classes of human Ig are N-glycosylated, with N-glycans attached to conserved glycosylation regions on the crystallizable fragment (Fc) and/or on the antigenic variable fragment (Fab) bond, where new glycosylation sites can be acquired during somatic hypermutation[687] .
N-glycans can influence the structural stability and conformation of immunoglobulins as well as their effector functions[688] .

N-glycan modifications of IgG

Insight

RESPIRATORY COMPLICATIONS- PART SECOND - Immunopathology from p. 15

Galactosylation

Increased IgG agalactosylated glycans are considered a hallmark of various diseases with an underlying inflammatory component[689] . Fc glycans lacking terminal galactosyl are thought to be proinflammatory and capable of activating complement through the alternative pathway, along with the lectin pathway via binding to mannose-binding lectin (MBL)[690] .
While agalactosylated glycans are considered strictly proinflammatory, terminal galactosylation seems to be rather controversial in this regard.

---

[686] Collins BE, Blixt O, DeSieno AR, Bovin N, Marth JD, Paulson JC.
Masking of CD22 by cis ligands does not prevent redistribution of CD22 to sites of cell contact.
Proc Natl Acad Sci U S A. 2004 Apr 20;101(16):6104-9. doi: 10.1073/pnas.0400851101.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC395930/

[687] Schroeder HW Jr, Cavacini L.
Structure and function of immunoglobulins.
J Allergy Clin Immunol. 2010 Feb;125(2 Suppl 2):S41-52. doi: 10.1016/j.jaci.2009.09.046.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3670108/

[688] Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA.
The impact of glycosylation on the biological function and structure of human immunoglobulins.
Annu Rev Immunol. 2007;25:21-50. doi: 10.1146/annurev.immunol.25.022106.141702.
https://pubmed.ncbi.nlm.nih.gov/17029568/

[689] Gudelj I, Lauc G, Pezer M.
Immunoglobulin G glycosylation in aging and diseases.
Cell Immunol. 2018 Nov;333:65-79. doi: 10.1016/j.cellimm.2018.07.009.
https://doi.org/10.1016/j.cellimm.2018.07.009

[690] Arnold JN, Dwek RA, Rudd PM, Sim RB.
Mannan binding lectin and its interaction with immunoglobulins in health and in disease.
Immunol Lett. 2006 Aug 15;106(2):103-10. doi: 10.1016/j.imlet.2006.05.007.
https://pubmed.ncbi.nlm.nih.gov/16814399/

Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB.
Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein.
Nat Med. 1995 Mar;1(3):237-43. doi: 10.1038/nm0395-237. Erratum in: Nat Med 1995 Jun;1(6):599.
https://pubmed.ncbi.nlm.nih.gov/7585040/

Galactose-decorated glycans have been found to be responsible for attenuating inflammation by binding to the inhibitor FcγRIIB, followed by inhibition of the proinflammatory activity of the C5a component of complement[691] .
On the other hand, galactosylation of Fc has been shown to activate the classical complement pathway by facilitating IgG hexamerization, thereby increasing C1q avidity and enhancing CDC[692] .
It has also been found to increase the affinity of IgG for FcγR activation, leading to ADCC[693] .

IgG seralization

The addition of sialic acid to the N-terminal end of IgG glycans is essential for the control of inflammatory immune responses. Highly sialylated IgGs have a lower affinity for FcγRIIIa activation, resulting in reduced ADCC[694] , while stimulating overregulation of inhibitory FcγRIIb and thus inhibition of CDC[695] .
In autoimmunity, hyposialylation is believed to be responsible for the development of chronic inflammation.

Fucosylation of IgG

More than 90% of IgG Fc glycans in healthy individuals have fucose bound to their nucleus, which acts as a "safety switch" and attenuates potentially harmful ADCC[696] .
A pathological example is reduced IgG core fucosylation in autoimmune thyroid diseases.

---

[691] Karsten CM, et al
Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and dectin-1.
Nat Med. 2012 Sep;18(9):1401-6. doi: 10.1038/nm.2862.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3492054/

[692] Wei B, Gao X, et al.
Fc galactosylation follows consecutive reaction kinetics and enhances immunoglobulin G hexamerization for complement activation.
MAbs. 2021 Jan-Dec;13(1):1893427. doi: 10.1080/19420862.2021.1893427.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7946005/

[693] Subedi GP, Barb AW.
The immunoglobulin G1 N-glycan composition affects binding to each low affinity Fc γ receptor.
MAbs. 2016 Nov/Dec;8(8):1512-1524. doi: 10.1080/19420862.2016.1218586.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5098437/

Dekkers G, et al
Decoding the Human Immunoglobulin G-Glycan Repertoire Reveals a Spectrum of Fc-Receptor- and Complement-Mediated-Effector Activities.
Front Immunol. 2017 Aug 2;8:877. doi: 10.3389/fimmu.2017.00877.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5539844/

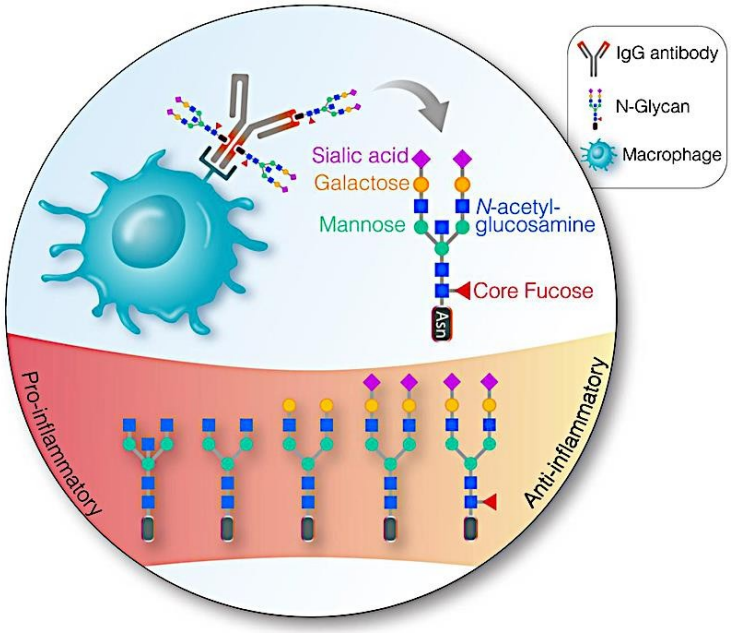
[694] Kaneko Y, Nimmerjahn F, Ravetch JV.
Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation.
Science. 2006 Aug 4;313(5787):670-3. doi: 10.1126/science.1129594.
https://pubmed.ncbi.nlm.nih.gov/16888140/

Raju TS.
Terminal sugars of Fc glycans influence antibody effector functions of IgGs.
Curr Opin Immunol. 2008 Aug;20(4):471-8. doi: 10.1016/j.coi.2008.06.007.
https://pubmed.ncbi.nlm.nih.gov/18606225/

[695] Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, Münz C, Nimmerjahn F, Dalakas MC, Lünemann JD.
Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity.
J Clin Invest. 2015 Nov 2;125(11):4160-70. doi: 10.1172/JCI82695
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4639970/

[696] Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG.
Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity.
J Biol Chem. 2002 Jul 26;277(30):26733-40. doi: 10.1074/jbc.M202069200.
https://doi.org/10.1074/jbc.M202069200

The underlying mechanism is believed to be the abnormal expression of the FUT8 and IKZF1 genes in B lymphocytes producing thyroid peroxidase antibody (TPOAb)[697] , both of which are associated with aphucosylated IgG N-glycans[698] . Another pathological example is aberrant methylation in the promoter region of the MGAT3 gene (which encodes for the MGAT3 enzyme responsible for the production of bisecting structures of GlcNAc) that results in an increased percentage of bisecting GlcNAc on IgG glycans in patients with celiac disease, suggesting a possible involvement of GlcNAc bisection in the pathogenesis of the disease[699] .



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6441623/

Immunoglobulin A

The prevailing concept regarding the immunological function of immunoglobulin A (IgA) is its ability to bind to and neutralize pathogens to prevent infection at the mucosal level of the body.
However, it has become clear over time that in humans IgA is also capable of actively contributing to the initiation of inflammation, both at the mucosal and nonmucosal levels.
This additional function of IgA is initiated by the formation of immune complexes, which activate the receptor Fc alpha I (FcαRI) and synergize with various other receptors to amplify inflammatory responses.
Recent findings have shown that co-stimulation of FcαRI strongly affects the production of pro-inflammatory cytokines by various myeloid cells, including several subgroups of dendritic cells, macrophages, monocytes, and Kupffer cells.
FcαRI-induced inflammation plays a crucial role in orchestrating human host defense against pathogens, as well as in the generation of tissue-specific immunity. In addition, it is believed that

[697] Martin TC, et al
Decreased Immunoglobulin G Core Fucosylation, A Player in Antibody-dependent Cell-mediated Cytotoxicity, is Associated with Autoimmune Thyroid Diseases.
Mol Cell Proteomics. 2020 May;19(5):774-792. doi: 10.1074/mcp.RA119.001860.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196582/

[698] Lauc G, et al
Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers.
PLoS Genet. 2013;9(1):e1003225. doi: 10.1371/journal.pgen.1003225.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3561084/

[699] Klasić M, et al
IBD consortium, Lauc G, Zoldoš V. Promoter methylation of the MGAT3 and BACH2genes correlates with the composition of the immunoglobulin G glycome in inflammatory bowel disease.
Clin Epigenetics. 2018 Jun 4;10:75. doi: 10.1186/s13148-018-0507-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5987481/

FcαRI-induced inflammation is involved in the pathogenesis of various chronic inflammatory disorders, including the inflammatory bowel disease, celiac disease, and rheumatoid arthritis. [700]
One of the best-studied chronic inflammatory diseases related to N-glycosylation of IgA is IgA nephropathy (IgAN). A study by Dotz et al. showed that decreased N-linked sialylation and galactosylation and increased bisection of IgAN are associated with worsened renal function.[701]

Immunoglobulin E

immunoglobulin E (IgE) is best known for its role in allergic immune responses.
Specifically, IgE binds to high-affinity IgE receptors (FcεRI) expressed on the surface of basophils and mast cells, triggering degranulation and release of proinflammatory mediators[702] .
IgE is the most glycosylated immunoglobulin, with seven N-glycosylation sites[703] and among these in particular the N-glycosylation site in Asn394, consisting exclusively of oligomannose N-glycans, is critical for the initiation of the IgE-mediated allergic cascade.
Specific amino acid mutations or complete deglycosylation of Asn394 alter IgE structure secondary, abolishing FcεRI binding and subsequent IgE-mediated degranulation and anaphylaxis[704] .
Interestingly, mutation of all other N-linked sites of IgE, which consist of complex N-glycans, have almost no effect on the ability of IgE to elicit an anaphylactic response[705] .

Immunoglobulin M

Immunoglobulin M (IgM) is the largest antibody in serum and its level is elevated in various inflammatory and autoimmune diseases[706] .
It is another highly N-glycosylated antibody, as its constant domain contains five N-linked glycosylation sites, three of which belong to the biantennary complex form (Asn171, Asn332, Asn395) and two to the oligomannose type (to Asn402, Asn563)[707] .

---

[700] Hansen IS, Baeten DLP, den Dunnen J.
The inflammatory function of human IgA. Cell Mol Life Sci. 2019 Mar;76(6):1041-1055. doi: 10.1007/s00018-018-2976-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6513800/

[701] Dotz V, Visconti A, Lomax-Browne HJ, Clerc F, Hipgrave Ederveen AL, Medjeral-Thomas NR, Cook HT, Pickering MC, Wuhrer M, Falchi M. O- and N-Glycosylation of Serum Immunoglobulin A is Associated with IgA Nephropathy and Glomerular Function.
J Am Soc Nephrol. 2021 Oct;32(10):2455-2465. doi: 10.1681/ASN.2020081208.
https://pubmed.ncbi.nlm.nih.gov/34127537/

[702] Gould HJ, Sutton BJ.
IgE in allergy and asthma today.
Nat Rev Immunol. 2008 Mar;8(3):205-17. doi: 10.1038/nri2273.
https://pubmed.ncbi.nlm.nih.gov/18301424/

[703] Dorrington KJ, Bennich HH.
Structure-function relationships in human immunoglobulin E.
Immunol Rev. 1978;41:3-25. doi: 10.1111/j.1600-065x.1978.tb01458.x.
https://pubmed.ncbi.nlm.nih.gov/100912/

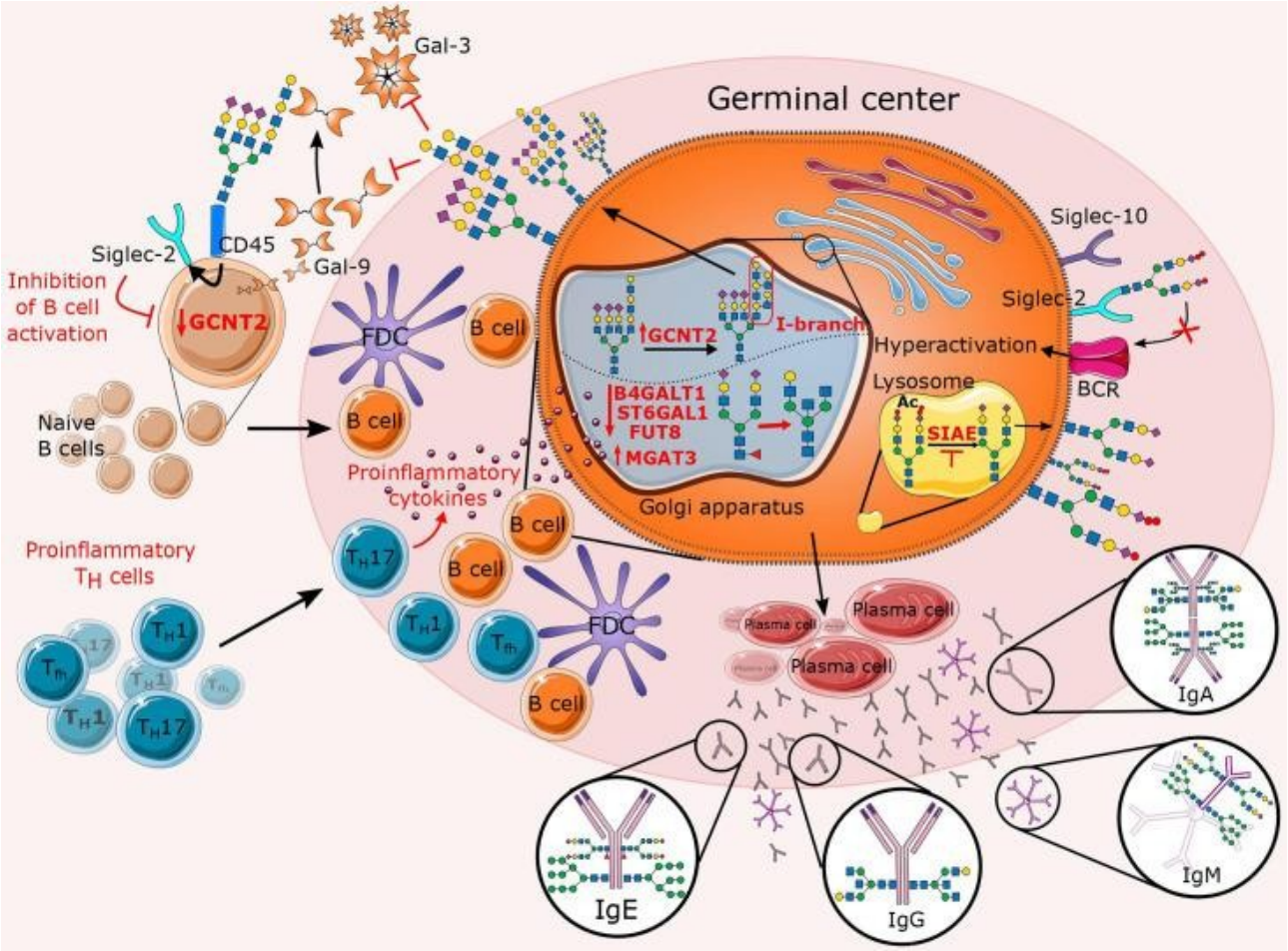
[704] Sayers I, Cain SA, Swan JR, Pickett MA, Watt PJ, Holgate ST, Padlan EA, Schuck P, Helm BA.
Amino acid residues that influence Fc epsilon RI-mediated effector functions of human immunoglobulin E.
Biochemistry. 1998 Nov 17;37(46):16152-64. doi: 10.1021/bi981456k.
https://pubmed.ncbi.nlm.nih.gov/9819207/

[705] Shade KT, Platzer B, Washburn N, Mani V, Bartsch YC, Conroy M, Pagan JD, Bosques C, Mempel TR, Fiebiger E, Anthony RM.
A single glycan on IgE is indispensable for initiation of anaphylaxis.
J Exp Med. 2015 Apr 6;212(4):457-67. doi: 10.1084/jem.20142182.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4387292/

[706] Karlsson M.
IgM and IgD in Infection and Inflammatory Diseases.
In: Molecular and Cellular Mechanisms of Antibody Activity. New York, NY: Springer; (2013). doi: 10.1007/978-1-4614-7107-3_1

[707] Arnold JN, Wormald MR, Sim RB, Rudd PM, Dwek RA.
The impact of glycosylation on the biological function and structure of human immunoglobulins.
Annu Rev Immunol. 2007;25:21-50. doi: 10.1146/annurev.immunol.25.022106.141702.

Oligomannous N-glycans have been shown to be important for MBL binding and subsequent elimination of IgM aggregates by opsonization[708] , whereas complex N-glycans are involved in T- and B-lymphocyte immunomodulation.
Colucci et al showed that sialylated N-linked glycans induce IgM internalization by T cells, which in turn causes inhibition of T-cell responses.
The authors therefore hypothesized that IgM-mediated immunosuppression occurs through the binding of sialylated IgM to the constitutively expressed IgM Fc receptor (FcμR) on the surface of T lymphocytes[709] .
These results support the concept that the presence of α2,6-sialic acid on Igs contributes to immunosuppression, as previously demonstrated for the anti-inflammatory effects of intravenous immunoglobulin therapy (IVIg)[710] .



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9272703/

https://pubmed.ncbi.nlm.nih.gov/17029568/

[708] Arnold JN, Wormald MR, Suter DM, Radcliffe CM, Harvey DJ, Dwek RA, Rudd PM, Sim RB.
Human serum IgM glycosylation: identification of glycoforms that can bind to mannan-binding lectin.
J Biol Chem. 2005 Aug 12;280(32):29080-7. doi: 10.1074/jbc.M504528200.
https://doi.org/10.1074/jbc.M504528200

[709] Colucci M, Stöckmann H, Butera A, Masotti A, Baldassarre A, Giorda E, Petrini S, Rudd PM, Sitia R, Emma F, Vivarelli M.
Sialylation of N-linked glycans influences the immunomodulatory effects of IgM on T cells.
J Immunol. 2015 Jan 1;194(1):151-7. doi: 10.4049/jimmunol.1402025.
https://doi.org/10.4049/jimmunol.1402025

[710] Schwab I, Nimmerjahn F.
Intravenous immunoglobulin therapy: how does IgG modulate the immune system?
Nat Rev Immunol. 2013 Mar;13(3):176-89. doi: 10.1038/nri3401.
https://pubmed.ncbi.nlm.nih.gov/23411799/

Overview of altered N-glycosylation pathways regarding B lymphocytes during chronic inflammation. In the presence of proinflammatory stimuli, inflammatory T cells significantly affect B-cell proliferation and their N-glycan profile by deregulating a specific subset of glycosyltransferases (B4GALT1, ST6GAL1, FUT8, MGAT3, and GCNT2). The latter is reflected in increased features such as bisection of GlcNAc, agalactosylation, aphucosylation, and presence of branch I that have been shown to inhibit Gal-3 and Gal-9 binding. In addition to the Golgi enzymes affected, lysosomal sialic acid acetyl esterase (SIAE) is also underregulated so that it is unable to deacetylate sialic acids, which is required for immunomodulation of B-cell receptor (BCR) signaling. This figure also summarizes the Fc N- glycome of secreted immunoglobulins, which reflects inflammation-related changes that may further contribute to disease progression.

Acute-phase proteins (APP)

APPs are proteins mainly synthesized and secreted by hepatocytes.

During inflammation, proinflammatory cytokines such as IL-1, IL-8, IL-6, and TNFalpha stimulate the acute phase response[711] , increasing serum levels of APP up to 1000-fold[712] .

Several APPs are glycoproteins, and changes in their N-glycans have been observed in chronic inflammation. The most significant N-glycosylation changes observed in APPs are high branching (tri- and tetra-antennary glycans) and increased levels of sLex epitope detected on haptoglobin (HPT), α1-acid glycoprotein (AGP-1), α1-antitrypsin (A1AT) and α1-antichymotrypsin (ACT)[713] .

---

[711] Hannoodee S, Nasuruddin DN.
Acute Inflammatory Response. [Updated 2021 Nov 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK556083/.

Anglin RE, Rosebush PI, Mazurek MF.
Neuroleptic malignant syndrome: a neuroimmunologic hypothesis.
CMAJ. 2010 Dec 14;182(18):E834-8. doi: 10.1503/cmaj.091442.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3001529/

Baumann H, Won KA, Jahreis GP.
Human hepatocyte-stimulating factor-III and interleukin-6 are structurally and immunologically distinct but regulate the production of the same acute phase plasma proteins.
J Biol Chem. 1989 May 15;264(14):8046-51.
https://www.jbc.org/article/S0021-9258(18)83148-9/pdf

Wigmore SJ, Fearon KC, Maingay JP, Lai PB, Ross JA.
Interleukin-8 can mediate acute-phase protein production by isolated human hepatocytes.
Am J Physiol. 1997 Oct;273(4):E720-6. doi: 10.1152/ajpendo.1997.273.4.E720.
https://pubmed.ncbi.nlm.nih.gov/9357801/

Ceciliani F, Giordano A, Spagnolo V.
The systemic reaction during inflammation: the acute-phase proteins.
Protein Pept Lett. 2002 Jun;9(3):211-23. doi: 10.2174/0929866023408779.
https://pubmed.ncbi.nlm.nih.gov/12144517/

[712] Gabay C, Kushner I.
Acute-phase proteins and other systemic responses to inflammation.
N Engl J Med. 1999 Feb 11;340(6):448-54. doi: 10.1056/NEJM199902113400607. Erratum in: N Engl J Med 1999 Apr 29;340(17):1376.
https://pubmed.ncbi.nlm.nih.gov/9971870/

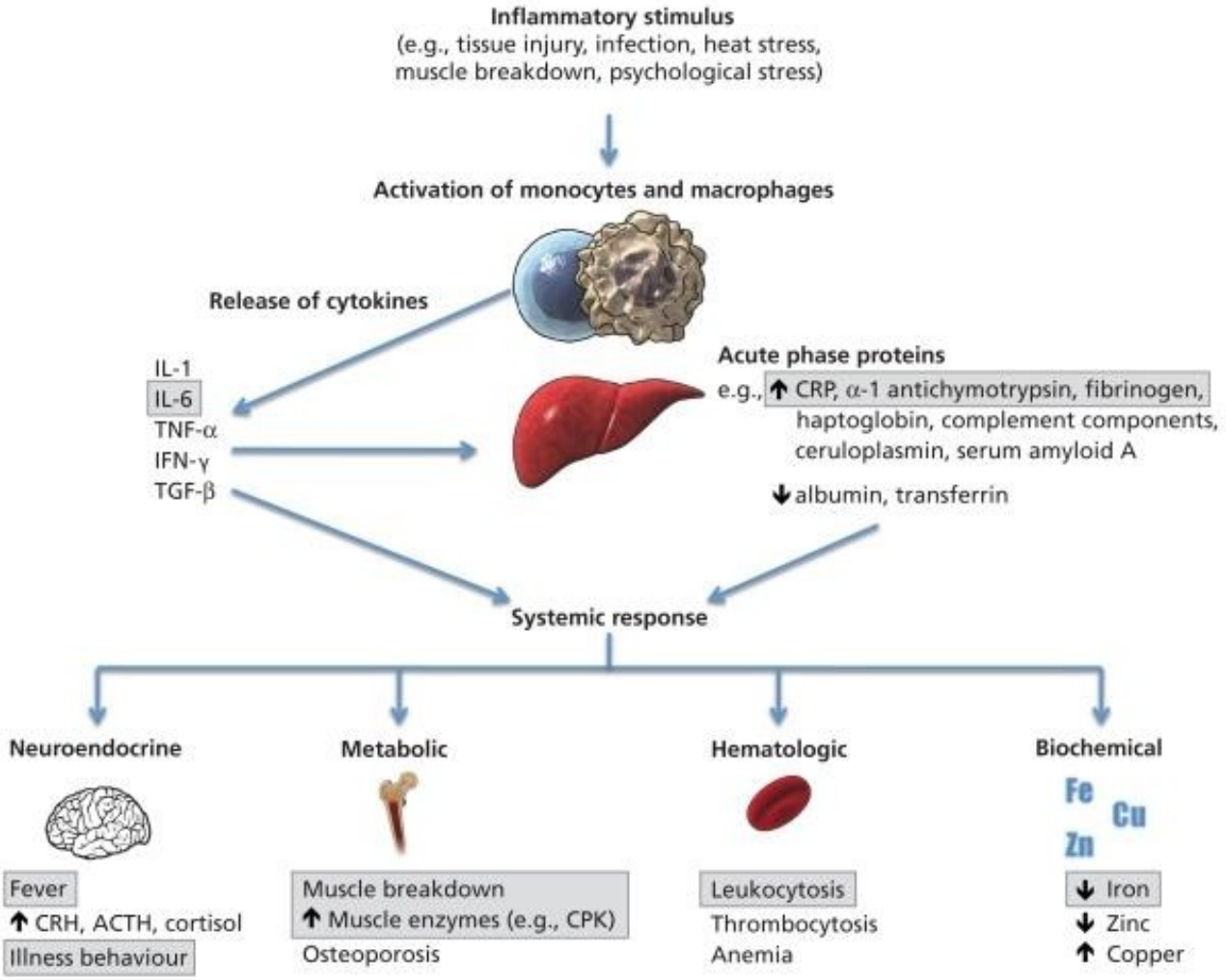
[713] Arnold JN, Saldova R, Hamid UM, Rudd PM.
Evaluation of the serum N-linked glycome for the diagnosis of cancer and chronic inflammation.
Proteomics. 2008 Aug;8(16):3284-93. doi: 10.1002/pmic.200800163.
https://pubmed.ncbi.nlm.nih.gov/18646009/

Gornik O, Lauc G.
Glycosylation of serum proteins in inflammatory diseases.
Dis Markers. 2008;25(4-5):267-78. doi: 10.1155/2008/493289.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3827815/

Higai K, Aoki Y, Azuma Y, Matsumoto K.
Glycosylation of site-specific glycans of alpha1-acid glycoprotein and alterations in acute and chronic inflammation.
Biochim Biophys Acta. 2005 Aug 30;1725(1):128-35. doi: 10.1016/j.bbagen.2005.03.012.
https://pubmed.ncbi.nlm.nih.gov/15863355/

Brinkman-van der Linden EC, de Haan PF, Havenaar EC, van Dijk W.
Inflammation-induced expression of sialyl LewisX is not restricted to alpha1-acid glycoprotein but also occurs to a lesser extent on alpha1- antichymotrypsin and haptoglobin.
Glycoconj J. 1998 Feb;15(2):177-82. doi: 10.1023/a:1006972307166.
https://pubmed.ncbi.nlm.nih.gov/9557878/

The proinflammatory cytokines IL-1β, IL-6, and TNFα, which are involved in the induction of the acute phase response, may also be involved in the regulation of APP glycan biosynthesis in hepatocytes. [714]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3001529/
The acute phase response. An inflammatory stimulus causes activation of monocytes and macrophages that release cytokines. Cytokines act on the liver to stimulate the production of acute phase proteins. Cytokines, together with acute phase proteins, generate a systemic response, with neuroendocrine, metabolic, hematologic, and biochemical changes. The features of the acute phase response that have been directly observed and measured in neuroleptic malignant syndrome are shown in the boxes. Note: ACTH = adrenocorticotropic hormone, CPK = creatine phosphokinase, CRH = corticotropin-releasing hormone, CRP = C-reactive protein, IFN = interferon, IL = interleukin, TGF = transforming growth factor, TNF = tumor necrosis factor.

---

[714] Azuma Y, Murata M, Matsumoto K.
Alteration of sugar chains on alpha(1)-acid glycoprotein secreted following cytokine stimulation of HuH-7 cells in vitro.
Clin Chim Acta. 2000 Apr;294(1-2):93-103. doi: 10.1016/s0009-8981(99)00248-x.
https://pubmed.ncbi.nlm.nih.gov/10727676/

Higai K, Miyazaki N, Azuma Y, Matsumoto K.
Interleukin-1beta induces sialyl Lewis X on hepatocellular carcinoma HuH-7 cells via enhanced expression of ST3Gal IV and FUT VI gene.
FEBS Lett. 2006 Nov 13;580(26):6069-75. doi: 10.1016/j.febslet.2006.09.073.
https://doi.org/10.1016/j.febslet.2006.09.073

Wang Y, Kinzie E, Berger FG, Lim SK, Baumann H.
Haptoglobin, an inflammation-inducible plasma protein.
Redox Rep. 2001;6(6):379-85. doi: 10.1179/135100001101536580.
https://pubmed.ncbi.nlm.nih.gov/11865981/

Glycosylation and intestinal immunity

Glycosylation is a central regulatory mechanism of intestinal homeostasis implicated in many different processes, ranging from epithelial self-renewal, maintenance of intestinal mucus barrier integrity to control of intestinal immunity.[715]

Glycosylation of intestinal epithelial cells (IECs) has been shown to be important in modulating the host immune response. Indeed, expression of α-1,2 fucose on the apical side of IECs has been shown to protect them from pathogenic bacteria.

This fucosylation process is essential in maintaining the commensal microbiota[716] and is mediated by innate lymphoid group 3 cells (ILC3)[717] .

Specifically, commensal and pathogenic bacteria and bacterial products (lipopolysaccharide (LPS)) stimulate ILC3s to produce IL-22, which in turn induces α-1,2 fucosylation of IECs.

Accordingly, inactivating polymorphisms of fucosyltransferase (FUT) 2 (non-secreting state) are associated with inflammatory diseases[718] .

Another component of the intestinal mucosa are Paneth cells (PCs), secretory epithelial cells that act as guardians of the small intestinal crypts, control the microbiota, and are also involved in the formation of the small intestinal crypt stem cell area and the morphogenesis of the crypt-villi axis (CVA).[719]

The crypt compartment houses abundant mannose-rich glycoproteins, while the mature villus zone contains mainly complex-type glycoproteins. [720]

---

[715] Dias AM, Pereira MS, Padrão NA, Alves I, Marcos-Pinto R, Lago P, Pinho SS.
Glycans as critical regulators of gut immunity in homeostasis and disease.
Cell Immunol. 2018 Nov;333:9-18. doi: 10.1016/j.cellimm.2018.07.007.
https://pubmed.ncbi.nlm.nih.gov/30049413/

[716] Goto Y, Obata T, et al
Innate lymphoid cells regulate intestinal epithelial cell glycosylation.
Science. 2014 Sep 12;345(6202):1254009. doi: 10.1126/science.1254009.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4774895/

[717] Artis D, Spits H.
The biology of innate lymphoid cells.
Nature. 2015 Jan 15;517(7534):293-301. doi: 10.1038/nature14189.
https://pubmed.ncbi.nlm.nih.gov/25592534/

[718] McGovern DP, et al
International IBD Genetics Consortium. Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease.
Hum Mol Genet. 2010 Sep 1;19(17):3468-76. doi: 10.1093/hmg/ddq248.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2916706/

Franke A, et al
Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci.
Nat Genet. 2010 Dec;42(12):1118-25. doi: 10.1038/ng.717. PMID: 2110246363;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3299551/

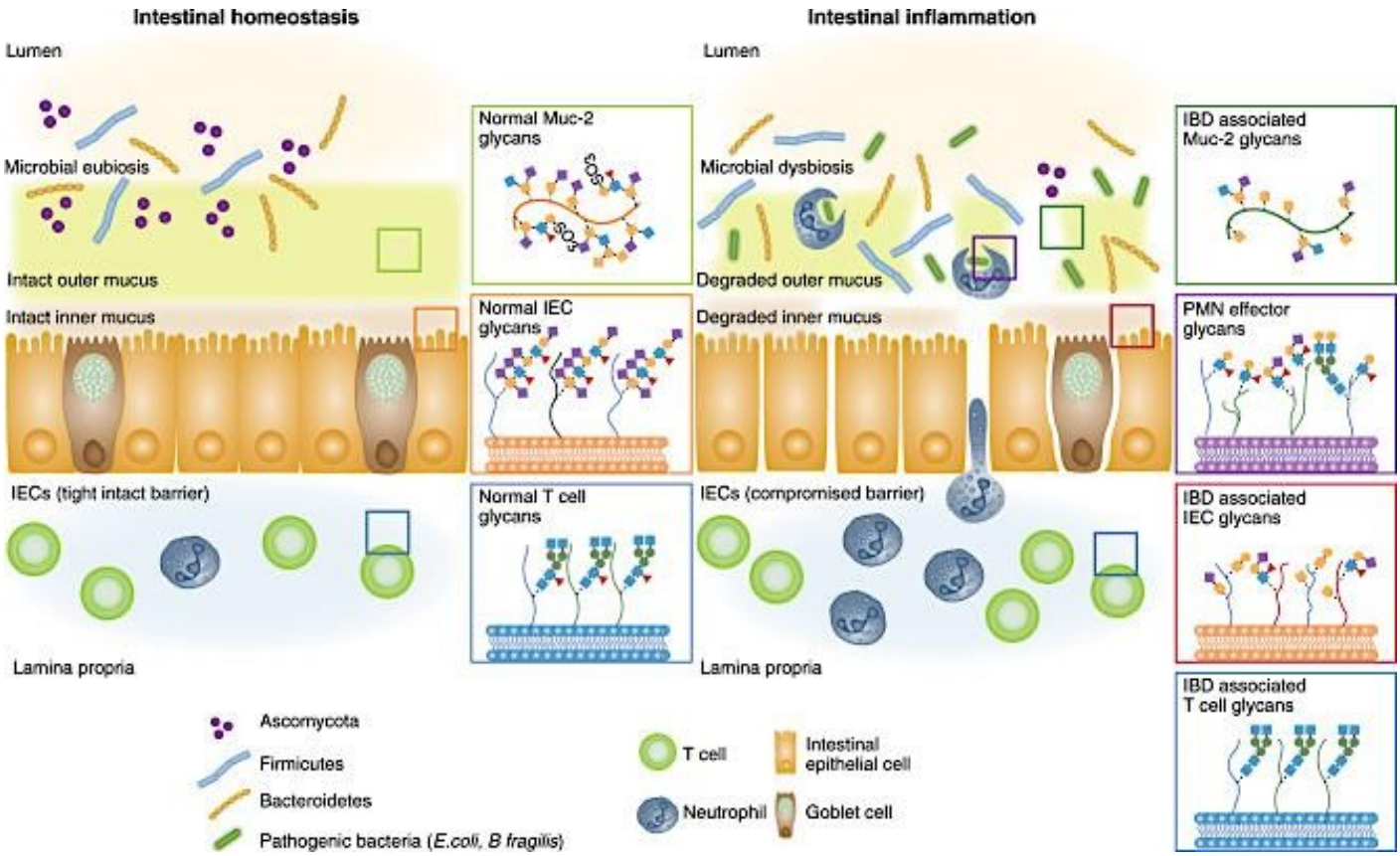
[719] Holly MK, Smith JG.
Paneth Cells during Viral Infection and Pathogenesis.
Viruses. 2018 Apr 26;10(5):225. doi: 10.3390/v10050225. PMID: 29701691;
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5977218/

Salzman NH, Underwood MA, Bevins CL.
Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa.
Semin Immunol. 2007 Apr;19(2):70-83. doi: 10.1016/j.smim.2007.04.002.
https://pubmed.ncbi.nlm.nih.gov/17485224/

[720] E. Köttgen E, Volk B, Kluge F, Gerok W.
Gluten, a lectin with oligomannosyl specificity and the causative agent of gluten-sensitive enteropathy.
Biochem Biophys Res Commun. 1982 Nov 16;109(1):168-73. doi: 10.1016/0006-291x(82)91580-7.
https://pubmed.ncbi.nlm.nih.gov/7159419/

Boniotto M, Braida L, Baldas V, Not T, Ventura A, Vatta S, Radillo O, Tedesco F, Percopo S, Montico M, Amoroso A, Crovella S.
Evidence of a correlation between mannose binding lectin and celiac disease: a model for other autoimmune diseases.
J Mol Med (Berl). 2005 Apr;83(4):308-15. doi: 10.1007/s00109-004-0623-3.
https://pubmed.ncbi.nlm.nih.gov/15645196/

Rouhanifard et al[721] demonstrated that the N-terminal acetylactosamine (LacNAc) of PCs contributes to the regulation of stem cell behavior in support of the central role of glycosylation in the maintenance of mucosal homeostasis through the control of stem cell growth and differentiation as a critical event for self-renewal of the intestinal epithelial layer.
One of the most common alterations in epithelial glycosylation observed in chronic intestinal inflammation is the upregulated expression of truncated or immature surface glycans.[722]
Ramarker et al[723] analyzed the luminal surface of healthy and neoplastic human colorectal tissues for the presence and architecture of the glycocalyx*, a dense network of highly glycosylated proteins, using transmission electron microscopy. Ultrastructural analysis showed that 93% of healthy mucosae were covered by an intact glycocalyx. In contrast, on more than 90% of the neoplastic cell surface, the glycocalyx was absent.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8591159/

Fälth-Magnusson K, Magnusson KE.
Elevated levels of serum antibodies to the lectin wheat germ agglutinin in celiac children lend support to the gluten-lectin theory of celiac disease.
Pediatr Allergy Immunol. 1995 May;6(2):98-102. doi: 10.1111/j.1399-3038.1995.tb00267.x.
https://pubmed.ncbi.nlm.nih.gov/7581728/

[721] Rouhanifard SH, Lopez Aguilar A, Meng L, Moremen KW, Wu P.
Engineered Glycocalyx Regulates Stem Cell Proliferation in Murine Crypt Organoids.
Cell Chem Biol. 2018 Apr 19;25(4):439-446.e5. doi: 10.1016/j.chembiol.2018.01.010.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5910180/

[722] Brazil JC, Parkos CA.
Finding the sweet spot: glycosylation mediated regulation of intestinal inflammation.
Mucosal Immunol. 2022 Feb;15(2):211-222. doi: 10.1038/s41385-021-00466-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8591159/

[723] Ramaker K, Bade S, Röckendorf N, Meckelein B, Vollmer E, Schultz H, Fröschle GW, Frey A.
Absence of the Epithelial Glycocalyx As Potential Tumor Marker for the Early Detection of Colorectal Cancer.
PLoS One. 2016 Dec 29;11(12):e0168801. doi: 10.1371/journal.pone.0168801.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5198998/

Model showing changes in mucosal glycosylation during intestinal inflammation.
The left panel shows intestinal homeostasis with intact mucus layers, microbial eubiosis, and expression of mature surface glycans. The right panel shows intestinal inflammation with degraded mucus layers, overgrowth of pathogenic bacteria, increased PMN trafficking to the lumen, and altered surface expression of glycan structures on epithelial cells, immune cells, and intestinal mucins

The Glycocalyx

To protect the intestinal mucosa from pathogens and mechanical stresses, epithelial cells generate protective layers consisting mainly of secreted and transmembrane glycoproteins that line the entire intestinal tract termed glycocalyx. [724]
In the small intestine, the glycocalyx layer directly covers the entire surface of epithelial cells, [725]
while the overlying lubricating mucus layer is thin and discontinuous[726] .
The glycocalyx comprises highly diverse glycoproteins and glycolipids expressed on the epithelial cell membrane, many of which act as receptors for bacterial adhesion[727] .
The glycocalyx thus serves as an attack site for normal flora to limit colonization by pathogens, as well as acting as a size-selective diffusion barrier to exclude deleterious bacteria and viruses.[728]

---

[724] Sun WW, Krystofiak ES, Leo-Macias A, Cui R, Sesso A, Weigert R, Ebrahim S, Kachar B.
Nanoarchitecture and dynamics of the mouse enteric glycocalyx examined by freeze-etching electron tomography and intravital microscopy.
Commun Biol. 2020 Jan 7;3(1):5. doi: 10.1038/s42003-019-0735-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6946683/

[725] Egberts HJ, Koninkx JF, van Dijk JE, Mouwen JM.
Biological and pathobiological aspects of the glycocalyx of the small intestinal epithelium. A review.
Vet Q. 1984 Sep;6(4):186-99. doi: 10.1080/01652176.1984.9693936.
https://pubmed.ncbi.nlm.nih.gov/6388138/

Pelaseyed T, et al
The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system.
Immunol Rev. 2014 Jul;260(1):8-20. doi: 10.1111/imr.12182.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4281373/

Moran AP, Gupta A, Joshi L.
Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract.
Gut. 2011 Oct;60(10):1412-25. doi: 10.1136/gut.2010.212704.
https://pubmed.ncbi.nlm.nih.gov/21228430/

[726] Linden SK, Sutton P, Karlsson NG, Korolik V, McGuckin MA.
Mucins in the mucosal barrier to infection.
Mucosal Immunol. 2008 May;1(3):183-97. doi: 10.1038/mi.2008.5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7100821/

[727] Hooper LV, Gordon JI.
Glycans as legislators of host-microbial interactions: spanning the spectrum from symbiosis to pathogenicity.
Glycobiology. 2001 Feb;11(2):1R-10R. doi: 10.1093/glycob/11.2.1r.
https://pubmed.ncbi.nlm.nih.gov/11287395/

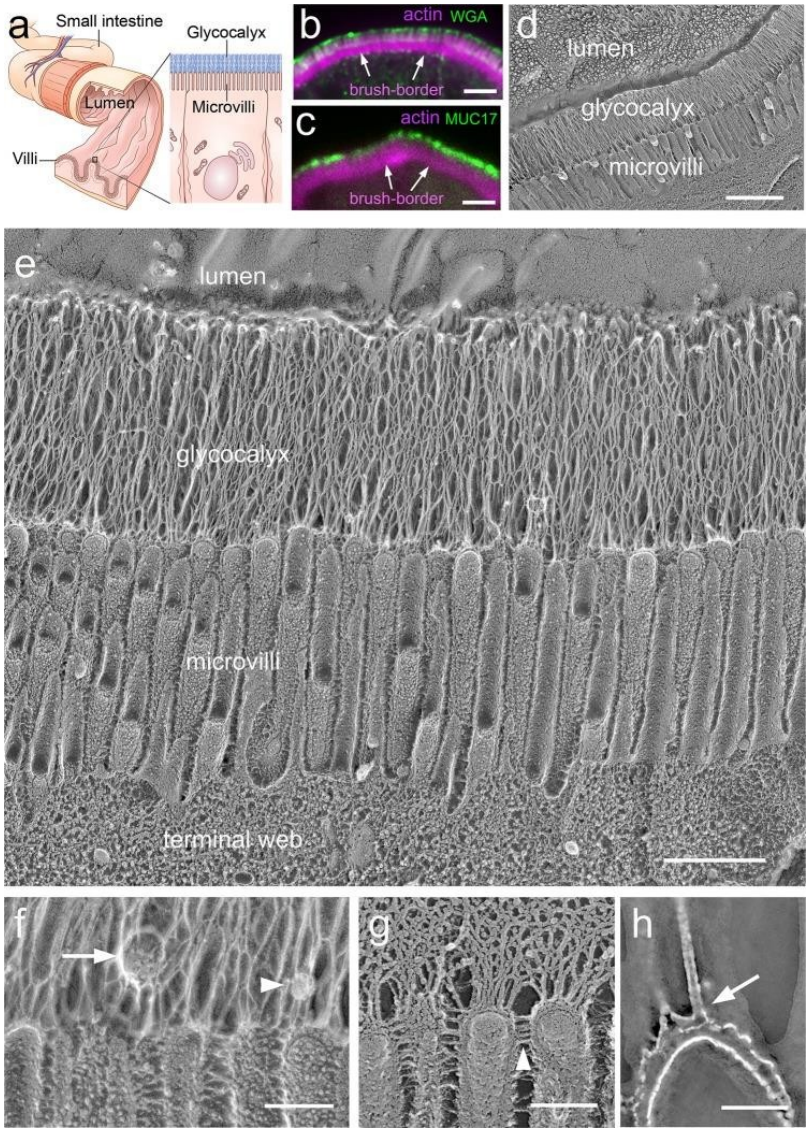
Corfield AP, Wiggins R, Edwards C, Myerscough N, Warren BF, Soothill P, Millar MR, Horner P.
A sweet coating--how bacteria deal with sugars.
Adv Exp Med Biol. 2003;535:3-15.
https://pubmed.ncbi.nlm.nih.gov/14714885/

Barnich N, Carvalho FA, Glasser AL, Darcha C, Jantscheff P, Allez M, Peeters H, Bommelaer G, Desreumaux P, Colombel JF, Darfeuille-Michaud A.
CEACAM6 acts as a receptor for adherent-invasive E. coli, supporting ileal mucosa colonization in Crohn's disease.
J Clin Invest. 2007 Jun;117(6):1566-74. doi: 10.1172/JCI30504.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1868786/

[728] Howe SE, Lickteig DJ, Plunkett KN, Ryerse JS, Konjufca V.
The uptake of soluble and particulate antigens by epithelial cells in the mouse small intestine.
PLoS One. 2014 Jan 27;9(1):e86656. doi: 10.1371/journal.pone.0086656.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3903549/

Stonebraker JR, Wagner D, Lefensty RW, Burns K, Gendler SJ, Bergelson JM, Boucher RC, O'Neal WK, Pickles RJ.
Glycocalyx restricts adenoviral vector access to apical receptors expressed on respiratory epithelium in vitro and in vivo: role for tethered mucins as barriers to lumenal infection.
J Virol. 2004 Dec;78(24):13755-68. doi: 10.1128/JVI.78.24.13755-13768.2004.

In addition to these protective roles, the intestinal glycocalyx contributes to the lubrication and hydrophobicity of the mucosal surface[729] , prevents mucosal self-digestion and ulceration, participates in cell signaling, and acts as a selective barrier to the diffusion of both endogenous and exogenous substances. Given these multiple roles in gut function and homeostasis, it is not surprising that glycocalyx impairment is implicated in a number of diseases of the intestinal tract, including inflammatory bowel disease and cancer.[730]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6946683/

The enteric glycocalyx forms a uniform transcellular layer covering the edge of the microvilli-rich brush.
(a) Schematic diagram of the lumen of the small intestine, which is twisted into villi. Close-up view of an enterocyte showing the glycocalyx layer above its microvilli. **b, c)** Cryo-sections of mouse small intestine were immunolabeled with (top) WGA (green) and (bottom) human anti-MUC17 (green) to highlight the glycocalyx basin above the actin-rich brush border (magenta).
(d) Electron micrograph of a freeze-etch replica of the mouse small intestine showing t h e layered organization of the microvilli-rich brush border and glycocalyx layers. **(e)** Close-up view of the apical region of an enterocyte, showing the glycocalyx separating the microvilli of the brush border from the intestinal lumen.
(f) Higher magnification of the microvilli highlighting glycocalyx filaments emerging from the distal tips of the microvilli. This panel also shows a vesicle (arrow) and a globular structure (arrowhead) embedded in the glycocalyx network.
g) Image of an unfixed specimen confirms a glycocalyx network emerging from the microvilli tips. Glycocalyx filaments emerge from the microvilli tips and can be distinguished from the lateral connections between microvilli (arrowheads). **h)** Single 2-nm tomographic slice through the tip of a microvilli showing that columnar filaments emerge from the membrane (arrow) consistent with a transmembrane mucin. Scale bars: b, c = 2 µm; d = 1 µm; e = 500 nm; f, h = 100 nm; g = 50 nm.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC533903/

Frey A, Giannasca KT, Weltzin R, Giannasca PJ, Reggio H, Lencer WI, Neutra MR.
Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting.
J Exp Med. 1996 Sep 1;184(3):1045-59. doi: 10.1084/jem.184.3.1045.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2192803/

[729] Butler BD, Lichtenberger LM, Hills BA.
Distribution of surfactants in the canine gastrointestinal tract and their ability to lubricate.
Am J Physiol. 1983 Jun;244(6):G645-51. doi: 10.1152/ajpgi.1983.244.6.G645.
https://pubmed.ncbi.nlm.nih.gov/6859272/

[730] van Putten JPM, Strijbis K.
Transmembrane Mucins: Signaling Receptors at the Intersection of Inflammation and Cancer.
J Innate Immun. 2017;9(3):281-299. doi: 10.1159/000453594.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5516414/

Whole-genome association studies (GWAS) have identified strong associations between susceptibility to IBD and polymorphisms in intestinal α1-2 fucosyltransferases (FUT1 and FUT2). [731]
Interestingly, ABO blood group antigens are derived from the modification of Gal terminal residues by the addition of α1,2 Fuc (H antigen) (see below), and FUT2 encodes for the H antigen in the intestinal epithelial cells lining the gastrointestinal tract, whereas FUT1 is required for the synthesis of these structures on erythrocytes.[732]
Various studies have established that ABO-related epithelial antigens strongly influence intestinal microbial composition by acting as binding epitopes for some intestinal microbes including *Helicobacter pylori* and *Norovirus,* and acting as a carbon source for bacterial species such as *Escherichia coli*.[733]

https://pubmed.ncbi.nlm.nih.gov/30049413/
Impact of glycosylation in intestinal homeostasis and inflammation. Changes in the glycan repertoire occur during the transition from normal to inflamed intestinal mucosa. Aberrant expression of specific glycans accompanying intestinal inflammation impacts not only the host immune response but also the content and functions of the microbiota. The composition of glycans, particularly in mucins, is critical for maintaining the protective function of the intestinal barrier against the pathogenic microbiota. In particular, α-1,2 fucosylation catalyzed by FUT2 in epithelial cells is critical for maintaining the commensal microbiota by protecting against pathogenic microorganisms. Loss of mucosal integrity results in the invasion of pathogens concomitant with a cascade of inflammatory events mediated by dendritic cells, macrophages, neutrophils, and subsequently T cells. These pathogenic microorganisms can take advantage of host glycans through glycosidase activity as strategies to "escape" recognition by the host immune system. This selective process gives rise to dysbiosis, which in intestinal disorders, as in IBD, is characterized by a decrease in microbiota diversity (fewer Firmicutes and more Proteobacteria) and a higher Basidiomycota/Ascomycota Fungi ratio. At the level of the lamina propria, alterations in glycosylation are known to regulate the T-lymphocyte-mediated immune response. In homeostasis, the expression of branched β1,6 GlcNAc-catalyzed N-glycans by GnT-V is crucial to control T cell receptor (TCR) function and signaling. In this process, specific carbohydrate recognition proteins such as galectins (Galectin-3) recognize the elongated chain (polylactosamine)

---

[731] McGovern DP, et al International IBD Genetics Consortium.
Fucosyltransferase 2 (FUT2) non-secretor status is associated with Crohn's disease.
Hum Mol Genet. 2010 Sep 1;19(17):3468-76. doi: 10.1093/hmg/ddq248.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2916706/

Franke A, et al
Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci.
Nat Genet. 2010 Dec;42(12):1118-25. doi: 10.1038/ng.717.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3299551/

Wu H, Sun L, Lin DP, Shao XX, Xia SL, Lv M.
Association of Fucosyltransferase 2 Gene Polymorphisms with Inflammatory Bowel Disease in Patients from Southeast China.
Gastroenterol Res Pract. 2017;2017:4148651. doi: 10.1155/2017/4148651.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5266819/

Parmar AS, et al
Association study of FUT2 (rs601338) with celiac disease and inflammatory bowel disease in the Finnish population.
Tissue Antigens. 2012 Dec;80(6):488-93. doi: 10.1111/tan.12016.
https://pubmed.ncbi.nlm.nih.gov/23075394/

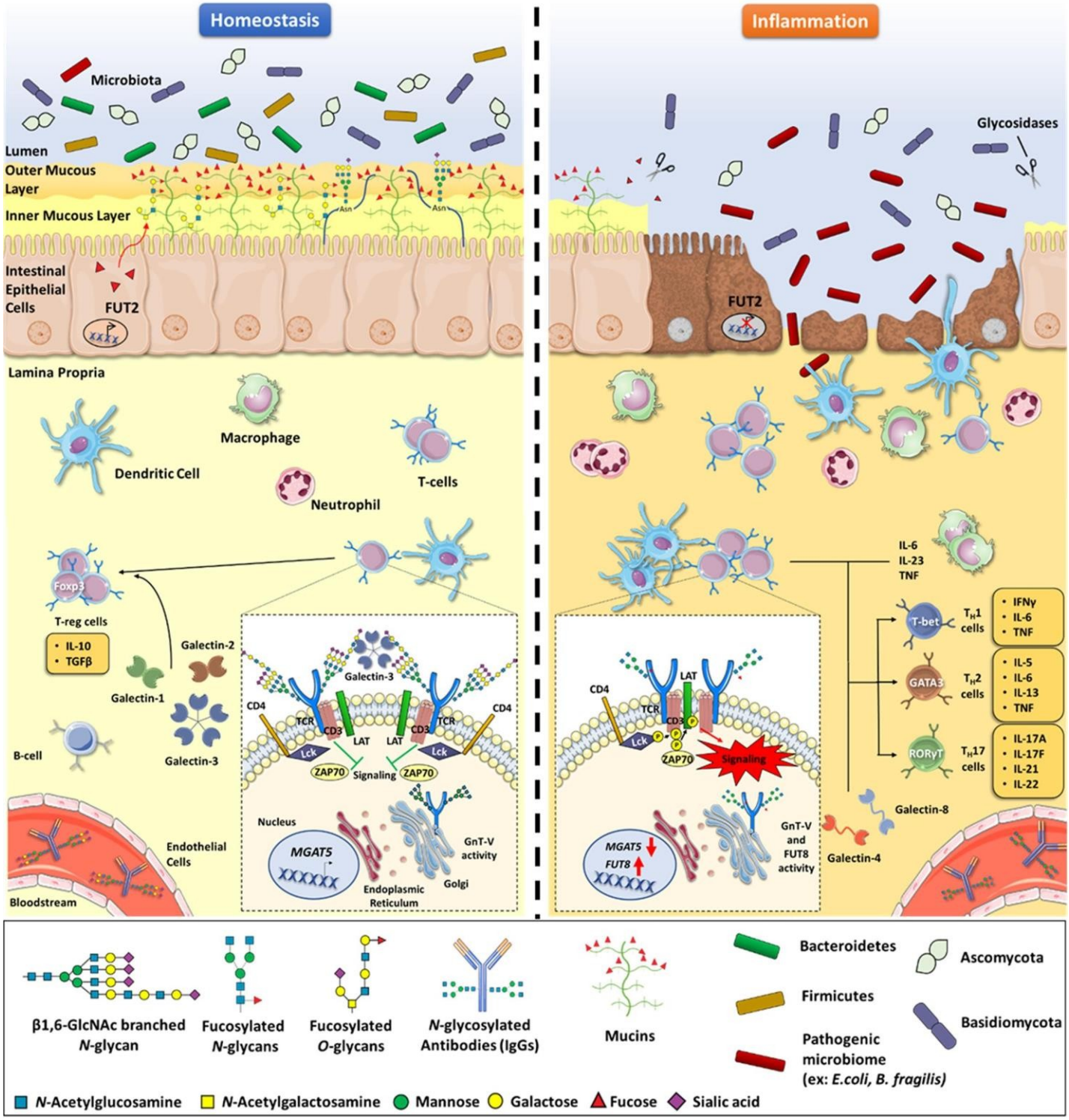
Hu D, Zhang D, Zheng S, Guo M, Lin X, Jiang Y.
Association of Ulcerative Colitis with FUT2 and FUT3 Polymorphisms in Patients from Southeast China.
PLoS One. 2016 Jan 14;11(1):e0146557. doi: 10.1371/journal.pone.0146557.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4713070/

[732] Stanley P, Cummings RD.
Structures Common to Different Glycans. 2017. In: Varki A, Cummings RD, Esko JD, et al, editors.
Essentials of Glycobiology [Internet]. 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015-2017. Chapter 14.
https://www.ncbi.nlm.nih.gov/books/NBK453042/ doi: 10.1101/glycobiology.3e.014

[733] Shirato H, Ogawa S, Ito H, Sato T, Kameyama A, Narimatsu H, Xiaofan Z, Miyamura T, Wakita T, Ishii K, Takeda N.
Noroviruses distinguish between type 1 and type 2 histo-blood group antigens for binding.
J Virol. 2008 Nov;82(21):10756-67. doi: 10.1128/JVI.00802-08.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2573190/

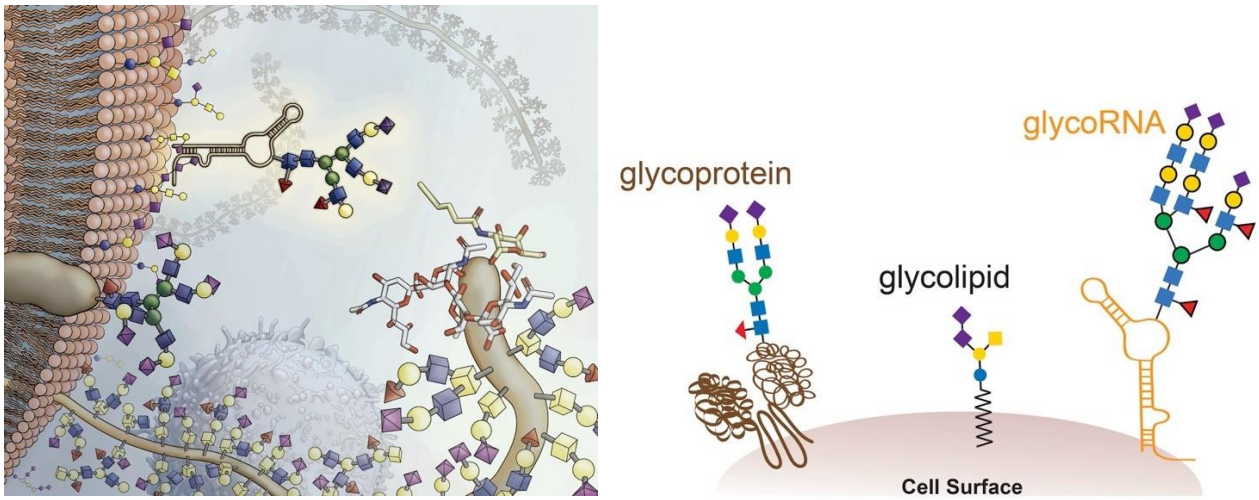
Pacheco AR, Curtis MM, Ritchie JM, Munera D, Waldor MK, Moreira CG, Sperandio V.
Fucose sensing regulates bacterial intestinal colonization.
Nature. 2012 Dec 6;492(7427):113-7. doi: 10.1038/nature11623.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3518558/

of branched N-glycans by forming a "lattice" that precludes TCR clustering and in this way controls the activation threshold of T lymphocytes . Other galectins, such as Galectin-1 and Galectin-2, are also important in the control of the T-lymphocyte-mediated immune response toward a T regulatory response. Importantly, under homeostatic conditions, it has been described that the profile of glycosylation of IgG released in serum shows galactosylated and terminal sialylated N-glycans in the Fc portion. In intestinal inflammation, specific alterations in the expression of glycans, particularly the overregulation of α1-6 fucosyltransferase (FUT8) with overexpression of fucose core and the downregulation of MGAT5 gene with reduced expression of GnT-V-mediated branched N-glycans, have a negative impact in T-lymphocyte response. This altered expression of N-glycans (branched and core-fucosylated) leads to T-cell hyperactivation and intestinal T-lymphocyte signaling, being also associated with T-cell differentiation toward Th1 and Th17 immune responses. In addition, the expression of galectins 4 and 8 further stimulates T-cell proliferation in colitis. Furthermore, in intestinal inflammation (as in IBD) the expression profile of serum IgG is different from homeostasis, being mainly characterized by agalatosylation.



Glyco-RNA

Glycans modify lipids and proteins to mediate intermolecular and intramolecular interactions in all domains of life, so much so that Rabinovich et al in their article *"Glycobiology opf immune response"*[734] call nucleic acids "cousins" of carbohydrates, as both DNA and RNA are essentially polysaccharides composed of phosphate-bound polyribose nuclei. Without carbohydrates, nucleic acids would not form the linear scaffold necessary for their function.
Similarly, they call proteins and carbohydrates "roommates" because in mammals carbohydrates are almost always associated with proteins or lipids.
Despite this nice metaphor, RNA was never considered to be one of the main targets of glycosylation.
Flynn et al, in their article "*Small RNAs are modified with N-glycans and displayed on the surface of living cells,*"[735] challenge this view with evidence that mammals use RNA as a third scaffold for glycosylation. Using a battery of chemical and biochemical approaches, they found that conserved small noncoding RNAs carry sialylated glycans.
These "glycoRNAs" were present in multiple cell types and mammalian species, in cultured cells and *in vivo*. GlycoRNA assembly depends on the canonical N-glycan biosynthesis machinery and results in structures enriched in sialic acid and fucose.
Analysis of living cells revealed that most glycoRNAs were present on the cell surface and can interact with anti-dsRNA antibodies and members of the Siglec receptor family.



https://www.hhmi.org/news/some-rna-molecules-have-unexpected-sugar-coating

The discovery of antibodies against an impressive number of RNAs, alone or in complex with glycosylated proteins, that cause various autoimmune diseases suggests the existence of a direct interface between RNA biology and glycobiology and an expanded role for RNA in extracellular biology. [736]

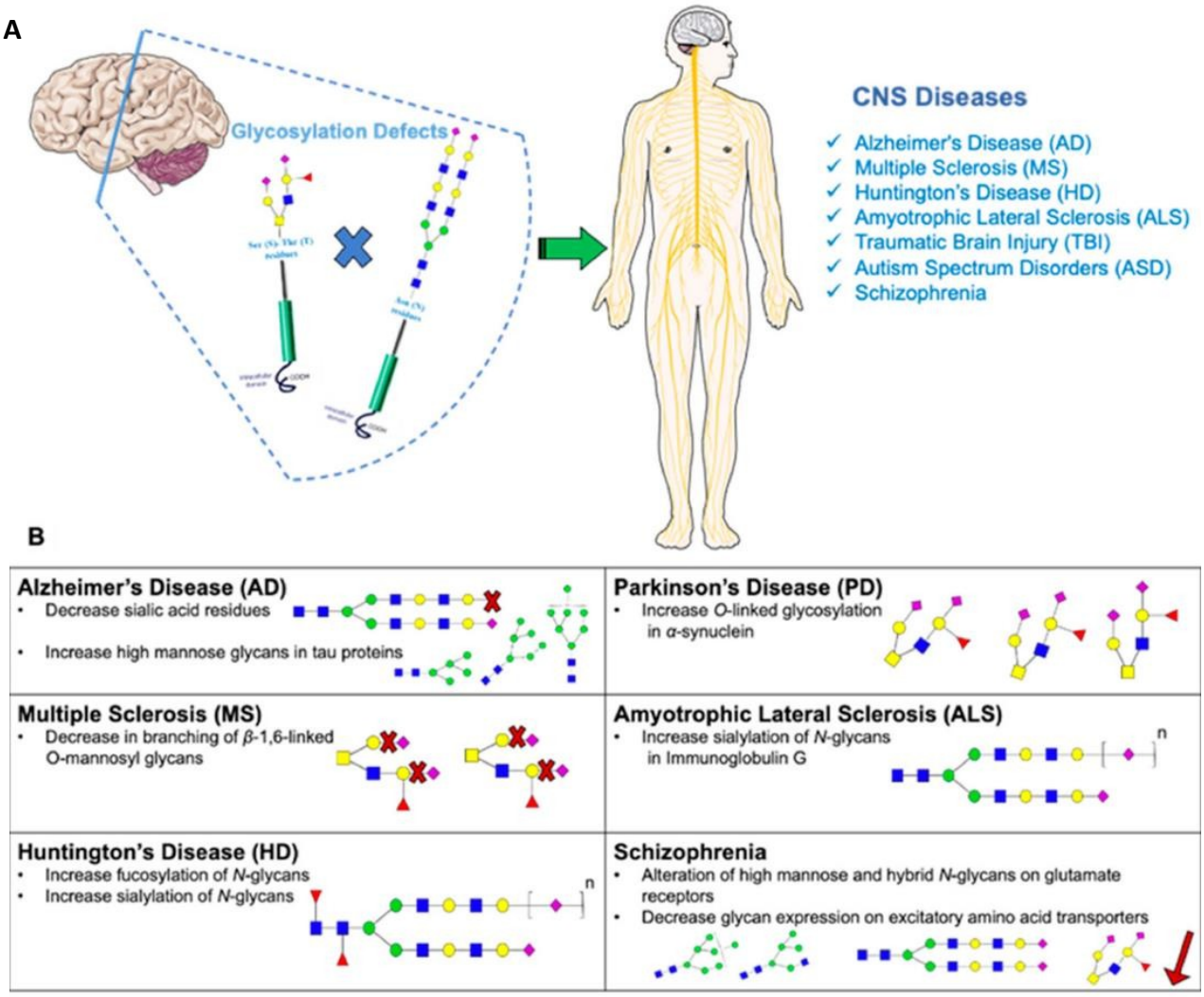
[734] Rabinovich GA, van Kooyk Y, Cobb BA.
Glycobiology of immune responses.
Ann N Y Acad Sci. 2012 Apr;1253:1-15. doi: 10.1111/j.1749-6632.2012.06492.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3884643/

[735] Flynn RA, Pedram K, Malaker SA, Batista PJ, Smith BAH, Johnson AG, George BM, Majzoub K, Villalta PW, Carette JE, Bertozzi CR.
Small RNAs are modified with N-glycans and displayed on the surface of living cells.
Cell. 2021 Jun 10;184(12):3109-3124.e22. doi: 10.1016/j.cell.2021.04.023.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9097497/

[736] Ryan A. Flynn, et al
Mammalian Y RNAs are modified at discrete guanosine residues with N-glycans
bioRxiv 787614; doi: https://doi.org/10.1101/787614
https://www.biorxiv.org/content/10.1101/787614v1.full.pdf

Glycosylation and neurodegenerative diseases

Over the past decade, researchers have worked extensively to unravel the relationships between glycoproteins and neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). [737]
The following figure shows the consequences of glycosylation defects.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8834236/
Correlation between alterations in glycosylation and CNS diseases. **(A)** Representation of the consequences of glycosylation defects occurring in different lobes of the human brain. **(B)** Characterization of the consequences of altered glycosylation according to the type of neurological or psychiatric disease formed.
See also **Table 3**

Glycosylation and prion diseases

Glycosylation also interacts with other factors that contribute to various neurodegenerative disorders such as prion disease and other disorders.
Prion disease involves the structural change of a specific prion protein PrP^{c} into its disease-associated isoform PrP^{Sc} [738] . PrP^{c} undergoes two PTMs (Post-Translational Modifications), the first

[737] Kobeissy F, et al
Glycomic and Glycoproteomic Techniques in Neurodegenerative Disorders and Neurotrauma: Towards Personalized Markers.
Cells. 2022 Feb 8;11(3):581. doi: 10.3390/cells11030581
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8834236/

[738] Prusiner SB.

is GPI attachment to its C-terminal residue Ser-231 and the other is glycosylation at residues Asn-181 and Asn197[739] . Studies have shown that when PrP^{C} is not glycosylated at its N-terminal, it becomes more susceptible to conversion to PrP^{Sc}[740] . This was observed in a study using murine neuroblastoma cells treated with tunicamycin to block N-glycosylation, and showed that reduced glycosylation of PrP^{C} predisposes it to convert to PrP^{Sc}[741] .
In particular, the immature high-mannose form of PrP^{C} , which has yet to undergo complex glycosylation, is the most susceptible to conversion to PrP^{Sc} .
Glycosylation at the N-terminus can contain up to five sialic acid residues[742] that alter the properties of the protein and play a role in the rate of infectivity of the misfolded PrP isoform[743] .
Because sialic acid is negatively charged and is directed outward to create a dense negative cloud,[744] Katorcha et al.[730] proposed that this could be an electrostatic obstacle for PrP replication^{Sc} , and in support of this they showed that the level of deglycosylation in the PrP form^{Sc} was lower than that in PrP^{C} .
In addition, among some mouse strains tested in the same study, partial desialylation caused an increase in the replication rate of the protein, adding evidence that this type of PTM forms a barrier to replication. In addition, changes in PrP^{C} sialylation levels influenced the formation of three different glycoforms[745] that can selectively give rise to PrP .[Sc746]

Novel proteinaceous infectious particles cause scrapie.
Science. 1982 Apr 9;216(4542):136-44. doi: 10.1126/science.6801762.
https://pubmed.ncbi.nlm.nih.gov/6801762/

[739] Katorcha E, Makarava N, Savtchenko R, Baskakov IV.
Sialylation of the prion protein glycans controls prion replication rate and glycoform ratio.
Sci Rep. 2015 Nov 18;5:16912. doi: 10.1038/srep16912.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4649626/

[740] Schachter H.
Congenital disorders involving defective N-glycosylation of proteins.
Cell Mol Life Sci. 2001 Jul;58(8):1085-104. doi: 10.1007/PL00000923.
https://pubmed.ncbi.nlm.nih.gov/11529501/

[741] Ma J, Lindquist S.
De novo generation of a PrPSc-like conformation in living cells.
Nat Cell Biol. 1999 Oct;1(6):358-61. doi: 10.1038/14053.
https://pubmed.ncbi.nlm.nih.gov/10559963/

[742] Rudd PM, Endo T, Colominas C, Groth D, Wheeler SF, Harvey DJ, Wormald MR, Serban H, Prusiner SB, Kobata A, Dwek RA.
Glycosylation differences between the normal and pathogenic prion protein isoforms.
Proc Natl Acad Sci U S A. 1999 Nov 9;96(23):13044-9. doi: 10.1073/pnas.96.23.13044.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC23897/

[743] Katorcha E, Makarava N, Savtchenko R, Baskakov IV.
Sialylation of the prion protein glycans controls prion replication rate and glycoform ratio.
Sci Rep. 2015 Nov 18;5:16912. doi: 10.1038/srep16912.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4649626/

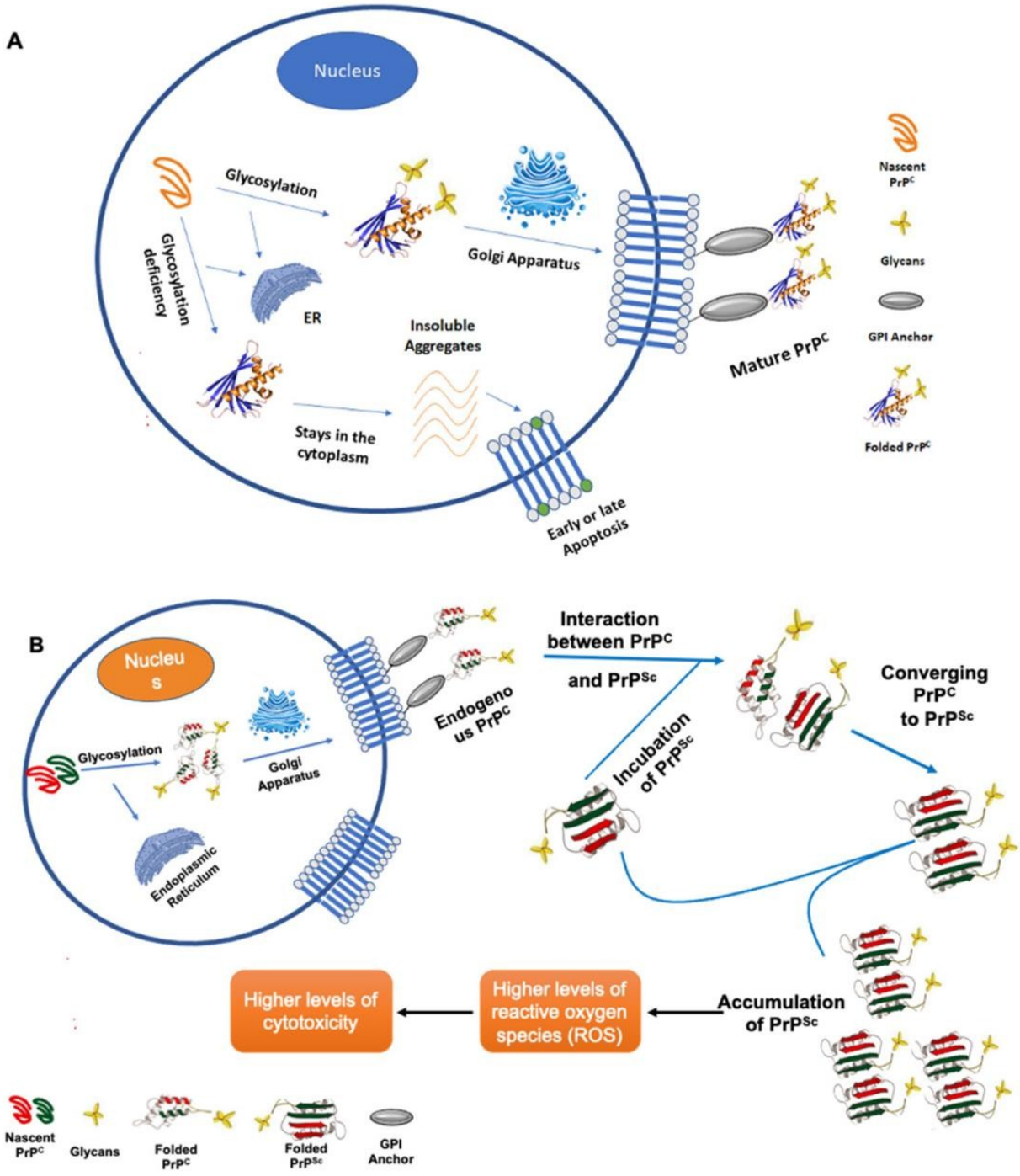
[744] Wille H, Michelitsch MD, Guenebaut V, Supattapone S, Serban A, Cohen FE, Agard DA, Prusiner SB.
Structural studies of the scrapie prion protein by electron crystallography.
Proc Natl Acad Sci U S A. 2002 Mar 19;99(6):3563-8. doi: 10.1073/pnas.052703499.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC122563/

Requena JR, Wille H.
The structure of the infectious prion protein: experimental data and molecular models.
Prion. 2014 Jan-Feb;8(1):60-6. doi: 10.4161/pri.28368.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7030906/

[745] Katorcha E, Makarava N, Savtchenko R, Baskakov IV.
Sialylation of the prion protein glycans controls prion replication rate and glycoform ratio.
Sci Rep. 2015 Nov 18;5:16912. doi: 10.1038/srep16912.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4649626/

[746] Xiao X, et al
Glycoform-selective prion formation in sporadic and familial forms of prion disease.
PLoS One. 2013;8(3):e58786. doi: 10.1371/journal.pone.0058786.

In another study, the role of glycosylation in the subcellular localization of PrP was investigated[C] ,[747] and it was found that it shows altered localization on the plasma membrane and that glycosylation enhances proteinase K resistance, protein aggregation capacity, increases ROS levels and cytotoxicity.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3602448/

Somerville RA.
Host and transmissible spongiform encephalopathy agent strain control glycosylation of PrP.
J Gen Virol. 1999 Jul;80(Pt 7):1865-1872. doi: 10.1099/0022-1317-80-7-1865.
https://pubmed.ncbi.nlm.nih.gov/10423157/

[747] Yi CW, Wang LQ, Huang JJ, Pan K, Chen J, Liang Y.
Glycosylation Significantly Inhibits the Aggregation of Human Prion Protein and Decreases Its Cytotoxicity.
Sci Rep. 2018 Aug 22;8(1):12603. doi: 10.1038/s41598-018-30770-6. Erratum in: Sci Rep. 2018 Sep 4;8(1):13486.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6105643/

https://www.mdpi.com/2073-4409/11/3/581/htm
The glycosylation process of normal PrP and the effect of glycosylation deficiency or conversion of PrP^{C} to PrP^{Sc} . **(A)** Under normal conditions, nascent PrP undergoes glycosylation that occurs in the endoplasmic reticulum (ER), then matures in the Golgi apparatus and eventually reaches the plasma with the help of the GPI anchor. However, when glycosylation deficiency occurs, nascent PrP becomes insoluble aggregates leading to early or late apoptosis (green label). **(B**) Plasma mature PrP^{C} may interact with PrP^{Sc} and this would lead to conversion and accumulation of PrP^{Sc} which in turn would increase the level of cytotoxicity due to the presence of this prion disease."

Other factors that induce amyloid protein aggregation.

Effect of electromagnetic radiation emitted by cell phones

It has been suggested that radiation emitted by cell phones may induce several health problems.[748] Various studies have reported headaches and sleep disturbances,[749] blood-brain barrier damage[750] and even genetic and proteomic alterations in humans.[751]

In vitro studies have also shown that exposure to ultra-low-frequency electromagnetic fields (ELF-EMFs) can affect modulation of heat shock proteins, apoptosis, and DNA damage.[752]

Neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD), are characterized by progressive degeneration of the central nervous system (CNS). The extracellular brain depositions

---

[748] Hardell L, Sage C.
Biological effects from electromagnetic field exposure and public exposure standards.
Biomed Pharmacother. 2008 Feb;62(2):104-9. doi: 10.1016/j.biopha.2007.12.004.
https://pubmed.ncbi.nlm.nih.gov/18242044/

[749] Hillert L, Akerstedt T, Lowden A, Wiholm C, Kuster N, Ebert S, Boutry C, Moffat SD, Berg M, Arnetz BB.
The effects of 884 MHz GSM wireless communication signals on headache and other symptoms: an experimental provocation study.
Bioelectromagnetics. 2008 Apr;29(3):185-96. doi: 10.1002/bem.20379.
https://pubmed.ncbi.nlm.nih.gov/18044740/

Schirmacher A, Winters S, Fischer S, Goeke J, Galla HJ, Kullnick U, Ringelstein EB, Stögbauer F.
Electromagnetic fields (1.8 GHz) increase the permeability to sucrose of the blood-brain barrier in vitro.
Bioelectromagnetics. 2000 Jul;21(5):338-45.
https://pubmed.ncbi.nlm.nih.gov/10899769/.

[751] Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS.
Qualitative effect on mRNAs of injury-associated proteins by cell phone like radiation in rat facial nerves.
Electromagn Biol Med. 2009;28(4):383-90. doi: 10.3109/15368370903287614.
https://pubmed.ncbi.nlm.nih.gov/20017629/

Misa-Agustiño MJ, Leiro-Vidal JM, Gomez-Amoza JL, Jorge-Mora MT, Jorge-Barreiro FJ, Salas-Sánchez AA, Ares-Pena FJ, López-Martín E.
EMF radiation at 2450 MHz triggers changes in the morphology and expression of heat shock proteins and glucocorticoid receptors in rat thymus.
Life Sci. 2015 Apr 15;127:1-11. doi: 10.1016/j.lfs.2015.01.027.
https://pubmed.ncbi.nlm.nih.gov/25731700/

[752] Kayhan H, Esmekaya MA, Saglam AS, Tuysuz MZ, Canseven AG, Yagci AM, Seyhan N.
Does MW Radiation Affect Gene Expression, Apoptotic Level, and Cell Cycle Progression of Human SH-SY5Y Neuroblastoma Cells?
Cell Biochem Biophys. 2016 Jun;74(2):99-107. doi: 10.1007/s12013-016-0734-9.
https://pubmed.ncbi.nlm.nih.gov/27260669/

Calabrò E, Condello S, Currò M, Ferlazzo N, Caccamo D, Magazù S, Ientile R.
Modulation of heat shock protein response in SH-SY5Y by mobile phone microwaves.
World J Biol Chem. 2012 Feb 26;3(2):34-40. doi: 10.4331/wjbc.v3.i2.34.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3286792/

Falone S, Grossi MR, Cinque B, D'Angelo B, Tettamanti E, Cimini A, Di Ilio C, Amicarelli F.
Fifty hertz extremely low-frequency electromagnetic field causes changes in redox and differentiative status in neuroblastoma cells.
Int J Biochem Cell Biol. 2007;39(11):2093-106. doi: 10.1016/j.biocel.2007.06.001.
https://pubmed.ncbi.nlm.nih.gov/17662640/

Seyyedi S, Mozdarani H, Rezaei Tavirani M, Heydari S.
Induction of chromosomal aberrations in human primary fibroblasts and immortalized cancer cells exposed to extremely-low-frequency electromagnetic fields.
Int J Radiat Res. 2010; 8 (1) :25-29
http://ijrr.com/article-1-606-en.pdf

of amyloid peptide ß (Aß) are considered the histopathological sign of AD, while Parkinson's disease is caused by the degeneration of dopaminergic neurons in the substantia nigra[753] .
The main histopathologic feature of PD is the formation of Lewy bodies (LBs) in the pigmented nuclei of the brainstem,[754] and it has been suggested that in normal brain tissue, alpha synuclein (α-syn) controls dopamine levels by decreasing dopamine transporter activity[755] and that alpha synuclein aggregates represent the main component of LBs.
It is also believed that free radicals generated by dopamine metabolism increase α-syn neurotoxicity in dopaminergic neurons,[756] in which toxic oligomeric α-syn protofibrils are implicated in disruption of cellular homeostasis, neuronal death, and total failure of synaptic function.[757]

Stefi et al, in their study "*Mobile phone electromagnetic radiation affects Amyloid Precursor Protein and α-synuclein metabolism in SH-SY5Y* cells,"[758] reported the results of the effects of electromagnetic field (EMF) emitted by low-level GSM on amyloid precursor protein (APP) and α-syn in human neuroblastoma cells.
The data indicated alterations on APP processing and cell topology after exposure to electromagnetic fields (Ɛ = 10.51 V/m, SAR = 0.23 W/kg, exposure time: 3 times, for 10 minutes, for 2 days), changes in accumulation and multimerization of monomeric α-syn, as well as induction
Of oxidative stress and cell death.
In particular, the increase in monomeric α-syn together with the generation of new amyloidogenic fragments possessing an alternative cellular topology, as well as the induction of cellular toxicity and oxidative stress, suggest that wireless communications may represent a new factor influencing human health, and that exposure to these devices may be involved in pathogenic mechanisms leading to neurodegeneration.

Prion diseases and spikes of natural SARS-Cov-2

The presence of prion-like sequences in the SARS-Cov-2 spike and in vaccines containing the whole spike sequence and the pathological consequences were discussed in detail in the chapter "Toxicology of the Spike" from p. 18.

[753] Spatula M, Wider C.
Genetics of Parkinson's disease: the yield.
Parkinsonism Relat Disord. 2014 Jan;20 Suppl 1:S35-8. doi: 10.1016/S1353-8020(13)70011-7.
https://pubmed.ncbi.nlm.nih.gov/24262184/

[754] Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M.
Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28;388(6645):839-40. doi: 10.1038/42166.
https://pubmed.ncbi.nlm.nih.gov/9278044/

[755] Little KY, McLaughlin DP, Zhang L, McFinton PR, Dalack GW, Cook EH Jr, Cassin BJ, Watson SJ.
Brain dopamine transporter messenger RNA and binding sites in cocaine users: a postmortem study. Arch Gen Psychiatry. 1998 Sep;55(9):793-9. doi: 10.1001/archpsyc.55.9.793.
https://pubmed.ncbi.nlm.nih.gov/9736005/

[756] Wersinger C, Sidhu A.
Attenuation of dopamine transporter activity by alpha-synuclein.
Neurosci Lett. 2003 Apr 17;340(3):189-92. doi: 10.1016/s0304-3940(03)00097-1.
https://pubmed.ncbi.nlm.nih.gov/12672538/

[757] Conway KA, Harper JD, Lansbury PT.
Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease.
Nat Med. 1998 Nov;4(11):1318-20. doi: 10.1038/3311.
https://pubmed.ncbi.nlm.nih.gov/9809558/

[758] Stefi AL, Margaritis LH, Skouroliakou AS, Vassilacopoulou D.
Mobile phone electromagnetic radiation affects Amyloid Precursor Protein and α-synuclein metabolism in SH-SY5Y cells.
Pathophysiology. 2019 Sep-Dec;26(3-4):203-212. doi: 10.1016/j.pathophys.2019.02.004.
https://pubmed.ncbi.nlm.nih.gov/30850244/

Further studies have confirmed the risk of prion disease occurrence following infection with SARS- Cov-2 [759] Specifically, Nyström et[760] al analyzed the amyloidogenic properties of the Spike protein and found seven amyloidogenic sequences capable of forming aggregates during incubation at 37°C.
Three synthetic peptides derived from the 20-amino-acid-long spike (sequence 192-211, 601-620, 1166-1185) fulfilled three criteria for the definition of amyloid fibril*: nucleation-dependent polymerization kinetics of ThT (thioflavin T), Congo red positivity, and ultrastructural fibrillar morphology.

** Amyloid is defined by the presence of deposits of fibrillar protein aggregates in tissues. Amyloid fibrils of different origins share common structural features, notably a unique quaternary conformation, called β-sheet (beta) structure, with an inter-sheet regularity of 4.7 Å. Tissue-type plasminogen activator (tPA), an activator of fibrinolysis, has been identified as a multiligand receptor for the amyloid cross-β structure in vitro[761] . Plasmin is a serine protease that mediates proteolysis of*

---

[759] Idrees D, Kumar V.
SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration.
Biochem Biophys Res Commun. 2021 May 21;554:94-98. doi: 10.1016/j.bbrc.2021.03.100.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7988450/

Bernardini A, Gigli GL, Janes F, Pellitteri G, Ciardi C, Fabris M, Valente M.
Creutzfeldt-Jakob disease after COVID-19: infection-induced prion protein misfolding? A case report.
Prion. 2022 Dec;16(1):78-83. doi: 10.1080/19336896.2022.2095185.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9255144/

Tetz G, Tetz V.
Prion-like Domains in Spike Protein of SARS-CoV-2 Differ across Its Variants and Enable Changes in Affinity to ACE2.
Microorganisms. 2022 Jan 25;10(2):280. doi: 10.3390/microorganisms10020280.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8878784/

Liu S, et al
Highly efficient intercellular spreading of protein misfolding mediated by viral ligand-receptor interactions.
Nat Commun. 2021 Oct 19;12(1):5739. doi: 10.1038/s41467-021-25855-2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8526834/

Camacho RC, Alabed S, Zhou H, Chang SL.
Network Meta-analysis on the Changes of Amyloid Precursor Protein Expression Following SARS-CoV-2 Infection.
J Neuroimmune Pharmacol. 2021 Dec;16(4):756-769. doi: 10.1007/s11481-021-10012-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8579188/

Semerdzhiev SA, Fakhree MAA, Segers-Nolten I, Blum C, Claessens MMAE.
Interactions between SARS-CoV-2 N-Protein and α-Synuclein Accelerate Amyloid Formation.
ACS Chem Neurosci. 2022 Jan 5;13(1):143-150. doi: 10.1021/acschemneuro.1c00666.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8739828/

Shahzad S, Willcox M.
The Possible Role of Prion-Like Viral Protein Domains on the Emergence of Novel Viruses as SARS-CoV-2.
J Mol Evol. 2022 Aug;90(3-4):227-230. doi: 10.1007/s00239-022-10054-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8972983/

Kozlov V.A., Sapozhnikov S.P.
Rapid Amyloid Formation and Thrombi Formation in COVID-19 (A brief literature review) [Electronic resource]
Acta medica Eurasica. - 2021. - №3. P. 1-9. - DOI: 10.47026/2413-4864-2021-3-1-9.
http://acta-medica-eurasica.ru/en/single/2021/3/1/.

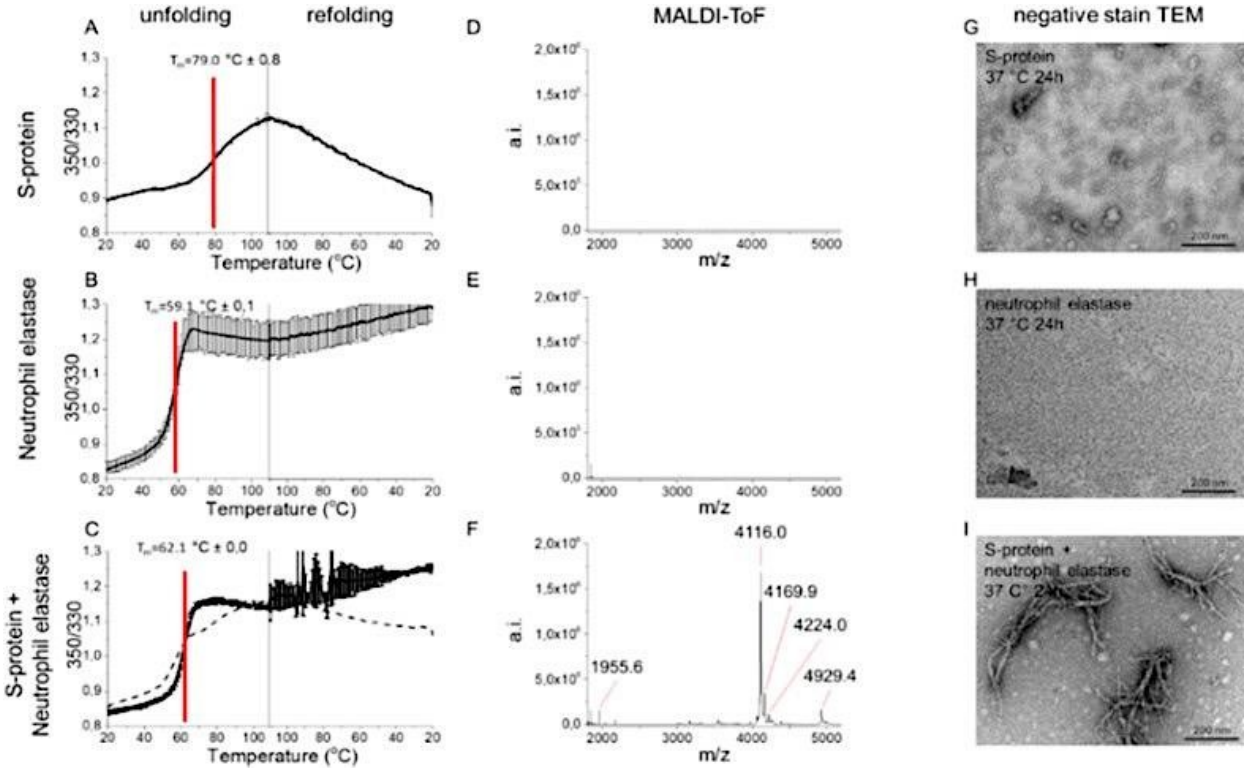
[760] Nyström S, Hammarström P.
Amyloidogenesis of SARS-CoV-2 Spike Protein.
J Am Chem Soc. 2022 May 25;144(20):8945-8950. doi: 10.1021/jacs.2c03925.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9136918/
https://www.biorxiv.org/content/10.1101/2021.12.16.472920v1.full.pdf

Taniya Bhardwaj, et al.
Amyloidogenic proteins in the SARS-CoV and SARS-CoV-2 proteomes
bioRxiv 2021.05.29.446267; doi: https://doi.org/10.1101/2021.05.29.446267
https://www.biorxiv.org/content/10.1101/2021.05.29.446267v2.full.pdf

[761] Kranenburg O, Bouma B, Kroon-Batenburg LM, Reijerkerk A, Wu YP, Voest EE, Gebbink MF.

many substrates, including fibrin, and increased plasmin activity has previously been implicated in bleeding[762] . The protein deposits accumulated in patients with systemic amyloidosis share structural features that activate plasmin formation. Dysregulated molecules include the acute-phase inflammatory molecule Serum Amyloid A (SAA) and α(2)-antiplasmin (α2AP)[763] . The levels of these circulating proteins were found to be predictive for the clinical outcome of diseased patients and iIn particular, plasma levels of PAP (plasmin-α2-antiplasmin) complexes were significantly elevated in patients with systemic amyloidosis, compared with healthy control subjects[764] and this is indicative of a shift in the hemostatic balance toward a fibrinolytic state in patients with systemic amyloidosis.

Full-length folded protein S did not form amyloid fibrils, but amyloid-like fibrils with obvious branching were formed during 24 h co-incubation of protein S with neutrophil elastase protease (NE) *in vitro*.
NE efficiently cleaved the S protein, promoting the exposure of amyloidogenic segments and the accumulation of the amyloidogenic peptide 194-203, unique to SARS-Cov-2 which, in combination with acute inflammation and neutrophil recruitment, known to be more prevalent in COVID-19 than in other viral infections, could explain the amyloid formation associated with COVID-19.



Tissue-type plasminogen activator is a multiligand cross-beta structure receptor.
Curr Biol. 2002 Oct 29;12(21):1833-9. doi: 10.1016/s0960-9822(02)01224-1.
https://doi.org/10.1016/S0960-9822(02)01224-1

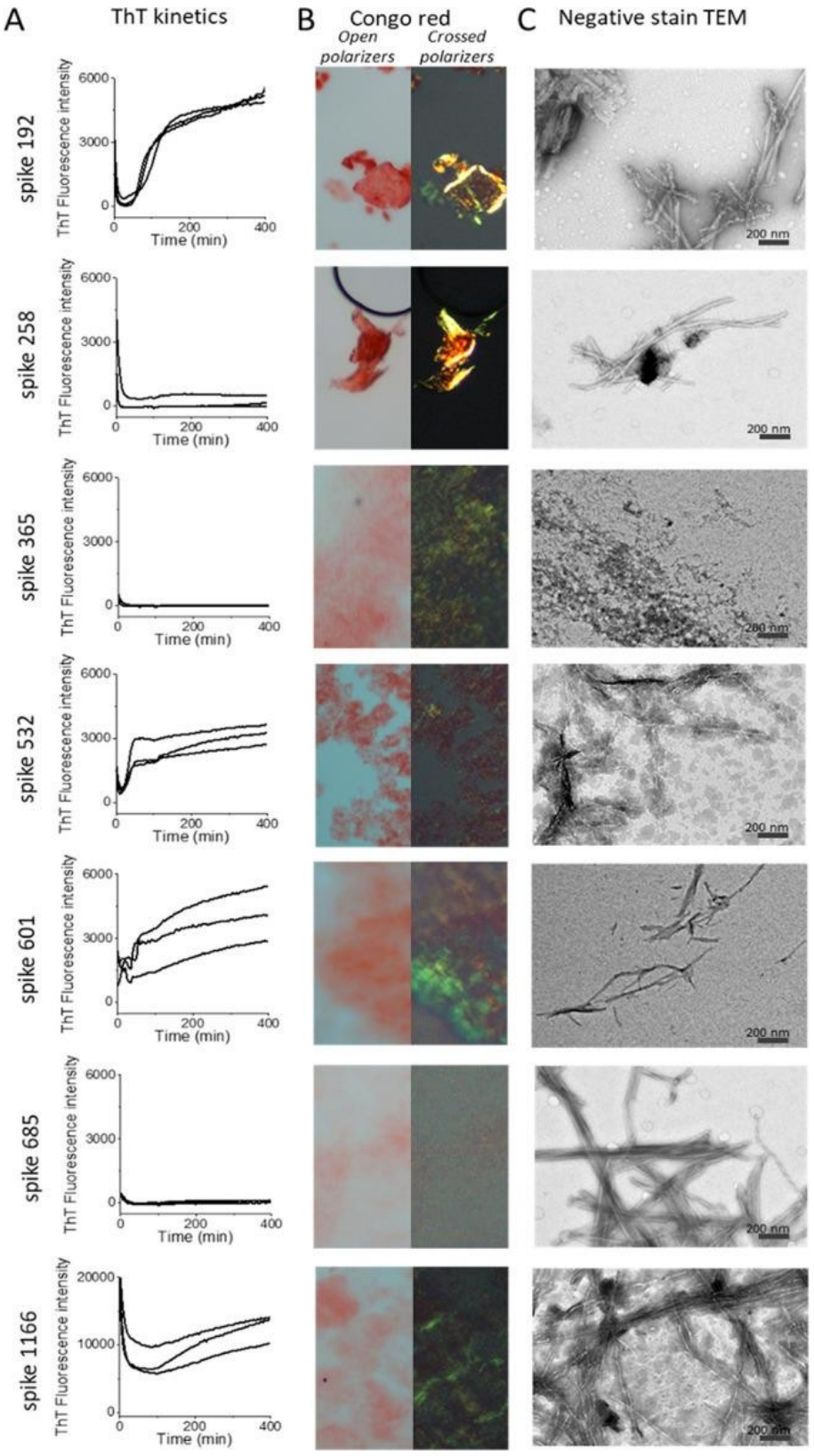
[762] Francis RB Jr.
Clinical disorders of fibrinolysis: a critical review.
Blut. 1989 Jul;59(1):1-14. doi: 10.1007/BF00320240.
https://pubmed.ncbi.nlm.nih.gov/2526671/

[763] Gillmore JD, Lovat LB, Persey MR, Pepys MB, Hawkins PN.
Amyloid load and clinical outcome in AA amyloidosis in relation to circulating concentration of serum amyloid A protein.
Lancet. 2001 Jul 7;358(9275):24-9. doi: 10.1016/S0140-6736(00)05252-1.
https://pubmed.ncbi.nlm.nih.gov/11454373/

[764] Bouma B, Maas C, Hazenberg BP, Lokhorst HM, Gebbink MF.
Increased plasmin-alpha2-antiplasmin levels indicate activation of the fibrinolytic system in systemic amyloidoses.
J Thromb Haemost. 2007 Jun;5(6):1139-42. doi: 10.1111/j.1538-7836.2007.02457.x.
https://doi.org/10.1111/j.1538-7836.2007.02457.x

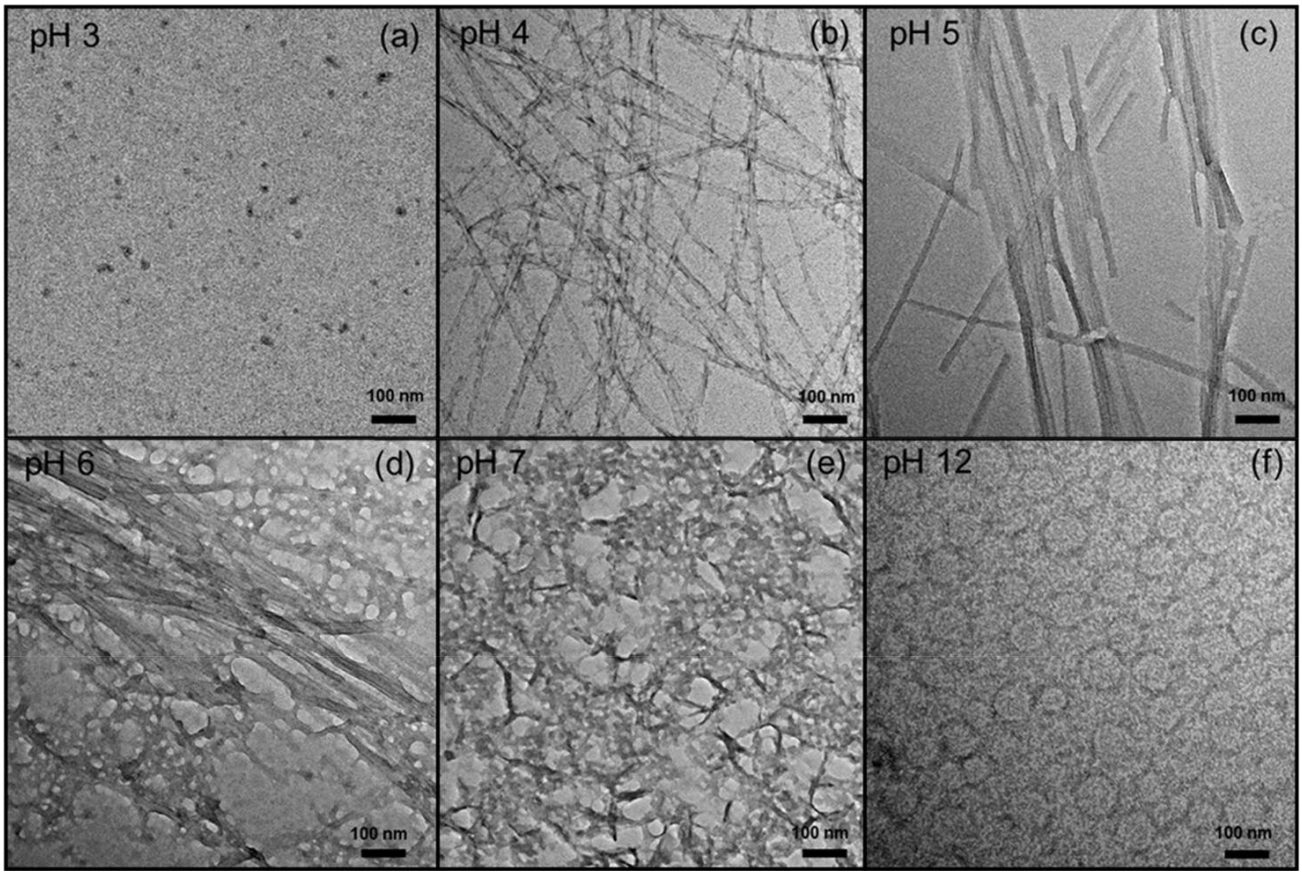
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9136918/

Proteolysis of S-protein by NE makes amyloid-like fibrils. Thermostability of (A) SARS-CoV-2 S-protein, (B) NE, (C) S-protein+NE, measured by DSF. The dotted line in (C) is the mathematical sum of S-protein and NE, respectively, from (A) and (B) supporting the cleavage of S-protein by NE. MALDI-ToF spectra of isolated C18 peptides of (D) S-protein, (E) NE and (F) S-protein + NE (6 h, 37°C). TEM micrographs of S-protein alone (G) depicting predicted trimers, (H) NE alone and (I) S-protein + NE coincubated at pH 8.4, 24 h, 37°C.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9136918/
Amyloid fibril assays of SARS-CoV-2 S peptides (0.1 mg/mL). (A) Kinetics of ThT fluorescence fibril formation. (B) Congo red birefringence microscopy. (C) Negative-staining TEM ultrastructure.

These findings were also confirmed by the study of Castelletto et al,[765] in which the formation of β-sheet fibril structures was demonstrated from the conserved sequence of the coronavirus spike protein, RSAIEDLLFDKV, and pH-dependent aggregation and hydrogelation behavior.



https://pubs.acs.org/doi/10.1021/acsnano.1c10658
TEM images for solutions of 1% by weight RSAIEDLLFDKV dissolved in (a) water (native; pH 3) or in aqueous solutions of NaOH adjusted to give pH (b) 4, (c) 5, (d) 6, (e) 7 and (f) 12.

It should be mentioned that amyloidosis* is quite common in the elderly population,[766] and it has been proposed that severe inflammatory disease, including ARDS in combination with SARS-CoV-2 protein aggregation, can induce systemic amyloidosis A. [767]

[765] Valeria Castelletto, Ian W. Hamley
Amyloid and Hydrogel Formation of a Peptide Sequence from a Coronavirus Spike Protein
ACS Nano 2022 16 (2), 1857-1867 DOI: 10.1021/acsnano.1c10658
https://pubs.acs.org/doi/10.1021/acsnano.1c10658

[766] Benson MD, Buxbaum JN, Eisenberg DS, Merlini G, Saraiva MJM, Sekijima Y, Sipe JD, Westermark P.
Amyloid nomenclature 2020: update and recommendations by the International Society of Amyloidosis (ISA) nomenclature committee.
Amyloid. 2020 Dec;27(4):217-222. doi: 10.1080/13506129.2020.1835263.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9136918/

[767] Sinha N, Thakur AK.
Likelihood of amyloid formation in COVID-19-induced ARDS.
Trends Microbiol. 2021 Nov;29(11):967-969. doi: 10.1016/j.tim.2021.03.008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8007089/

** Primary systemic amyloidosis (AL) is caused by the accumulation of monoclonal immunoglobulin (LC) light chains, which are overproduced by plasma cells[768] . AL is the most common form of systemic amyloidosis. Secondary systemic amyloidosis (AA) can be recognized by the accumulation of serum amyloid protein A, a process believed to be a dangerous side effect of chronic inflammation[769] . Third, there are hereditary forms of systemic amyloidosis caused by mutations in transthyretin (ATTR[770]) or, more rarely, by lysozyme, fibrinogen, or other proteins[771] , which cause the accumulation of these proteins in amyloid deposits. Amyloidosis is associated with amyloid cerebral angiopathy, altered blood coagulation, fibrinolytic disorders,[772] activation of FXII kallikrein/kinin, and thromboinflammation. [773]*

Neurotropic colonization and cross-seeding of amyloid S protein fibrils to induce aggregation of endogenous proteins were discussed above in the context of neurodegeneration.[774]

---

[768] Comenzo RL.
Amyloidosis.
Curr Treat Options Oncol. 2006 May;7(3):225-36. doi: 10.1007/s11864-006-0015-8.
https://pubmed.ncbi.nlm.nih.gov/16615878/

[769] Suzuki A, Ohosone Y, Obana M, Mita S, Matsuoka Y, Irimajiri S, Fukuda J.
Cause of death in 81 autopsied patients with rheumatoid arthritis.
J Rheumatol. 1994 Jan;21(1):33-6.
https://pubmed.ncbi.nlm.nih.gov/8151583/

[770] Benson MD.
The hereditary amyloidoses.
Best Pract Res Clin Rheumatol. 2003 Dec;17(6):909-27. doi: 10.1016/j.berh.2003.09.001.
https://pubmed.ncbi.nlm.nih.gov/15123043/

[771] Granel B, Valleix S, Serratrice J, Chérin P, Texeira A, Disdier P, Weiller PJ, Grateau G.
Lysozyme amyloidosis: report of 4 cases and a review of the literature.
Medicine (Baltimore). 2006 Jan;85(1):66-73. doi: 10.1097/01.md.0000200467.51816.6d.
https://journals.lww.com/md-journal/Fulltext/2006/01000/Lysozyme_Amyloidosis_Report_of_4_Cases_and_a.7.aspx

Tennent GA, Brennan SO, Stangou AJ, O'Grady J, Hawkins PN, Pepys MB.
Human plasma fibrinogen is synthesized in the liver.
Blood. 2007 Mar 1;109(5):1971-4. doi: 10.1182/blood-2006-08-040956.
https://doi.org/10.1182/blood-2006-08-040956

Merlini G, Westermark P.
The systemic amyloidoses: clearer understanding of the molecular mechanisms offers hope for more effective therapies.
J Intern Med. 2004 Feb;255(2):159-78. doi: 10.1046/j.1365-2796.2003.01262.x.
https://doi.org/10.1046/j.1365-2796.2003.01262.x

de Carvalho M, Linke RP, Domingos F, Evangelista T, Ducla-Soares JL, Nathrath WB, Azevedo-Coutinho C, Lima R, Saraiva MJ.
Mutant fibrinogen A-alpha-chain associated with hereditary renal amyloidosis and peripheral neuropathy.
Amyloid. 2004 Sep;11(3):200-7. doi: 10.1080/13506120400000772.
https://pubmed.ncbi.nlm.nih.gov/15523923/

Sucker C, Hetzel GR, Grabensee B, Stockschlaeder M, Scharf RE.
Amyloidosis and bleeding: pathophysiology, diagnosis, and therapy.
Am J Kidney Dis. 2006 Jun;47(6):947-55. doi: 10.1053/j.ajkd.2006.03.036.
https://pubmed.ncbi.nlm.nih.gov/16731289/

[772] Hammarström P.
The bloody path of amyloids and prions.
J Thromb Haemost. 2007 Jun;5(6):1136-8. doi: 10.1111/j.1538-7836.2007.02575.x.
https://doi.org/10.1111/j.1538-7836.2007.02575.x

[773] Maas C, Govers-Riemslag JW, Bouma B, Schiks B, Hazenberg BP, Lokhorst HM, Hammarström P, ten Cate H, de Groot PG, Bouma BN, Gebbink MF. Misfolded proteins activate factor XII in humans, leading to kallikrein formation without initiating coagulation.
J Clin Invest. 2008 Sep;118(9):3208-18. doi: 10.1172/JCI35424.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2518075/

[774] Tavassoly O, Safavi F, Tavassoly I.
Seeding Brain Protein Aggregation by SARS-CoV-2 as a Possible Long-Term Complication of COVID-19 Infection.
ACS Chem Neurosci. 2020 Nov 18;11(22):3704-3706. doi: 10.1021/acschemneuro.0c00676
https://pubmed.ncbi.nlm.nih.gov/33147014/

In particular, blood clotting associated with extracellular amyloid fibrillar aggregates in the bloodstream has been reported in patients with COVID-19.
In addition, impaired hypercoagulation/fibrinolysis has been demonstrated in the blood plasma of healthy donors experimentally supplemented with protein S*.[775]

** This study shows that blood from vaccinated and long-Covid donors can induce clotting in recipients.*

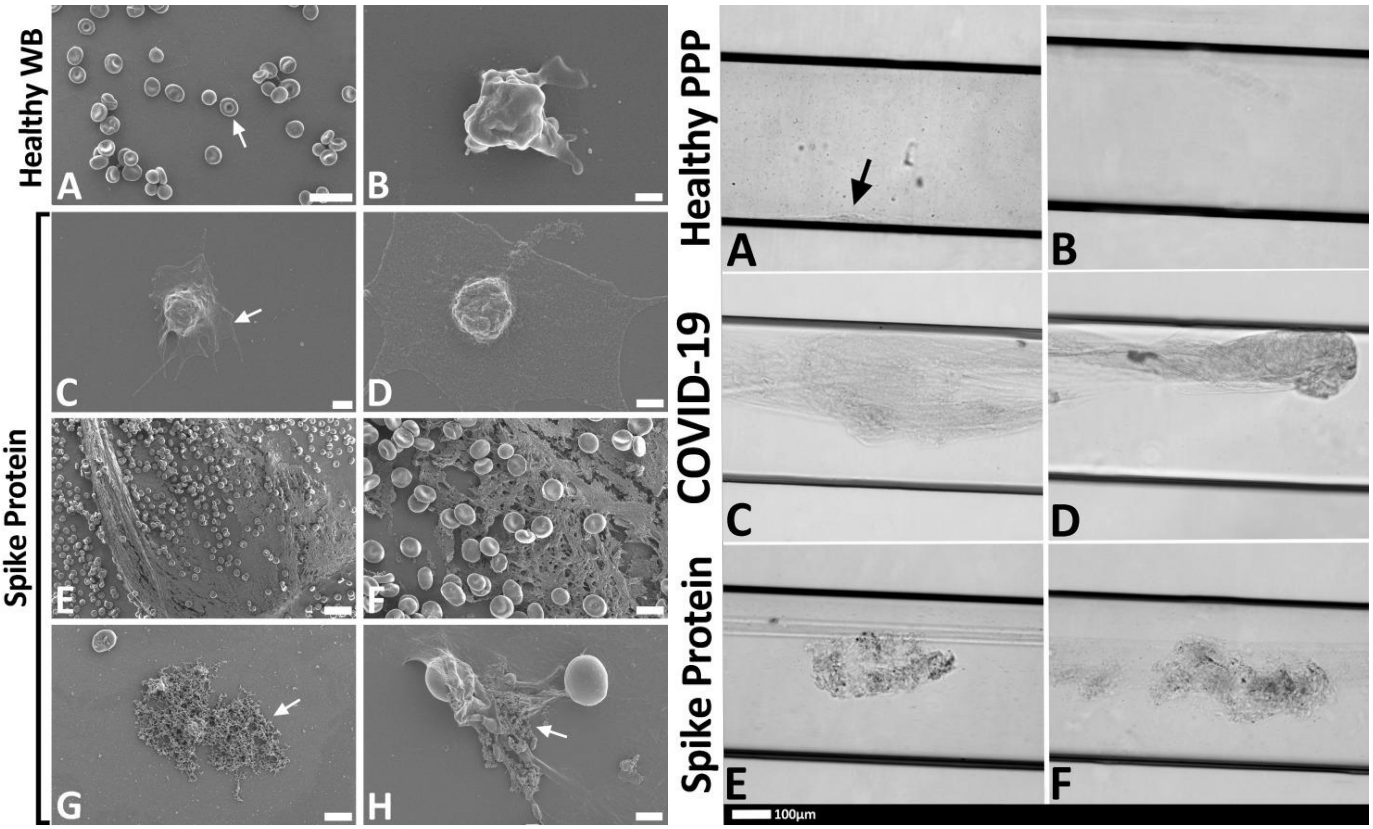


Fig. 1Fig . 2

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8380922/

Fig. 1 Whole blood sample of healthy volunteers, before and after exposure to spike protein
(A-H) Representative scanning electron micrographs of a healthy control WB, with and without protein spike. (A, B) Healthy WB smears, with an arrow indicating normal erythrocyte ultrastructure. (C-H) Healthy WB exposed to protein spike (1 ng.ml-1 final concentration), with (C, D) indicating activated platelets (arrow), (E, F) showing spontaneously formed fibrin network and (G ,H) abnormal deposits of amyloid nature (arrows) (scale bars: (E) 20 µm; (A) 10 µm; (F,G) 5 µm; (H) 2 µm; (C) 1 µm; (B,D) 500 nm).

Fig. 2 Representative micrographs of PPP clots in microfluidic chambers (black horizontal lines are the contours of the chambers) that were coated with thrombin
(A) Healthy PPP clot with small clot formation (arrow), with (B) no clot formed in healthy PPP sample. (C,D) examples of clots from COVID-19 PPP samples and healthy PPP clot (E,F) with spike protein. Black arrow = small clot formed in control sample; red arrows large clots in COVID-19 sample.

A few years ago, Kell et al[776] found that fibrinogen in the blood can coagulate into an abnormal form of "amyloid" fibrin that (like other amyloids and β-rich prions) is relatively resistant to proteolysis (fibrinolysis).

[775] Grobbelaar LM, Venter C, Vlok M, Ngoepe M, Laubscher GJ, Lourens PJ, Steenkamp J, Kell DB, Pretorius E.
SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19.
Biosci Rep. 2021 Aug 27;41(8):BSR20210611. doi: 10.1042/BSR20210611.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8380922/

[776] Kell DB, Laubscher GJ, Pretorius E.
A central role for amyloid fibrin microclots in long COVID/PASC: origins and therapeutic implications.
Biochem J. 2022 Feb 17;479(4):537-559. doi: 10.1042/BCJ20220016.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8883497/

The result, as occurs in the platelet-poor plasma (PPP) of individuals with long-COVID, are extensive fibrin amyloid microclots that can persist, trap other proteins, and lead to the production of various autoantibodies. These microclots are more or less easily measured in PPP with thioflavin T staining and a simple fluorescence microscope.

Another feature of COVID-19 is extremely high levels of platelet activation[777] due to dysregulation of P-selectin[778] (P-selectin is an inflammatory biomarker of coagulation and is known to modulate interactions between blood cells and endothelial cells[779]), and hyperferritinemia[780] .

This leads to an apparent paradox, where both clotting and bleeding can be observed as part of the pathology, and it is believed that the resolution of the paradox is that these stages of clotting and bleeding are separated in time.

Specifically, bleeding propensity is mediated by the previous coagulation-induced depletion of both fibrinogen and von Willebrand factor (VWF).

In addition, P-selectin can significantly contribute to the adhesion of both pathological and healthy erythrocytes to the damaged endothelium as well as to adjacent erythrocytes.

Regarding hyperferritinemia, it has long been known that excess circulating iron causes the blood to clot in an abnormal form[781] , which was later found to be amyloid in nature[782] (it is

---

[777] Venter C, Bezuidenhout JA, Laubscher GJ, Lourens PJ, Steenkamp J, Kell DB, Pretorius E.
Erythrocyte, Platelet, Serum Ferritin, and P-Selectin Pathophysiology Implicated in Severe Hypercoagulation and Vascular Complications in COVID-19. Int J Mol Sci. 2020 Nov 3;21(21):8234. doi: 10.3390/ijms21218234.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7662625/

Grobler C, Maphumulo SC, Grobbelaar LM, Bredenkamp JC, Laubscher GJ, Lourens PJ, Steenkamp J, Kell DB, Pretorius E.
Covid-19: The Rollercoaster of Fibrin(Ogen), D-Dimer, Von Willebrand Factor, P-Selectin and Their Interactions with Endothelial Cells, Platelets and Erythrocytes.
Int J Mol Sci. 2020 Jul 21;21(14):5168. doi: 10.3390/ijms21145168.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7403995/

[778] Neri T., Nieri D., Celi A.
P-selectin blockade in COVID-19-related ARDS.
Am. J. Physiol. Lung Cell. Mol. Physiol. 2020;318:L1237-L1238. doi: 10.1152/ajplung.00202.2020
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7276981/

Goshua G., Pine A.B., Meizlish M.L., Chang C.H., Zhang H., Bahel P., Baluha A., Bar N., Bona R.D., Burns A.J., et al.
Endotheliopathy in COVID-19-associated coagulopathy: Evidence from a single-center, cross-sectional study.
Lancet Haematol. 2020;7:e575-e582. doi: 10.1016/S2352-3026(20)30216-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7326446/

[779] Neri T., Nieri D., Celi A.
P-selectin blockade in COVID-19-related ARDS.
Am. J. Physiol. Lung Cell. Mol. Physiol. 2020;318:L1237-L1238. doi: 10.1152/ajplung.00202.2020
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7276981/

[780] Perricone C, Bartoloni E, Bursi R, Cafaro G, Guidelli GM, Shoenfeld Y, Gerli R.
COVID-19 as part of the hyperferritinemic syndromes: the role of iron depletion therapy.
Immunol Res. 2020 Aug;68(4):213-224. doi: 10.1007/s12026-020-09145-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7366458/

Gómez-Pastora J, Weigand M, Kim J, Wu X, Strayer J, Palmer AF, Zborowski M, Yazer M, Chalmers JJ.
Hyperferritinemia in critically ill COVID-19 patients - Is ferritin the product of inflammation or a pathogenic mediator?
Clin Chim Acta. 2020 Oct;509:249-251. doi: 10.1016/j.cca.2020.06.033
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7306200/

[781] Pretorius E., Vermeulen N., Bester J., Lipinski B., Kell D.B.
A novel method for assessing the role of iron and its functional chelation in fibrin fibril formation: The use of scanning electron microscopy.
Toxicol. Mech. Methods. 2013;23:352-359. doi: 10.3109/15376516.2012.762082.
https://pubmed.ncbi.nlm.nih.gov/23278212/

[782] Kell D.B., Pretorius E.
Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: Lessons from and for blood clotting.
Prog. Biophys. Mol. Biol. 2017;123:16-41. doi: 10.1016/j.pbiomolbio.2016.08.006.
https://doi.org/10.1016/j.pbiomolbio.2016.08.006

important to report that LPS produced by Gram-negative bacteria is also capable of forming amyloid-like clots and that it is possible to inhibit and reverse amyloid structures *in vitro* by administering small amounts of LBP (lipopolysaccharide-binding protein)).[783]
Both platelets and erythrocytes manifest pathological ultrastructural changes[784] in the presence of increased serum ferritin and iron[785] .

Kell DB, Pretorius E.
To What Extent Are the Terminal Stages of Sepsis, Septic Shock, Systemic Inflammatory Response Syndrome, and Multiple Organ Dysfunction Syndrome Actually Driven by a Prion/Amyloid Form of Fibrin?
Semin Thromb Hemost. 2018 Apr;44(3):224-238. doi: 10.1055/s-0037-1604108.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6193370/

Pretorius E, Bester J, Page MJ, Kell DB.
The Potential of LPS-Binding Protein to Reverse Amyloid Formation in Plasma Fibrin of Individuals With Alzheimer-Type Dementia.
Front Aging Neurosci. 2018 Aug 22;10:257. doi: 10.3389/fnagi.2018.00257.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6113936/

Pretorius E., Mbotwe S., Bester J., Robinson C.J., Kell D.B.
Acute induction of anomalous and amyloidogenic blood clotting by molecular amplification of highly substoichiometric levels of bacterial lipopolysaccharide.
J. R. Soc. Interface. 2016;123:20160539. doi: 10.1098/rsif.2016.0539
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5046953/

Pretorius E, Page MJ, Hendricks L, Nkosi NB, Benson SR, Kell DB.
Both lipopolysaccharide and lipoteichoic acids potently induce anomalous fibrin amyloid formation: assessment with novel Amytracker™ stains.
J R Soc Interface. 2018 Feb;15(139):20170941. doi: 10.1098/rsif.2017.0941.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5832738/

De Villiers S., Bester J., Kell D.B., Pretorius E.
Erythrocyte health and the possible role of amyloidogenic blood clotting in the evolving haemodynamics of female migraine-with-aura pathophysiology: Results from a pilot study.
Front. Neurol. 2019;10:1262. doi: 10.3389/fneur.2019.01262.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6887655/

[783] Pretorius E, Bester J, Page MJ, Kell DB.
The Potential of LPS-Binding Protein to Reverse Amyloid Formation in Plasma Fibrin of Individuals With Alzheimer-Type Dementia.
Front Aging Neurosci. 2018 Aug 22;10:257. doi: 10.3389/fnagi.2018.00257.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6113936/

[784] Du Plooy J.N., Bester J., Pretorius E.
Eryptosis in Haemochromatosis: Implications for rheology.
Clin. Hemorheol. microcirc. 2018;69:457-469. doi: 10.3233/CH-170325.
https://pubmed.ncbi.nlm.nih.gov/29710680/

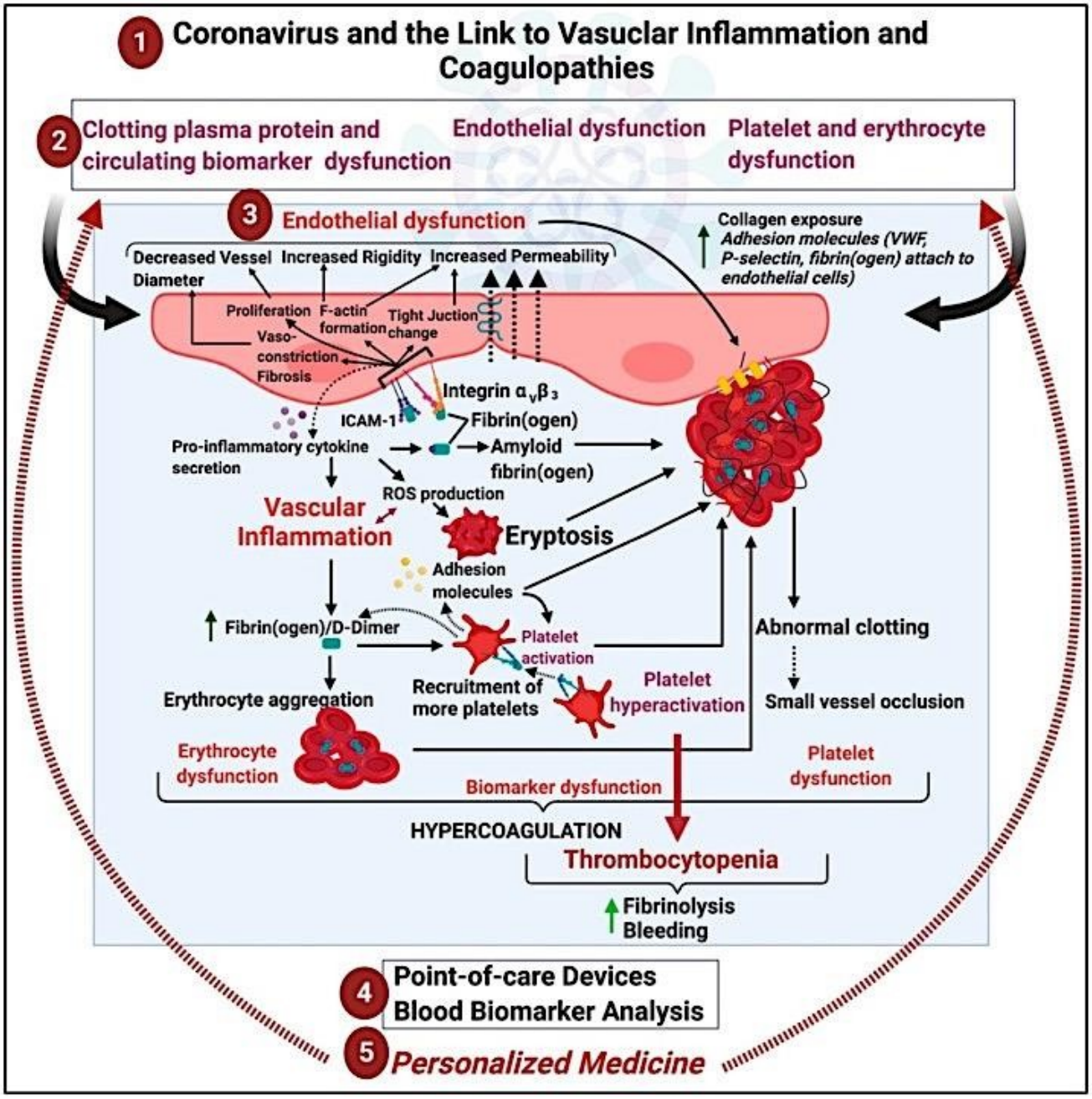
Lipinski B, Pretorius E.
Novel pathway of iron-induced blood coagulation: implications for diabetes mellitus and its complications.
Pol Arch Med Wewn. 2012;122(3):115-22.
http://pamw.pl/en/node/1201/pdf

Olumuyiwa-Akeredolu OO, Page MJ, Soma P, Pretorius E.
Platelets: emerging facilitators of cellular crosstalk in rheumatoid arthritis.
Nat Rev Rheumatol. 2019 Apr;15(4):237-248. doi: 10.1038/s41584-019-0187-9.
https://pubmed.ncbi.nlm.nih.gov/30824879/

Van Rooy M.J., Duim W., Ehlers R., Buys A.V., Pretorius E.
Platelet hyperactivity and fibrin clot structure in transient ischemic attack individuals in the presence of metabolic syndrome: A microscopy and thromboelastography study.
Cardiovasc. Diabetol. 2015;14:86. doi: 10.1186/s12933-015-0249-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4542104/

[785] Pretorius E.
The adaptability of red blood cells. Cardiovasc.
Diabetol. 2013;12:63. doi: 10.1186/1475-2840-12-63
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3637111/

Lipinski B., Pretorius E., Oberholzer H.M., van der Spuy W.J.
Interaction of fibrin with red blood cells: The role of iron.
Ultrastruct. Pathol. 2012;36:79-84. doi: 10.3109/01913123.2011.627491.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7403995/

(1) The vascular implications of coronavirus acute respiratory syndrome 2 (COVID-19) (2) may result in protein coagulation and dysfunction of circulating, endothelial, erythrocyte, and platelet biomarkers. (3) We examine the various biochemical processes associated with vascular dysfunction, focusing on fibrin (ogen), D-dimer, P-selectin, and von Willebrand factor. (4) We conclude by reviewing point-of-care devices and methodologies in the treatment of COVID-19 and suggest that each patient should be treated using a (5) personalized medicine approach.

Together with platelet pathology and the presence of circulating microclots, endothelial damage may be the key factor in the persistent symptoms of long-COVID/PASC[786] .

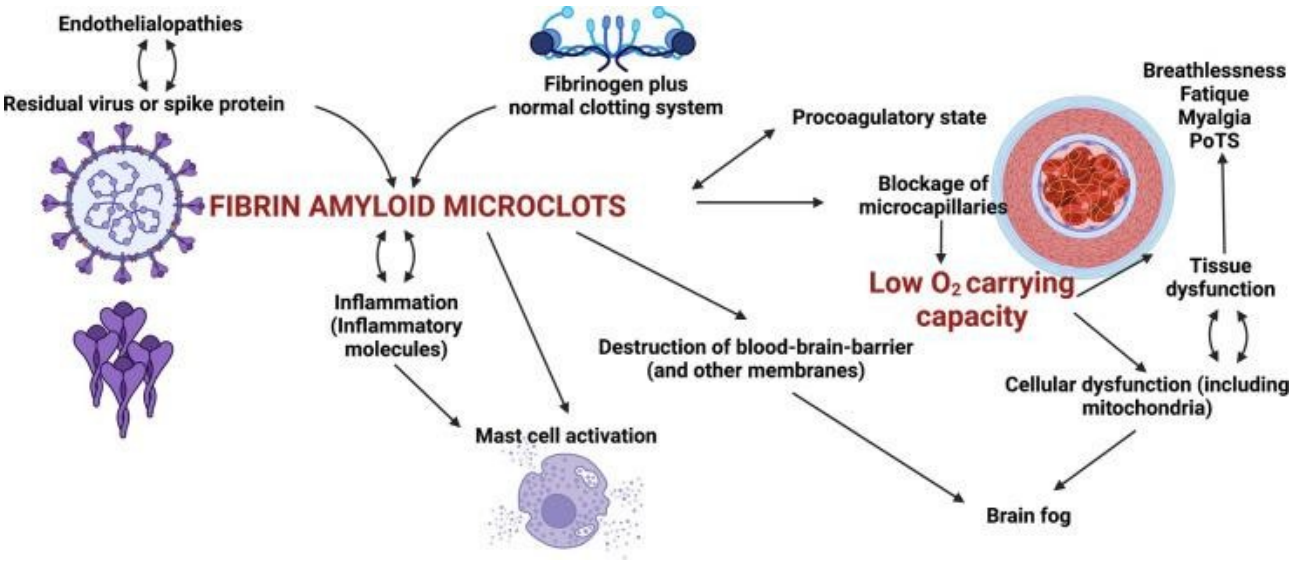
https://pubmed.ncbi.nlm.nih.gov/22471429/

[786] Pretorius E, Vlok M, Venter C, Bezuidenhout JA, Laubscher GJ, Steenkamp J, Kell DB.
Persistent clotting protein pathology in Long COVID/Post-Acute Sequelae of COVID-19 (PASC) is accompanied by increased levels of antiplasmin.
Cardiovasc Diabetol. 2021 Aug 23;20(1):172. doi: 10.1186/s12933-021-01359-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8381139/.

Pretorius E, Venter C, Laubscher GJ, Kotze MJ, Oladejo SO, Watson LR, Rajaratnam K, Watson BW, Kell DB.

In particular, the ability of these amyloid fibrin microclots (fibrinaloids) to block capillaries, to restrict the passage of red blood cells and consequently the exchange of O_2, may actually underlie most of these symptoms.
Consistent with this, in a preliminary report[787] , Pretorius et al demonstrated that adequate and carefully monitored "triple" anticoagulation therapy* leading to microclot removal removes persistent symptoms.

** Tested anticoagulation therapy: one month of dual antiplatelet therapy (DAPT) (Clopidogrel 75 mg/Aspirin 75 mg) once daily and a direct oral anticoagulant (DOAC) (Apixiban) 5 mg twice daily. A proton pump inhibitor (PPI) pantoprazole 40 mg/day was also prescribed for gastric protection. This regimen should be followed only under strict and qualified medical guidance to obviate any hazards, particularly bleeding losses, and of the therapy as a whole.*



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8883497/

Prion diseases and SARS-Cov-2 vaccine spike

What has been reported in the literature regarding the mechanism of induction of prion damage by the natural spike of SARS-Cov-2 can also be demonstrated for its vaccines[788] .

Prevalence of symptoms, comorbidities, fibrin amyloid microclots and platelet pathology in individuals with Long COVID/Post-Acute Sequelae of COVID-19 (PASC).
Cardiovasc Diabetol. 2022 Aug 6;21(1):148. doi: 10.1186/s12933-022-01579-5.
https://cardiab.biomedcentral.com/articles/10.1186/s12933-022-01579-5

[787] Pretorius, et al (2021).
Combined triple treatment of fibrin amyloid microclots and platelet pathology in individuals with Long COVID/ Post-Acute Sequelae of COVID-19 (PASC) can resolve their persistent symptoms.
https://www.researchgate.net/publication/357428572_Combined_triple_treatment_of_fibrin_amyloid_microclots_and_platelet_pathology_in_individuals_with_Long_COVID_Post-Acute_Sequelae_of_COVID-19_PASC_can_resolve_their_persistent_symptoms

[788] Seneff, S., & Nigh, G. (2021).
Worse Than the Disease? Reviewing Some Possible Unintended Consequences of the mRNA Vaccines Against COVID-19.
International Journal of Vaccine Theory, Practice, and Research, 2(1), 38-79. https://doi.org/10.56098/ijvtpr.v2i1.23 (Original work published May 10, 2021)
https://dpbh.nv.gov/uploadedFiles/dpbhnvgov/content/Boards/BOH/Meetings/2021/SENEFF~1.PDF

Actis GC, Ribaldone DG, Pellicano R.
COVID vaccine's hot issues: erratic serious blood clotting, ill-defined prion-like reactogenicity of the spike, unclear roles of other factors.
Minerva Med. 2021 Dec;112(6):695-697. doi: 10.23736/S0026-4806.21.07769-7.
https://www.minervamedica.it/en/journals/minerva-medica/article.php?cod=R10Y2021N06A0695

Classen JB.
COVID-19 Vaccine Associated Parkinson's Disease, A Prion Disease Signal in the UK Yellow Card Adverse Event Database.

It should be kept in mind that the mRNA vaccines, in addition to having the same prion sequences as the original virus, are designed with an altered sequence that replaces two adjacent amino acids in the fusion domain with a proline pair.
This is done intentionally to force the protein to remain in its open state and make it more difficult for it to fuse with the membrane. This can be a dangerous step toward misfolding that potentially leads to prion disease.
A paper published by J. Bart Classen[789] proposed that the spike protein in mRNA vaccines could cause prion-like diseases, in part through its ability to bind to many known prion proteins and induce their misfolding into pathological prions.
As previously discussed, Idrees and Kumar[790] proposed that the S1 component of the spike protein is prone to act as a functional amyloid and form toxic aggregates. These authors pointed out that S1 has the ability "to form amyloid and toxic aggregates that can act as seeds to aggregate many of the misfolded brain proteins and eventually can lead to neurodegeneration."
In support of this hypothesis, $A\beta 1_{-42}$ (the 42-amino acid form of amyloid β in cerebrospinal fluid) was found to bind with high affinity to the S1 and ACE2 subunits, enhancing S1 binding to ACE2, and increasing viral entry and IL-6 production in a mouse model of infection with SARS- CoV-2 pseudovirus.
Data from this surrogate mouse model with intravenous inoculation of $_{A\beta 1-42}$ showed that the clearance of $_{A\beta 1-42}$ in blood was reduced in the presence of the extracellular trimer domain of the protein S. [791]
Given the wide expression of ACE2 in the human brain[792] , a study of particular interest showed that intravenously injected radioiodinated S1 subunit (I-S1) readily crossed the blood-brain barrier by transcytosis in male mice, was absorbed in various regions of the brain, and entered the parenchymal brain space. I-S1 was also absorbed in the lung, spleen, kidney and liver,and intranasally administered I-S1 also entered the brain, although at lower levels than after intravenous administration. [793]

J Med - Clin Res & Rev. 2021; 5(7): 1-6.
https://scivisionpub.com/pdfs/covid19-vaccine-associated-parkinsons-disease-a-prion-disease-signal-in-the-uk-yellow-card-adverse-event-database-1746.pdf

"Disinfection" Notebook 01/2022 with dark-field microscopy analysis of blood from more than a thousand subjects treated with mRNA vaccines. https://www.ndmagazine.it/analisi-soggetti-trattati/

[789] Classen, J. B. (2021).
Review of COVID-19 Vaccines and the Risk of Chronic Adverse Events Including Neurological Degeneration.
Journal of Medical-Clinical Research and Reviews 5(4): 1-7.
https://foundationforhealthresearch.org/review-of-covid-19-vaccines-and-the-risk-of-chronic-adverse-events/.

Classen JB.
COVID-19 RNA Based Vaccines and the Risk of Prion Disease.
Microbiol Infect Dis. 2021; 5(1): 1-3
https://scivisionpub.com/pdfs/covid19-rna-based-vaccines-and-the-risk-of-prion-disease-1503.pdf

[790] Idrees D, Kumar V.
SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration.
Biochem Biophys Res Commun. 2021 May 21;554:94-98. doi: 10.1016/j.bbrc.2021.03.100
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7988450/

[791] Hsu JT, Tien CF, Yu GY, Shen S, Lee YH, Hsu PC, Wang Y, Chao PK, Tsay HJ, Shie FS.
The Effects of Aβ1-42 Binding to the SARS-CoV-2 Spike Protein S1 Subunit and Angiotensin-Converting Enzyme 2.
Int J Mol Sci. 2021 Jul 30;22(15):8226. doi: 10.3390/ijms22158226.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8347908/

[792] Chen R, Wang K, Yu J, Howard D, French L, Chen Z, Wen C, Xu Z.
The Spatial and Cell-Type Distribution of SARS-CoV-2 Receptor ACE2 in the Human and Mouse Brains.
Front Neurol. 2021 Jan 20;11:573095. doi: 10.3389/fneur.2020.573095.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7855591/

[793] Rhea EM, Logsdon AF, Hansen KM, Williams LM, Reed MJ, Baumann KK, Holden SJ, Raber J, Banks WA, Erickson MA.
The S1 protein of SARS-CoV-2 crosses the blood-brain barrier in mice.
Nat Neurosci. 2021 Mar;24(3):368-378. doi: 10.1038/s41593-020-00771-8.

Furthermore, S1 was found to disrupt the integrity of the blood-brain barrier in a 3D microfluidic blood-brain barrier model. [794]
Regarding vaccine safety, low levels of mRNA-LNP platform biodistribution studies have been demonstrated in the brain, indicating that mRNA-LNPs can cross the blood-brain barrier and produce free spikes.[795]

To date, several cases of Creutzfeldt-Jakob Disease (CJD - spongiform encephalopathy also known as mad cow disease) have been documented from the vaccine[796] ; in particular, Prof. Montagnier's research group[797] recently published a preprint with a study of 26 cases of CJD, in which the first symptoms appeared on average 11.38 days after vaccination. Of these 26 cases, 20 died only 4.76 months after the injection. Among them, 8 of them died of sudden death (2.5 months). All this confirms the radically different nature of this new form of CJD, compared with the classic form takes several decades.
The group summarizes the most important findings of the case study in the following points:
"- *First*, we demonstrate the existence of a prion region in all spikes of the original Wuhan strain of SARS-CoV2, all variants, and all vaccines, as they were all constructed from this original Wuhan spike.
- *Second*, we show that this prion region completely disappeared in the last Omicron variant. This can be explained by the phylogenetic tree of SARS-CoV-2 viruses, of which Omicron is the result of one of the very first branches, then would have evolved slowly in South Africa, eventually emerging in November 2021, in a form that became dominant.
-Finally*, and this is the third notable finding*, if the presence of this prion region in all COVID- 19 vaccines constituted "a necessary but not sufficient reason" for the emergence of possible prion disease, we report here official evidence of this new form of CJD immediately after injection."

Another severely disabling neurodegenerative disease reported after vaccination is Parkinson's disease, either new onset or aggravation of previous disease.[798] This disease can be

---

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8793077/

[794] DeOre BJ, Tran KA, Andrews AM, Ramirez SH, Galie PA.
SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation.
J Neuroimmune Pharmacol. 2021 Dec;16(4):722-728. doi: 10.1007/s11481-021-10029-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8536479/

[795] https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf

Palmer, Michael and Sucharit Bhakdi. "The Pfizer mRNA vaccine: pharmacokinetics and toxicity." (2021).
https://doctors4covidethics.org/wp-content/uploads/2021/07/Pfizer-pharmacokinetics-and-toxicity.pdf

[796] Kuvanfık, Anıl & Özcan, Ecenur & Serin, Simay & Sungurtekin, Hulya. (2021).
Creutzfeldt-Jakob Disease After the COVID-19 Vaccination.
Turkish Journal of Intensive Care. 20. 10.4274/tybd.galenos.2021.91885.
https://cms.galenos.com.tr/Uploads/Article_50671/TYBD-0-0.pdf

[797] Jean claude Perez, claire Moret-Chalmin, & RIP Luc Montagnier. (2022).
Towards the emergence of a new form of the neurodegenerative Creutzfeldt-Jakob disease: Twenty six cases of CJD declared a few days after a COVID-19 "vaccine" Jab (Version V4).
Zenodo. https://doi.org/10.5281/zenodo.6641999
https://zenodo.org/record/6641999#.YthiEexBz9E

[798] Erro R, Buonomo AR, Barone P, Pellecchia MT.
Severe Dyskinesia After Administration of SARS-CoV2 mRNA Vaccine in Parkinson's Disease.
Mov Disord. 2021 Oct;36(10):2219. doi: 10.1002/mds.28772.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8441657/

Cosentino C, Torres L, Vélez M, Nuñez Y, Sánchez D, Armas C, Alvarado M.
SARS-CoV-2 Vaccines and Motor Symptoms in Parkinson's Disease.
Mov Disord. 2022 Jan;37(1):233. doi: 10.1002/mds.28851.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8661843/

Salinas MR, Dieppa M.

also manifest after infection with SARS-Cov-2[799] and is associated with Lewy body deposits in the brain, in which the main protein found is α-synuclein. This protein behaves like a prion when it aggregates into toxic soluble oligomers and fibrils under certain conditions.[800]
Research has shown that misfolded α-synuclein can form first in the intestine and then travel from there to the brain along the vagus nerve, probably in the form of exosomes, released from dying cells where the misfolded protein originated[801] .
Cellular conditions that promote misfolding include both an acidic pH and high expression of inflammatory cytokines. It is now known that the vagus nerve is critical for the transmission of misfolded proteins to the brain, because disruption of the vagus nerve protects against Parkinson's disease.
Vagus nerve atrophy in association with Parkinson's disease provides further evidence of vagus nerve involvement in the transport of misfolded α-synuclein oligomers from the gut to the brain.[802] Another pathway is through the olfactory nerve, and loss of sense of smell is an early sign of Parkinson's disease, as well as a common, often temporary, symptom of SARS-CoV-2 infection.

Transient akathisia after the SARS-Cov-2 vaccine.
Clin Park Relat Disord. 2021;4:100098. doi: 10.1016/j.prdoa.2021.100098.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8178529/

[799] Kamel WA, Ismail II, Ibrahim M, Al-Hashel JY.
Unexplained worsening of parkinsonian symptoms in a patient with advanced Parkinson's disease as the sole initial presentation of COVID-19 infection: a case report.
Egypt J Neurol Psychiatr Neurosurg. 2021;57(1):60. doi: 10.1186/s41983-021-00314-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8127432/

Bouali-Benazzouz R, Benazzouz A.
Covid-19 Infection and Parkinsonism: Is There a Link?
Mov Disord. 2021 Aug;36(8):1737-1743. doi: 10.1002/mds.28680.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8242862/

Imbalzano G, Ledda C, Artusi CA, Romagnolo A, Montanaro E, Rizzone MG, Lopiano L, Zibetti M.
SARS-CoV-2 vaccination, Parkinson's disease, and other movement disorders: case series and short literature review.
Neurol Sci. 2022 Jun 6:1-4. doi: 10.1007/s10072-022-06182-w.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9167915/

Gultekin M, Tufekcioglu Z.
COVID-19 infection presented as severe dyskinesia in a patient with Parkinson's disease: a case with daily video recording.
Neurol Sci. 2022 May;43(5):2961-2963. doi: 10.1007/s10072-022-05912-4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9023739/

Jaiswal V, et al
The Influence of Coronavirus Disease-2019 (COVID-19) On Parkinson's Disease: An Updated Systematic Review.
J Prim Care Community Health. 2021 Jan-Dec;12:21501327211039709. doi: 10.1177/21501327211039709.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8377313/

Count C.
Possible Link between SARS-CoV-2 Infection and Parkinson's Disease: The Role of Toll-Like Receptor 4.
Int J Mol Sci. 2021 Jul 1;22(13):7135. doi: 10.3390/ijms22137135.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8269350/

[800] Lema Tomé CM, Tyson T, Rey NL, Grathwohl S, Britschgi M, Brundin P.
Inflammation and α-synuclein's prion-like behavior in Parkinson's disease--is there a link?
Mol Neurobiol. 2013 Apr;47(2):561-74. doi: 10.1007/s12035-012-8267-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3589652/.

[801] Kakarla R, Hur J, Kim YJ, Chwae YJ.
Apoptotic cell-derived exosomes: messages from dying cells.
Exp Mol Med. 2020 Jan;52(1):1-6. doi: 10.1038/s12276-019-0362-8.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7000698/

Steiner JA, Angot E, Brundin P.
A deadly spread: cellular mechanisms of α-synuclein transfer.
Cell Death Differ. 2011 Sep;18(9):1425-33. doi: 10.1038/cdd.2011.53.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3178422/

[802] Walter U, Tsiberidou P, Kersten M, Storch A, Löhle M.
Atrophy of the Vagus Nerve in Parkinson's Disease Revealed by High-Resolution Ultrasonography.

There are many parallels between α-synuclein and the spike protein, which confirms the possibility of prion-like disease after vaccination.
In addition, the cationic lipids in the vaccine create an acidic pH conducive to misfolding and also induce a strong inflammatory response, another predisposing condition.
Germinal centers are structures within the spleen and other secondary lymphoid organs in which follicular dendritic cells present antigens to B cells, which in turn refine their antibody response.
Researchers have shown that mRNA vaccines, in contrast to recombinant protein vaccines, elicit a robust development of neutralizing antibodies in these germinal centers in the spleen.[803] However, this also means that mRNA vaccines induce an ideal situation for prion formation from the spike protein[804] and its transport through exosomes along the vagus nerve to the brain. [805]
Studies have shown that prion transmission from animal to animal first appears in lymphoid tissues, particularly the spleen. Differentiated follicular dendritic cells are central to the process as they accumulate misfolded prion proteins.[806]

Also Classen in his article "*COVID-19 Vaccine Associated Parkinson's Disease, A Prion Disease Signal in the UK Yellow Card Adverse Event* Database"[807] documents, from his review of adverse reactions published in the UK Yellow Card database, a specific signal for an increased risk of Parkinson's disease. Another prion disease is fatal familial insomnia, a rare genetic disorder characterized by the inability to sleep[808] .
It was noted in the analysis of the database data on nervous disorders that there were reports of sleep dysregulation among the vaccine groups. This could be explained by the spike protein that aggregates prion molecules already in cells, as discussed above with Parkinson's disease symptoms.

Front Neurol. 2018 Sep 27;9:805. doi: 10.3389/fneur.2018.00805.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6170613/

[803] Lederer K, et al
SARS-CoV-2 mRNA Vaccines Foster Potent Antigen-Specific Germinal Center Responses Associated with Neutralizing Antibody Generation.
Immunity. 2020 Dec 15;53(6):1281-1295.e5. doi: 10.1016/j.immuni.2020.11.009.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7680029/

[804] Rossato F, Gazzola G, Carlucci V, Bisogno A, Crivellari S, Mozzetta S, Boso F, Cagnin A.
Cerebral amyloid angiopathy - Related inflammation after COVID-19 vaccination: Case or causality?
J Neurol Sci. 2021 Oct;429:119917. doi: 10.1016/j.jns.2021.119917.
Mai Yamakawa, Sharon Lynch, Ryan Townley,
Neuroimmunology Reports, Volume 2, 2022, 100120, ISSN 2667-257X,
https://doi.org/10.1016/j.nerep.2022.100120.
(https://www.sciencedirect.com/science/article/pii/S2667257X22000663)

[805] Liu S, Hossinger A, Göbbels S, Vorberg IM.
Prions on the run: How extracellular vesicles serve as delivery vehicles for self-templating protein aggregates.
Prion. 2017 Mar 4;11(2):98-112. doi: 10.1080/19336896.2017.1306162.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5399892/

[806] Al-Dybiat I, Moudjou M, Martin D, Reine F, Herzog L, Truchet S, Berthon P, Laude H, Rezaei H, Andréoletti O, Béringue V, Sibille P.
Prion strain-dependent tropism is maintained between spleen and granuloma and relies on lymphofollicular structures.
Sci Rep. 2019 Oct 10;9(1):14656. doi: 10.1038/s41598-019-51084-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6787085/

[807] Classen JB.
COVID-19 Vaccine Associated Parkinson's Disease, A Prion Disease Signal in the UK Yellow Card Adverse Event Database.
J Med - Clin Res & Rev. 2021; 5(7): 1-6.
https://scivisionpub.com/pdfs/covid19-vaccine-associated-parkinsons-disease-a-prion-disease-signal-in-the-uk-yellow-card-adverse-event-database-1746.pdf

[808] He R, Hu Y, Yao L, Tian Y, Zhou Y, Yi F, Zhou L, Xu H, Sun Q.
Clinical features and genetic characteristics of two Chinese pedigrees with fatal family insomnia.
Prion. 2019 Jan;13(1):116-123. doi: 10.1080/19336896.2019.1617027.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6629183/

Further confirmation of amyloid formation after vaccination comes from recent research by Drs. Benzi Cipelli, F. Giovannini and GP. Pisano disclosed in Issue 01/2022 of "Disinfection" with *dark field microscopy analysis of the blood of more than a thousand subjects treated with mRNA vaccines*.[809]
Specifically, the authors report that of the 1006 subjects tested, 48 or 94 percent of the total showed, after inoculation of mRNA vaccines [Pfizer or Moderna] one month later, various alterations in the aggregation state of erythrocytes and the presence in the peripheral blood of particles of various shapes and sizes of dubious nature that, however, have the characteristics of amyloid fibrils.

Glycosylation of the SARS-Cov-2 spike

Envelope viruses have evolved with envelope proteins that have several glycosylation sites linked to the N-X-S/T amino acid sequon, i.e., asparagine-any amino acid (except proline)-serine or threonine and in rare cases to asparagine in the asparagine-any amino acid-cysteine sequon.
Asparagine (N) in these sequences acts as an amino acid to which are linked chains of high mannose carbohydrates, or complex-type carbohydrate chains (glycans) synthesized by the host cell's glycosylation machinery.[810]
The presence of multiple glycans on viral envelope glycoproteins suggests that they may contribute to virus survival in hosts. The roles of these glycans may vary and include:

1. Formation of a protective hydration layer around the virus due to the hydrophilic nature of glycans.

2. In some viruses, sialic acid on these glycoproteins confers a negative electrostatic charge that surrounds the virus and prevents nonspecific adhesion to cell membranes because of electrostatic repulsion by the negative charges of sialic acid on cell membrane glycans.

3. Some glycans bind to a variety of cell surface receptors.

4. Glycans act as a shield (called a "glycan shield") that masks various peptide antigens and prevents the binding of neutralizing antibodies, thereby decreasing the effectiveness of the protective immune response mounted by the host. [811]

---

[809] "Disinfection" Notebook 01/2022 with dark-field microscopy analysis of blood from over a thousand subjects treated with mRNA vaccines.
https://www.ndmagazine.it/analisi-soggetti-trattati/

[810] Vigerust DJ, Shepherd VL.
Virus glycosylation: role in virulence and immune interactions.
Trends Microbiol. 2007 May;15(5):211-8. doi: 10.1016/j.tim.2007.03.003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7127133/

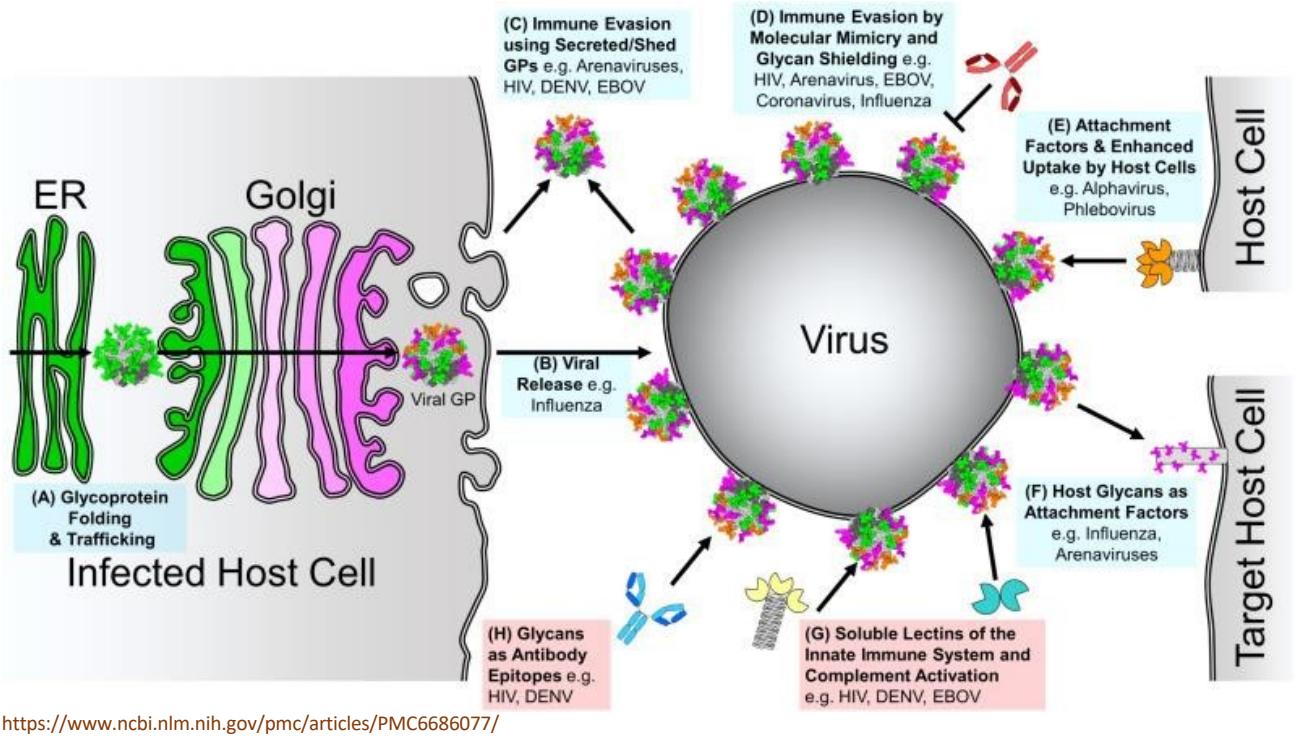
[811] Walls AC, Tortorici MA, Frenz B, Snijder J, Li W, Rey FA, DiMaio F, Bosch BJ, Veesler D.
Glycan shield and epitope masking of a coronavirus spike protein observed by cryo-electron microscopy.
Nat Struct Mol Biol. 2016 Oct;23(10):899-905. doi: 10.1038/nsmb.3293.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5515730/

Xiong X, Tortorici MA, Snijder J, Yoshioka C, Walls AC, Li W, McGuire AT, Rey FA, Bosch BJ, Veesler D.
Glycan Shield and Fusion Activation of a Deltacoronavirus Spike Glycoprotein Fine-Tuned for Enteric Infections.
J Virol. 2018 Jan 30;92(4):e01628-17. doi: 10.1128/JVI.01628-17.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5790929/

Wei X, et al
Antibody neutralization and escape by HIV-1.
Nature. 2003 Mar 20;422(6929):307-12. doi: 10.1038/nature01470.
https://pubmed.ncbi.nlm.nih.gov/12646921/

Crooks ET, et al
Vaccine-Elicited Tier 2 HIV-1 Neutralizing Antibodies Bind to Quaternary Epitopes Involving Glycan-Deficient Patches Proximal to the CD4 Binding Site.
PLoS Pathog. 2015 May 29;11(5):e1004932. doi: 10.1371/journal.ppat.1004932.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4449185/

Lavie M, Hanoulle X, Dubuisson J.
Glycan Shielding and Modulation of Hepatitis C Virus Neutralizing Antibodies.
Front Immunol. 2018 Apr 27;9:910. doi: 10.3389/fimmu.2018.00910.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5934428/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6686077/
Roles of glycosylation in viral pathogenesis. Roles contributing to viral pathogenesis and host cell strategies used to respond to viral infection are blue and red, respectively. Green and pink indicate the processing states of complex-type N-linked oligomannoses and glycans, respectively. (A) Folding and trafficking of glycoproteins. As with all glycoproteins, glycans on viral glycoproteins aid in folding and trafficking through the host secretory pathway. (B) Glycosylation in viral spreading. Glycosylation of infected host cell proteins can influence viral spread. (C) Immune evasion by secreted/spilled glycoproteins. Viruses can release or secrete glycoproteins that act as immune boosters. (D) Immune evasion by molecular mimicry and glycan shielding. Extensively glycosylated viral proteins protect themselves from the host immune response by occluding the immunogenic protein surface with a dense layer of host-derived glycans. (E) Glycans as attachment factors and increased uptake by immune cells. Some glycoproteins displayed by the virus envelope contain subprocessed oligomannose-type glycans that function as host cell attachment factors to increase or facilitate immune cell infection. (F) Host glycans as attachment factors. Viruses can recognize glycans presented on host cell surface proteins t o facilitate host cell attachment. (G) Soluble lectins of the innate immune system and complement activation. Because subprocessed glycans are rarely presented on the glycoproteins of mature host cells [67,68], the innate immune system is able to recognize these glycans as pathogen-associated molecular patterns (PAMPs) using soluble lectins. (H) Glycans as epitopes of antibodies. Where the shielding of glycans is conserved on viral glycoproteins, it is possible for the humoral immune response, in rare cases, to elicit neutralizing antibodies that target sugars as part of their epitopes.

However, since the glycans of enveloped viruses are synthesized by the host cellular apparatus, the glycans themselves can also be considered the "Achilles' heel" of the virus, as they can cause its neutralization and destruction by natural antibodies that bind to carbohydrate antigens on the virus' glycan shield.
Production of these natural antibodies in humans (and other mammals) occurs throughout life without active immunization, as it is the result of the continuous immune response against a wide range of environmental glycans, most of which occur on the wall of bacteria that colonize the gastrointestinal tract.[812]

Ward AB, Wilson IA.
The HIV-1 envelope glycoprotein structure: nailing down a moving target.
Immunol Rev. 2017 Jan;275(1):21-32. doi: 10.1111/imr.12507.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5300090/

York IA, Stevens J, Alymova IV.
Influenza virus N-linked glycosylation and innate immunity.
Biosci Rep. 2019 Jan 8;39(1):BSR20171505. doi: 10.1042/BSR20171505.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6328934/

[812] Wiener AS.
Origin of naturally occurring hemagglutinins and hemolysins; a review.

The variety and quantity of bacterial carbohydrate antigens that consistently stimulate the human immune system is enormous, as there are about 400 strains of bacteria that make up more than 25% of fecal material.[813] Fortunately, some of the immunogenic bacterial carbohydrate antigens have similar structures to the major carbohydrate antigens on other mammals (e.g., the α-gal epitope and the N-glycolyl-neuraminic acid (Neu5Gc) antigens in nonhuman primates), resulting in the production of natural anti-carbohydrate antibodies that cross-react with these carbohydrate antigens.

Therefore, a zoonotic virus that replicates in any of these nonhuman hosts has carbohydrate antigens that are synthesized by the host's glycosylation machinery and that bind human natural anti-carbohydrate antibodies that can neutralize and destroy the virus when it passes from the animal host to humans (species hopping).

In some cases, protection by these antibodies may result in complete destruction of the virus, and the infected individual may be asymptomatic.

In other cases, protection may be partial; if the neutralization and destruction of the invading virus by natural anti-carbohydrate antibodies is not complete, there is a "race" within the infected individual between the replicating virus damaging various tissues and the specific anti-virus immune response, which attempts to prevent the viruses from reaching a lethal mass.

In some infected individuals, even partial protection by natural anti-carbohydrate antibodies may allow the immune system to "catch up" with the expanding viral population and prevent progression to a lethal stage.

However, in outbreaks of viruses with very high virulence, penetration of even a few virions into human cells may be sufficient to cause the virus to replicate with a human glycan shield, which does not bind any natural human anti-carbohydrate antibody.

In this scenario, the subsequent rapid replication of the virus causes most of the infected hosts to die before a protective immune response can be elicited.[814]

J Immunol. 1951 Feb;66(2):287-95.
https://pubmed.ncbi.nlm.nih.gov/14814309/

Springer GF.
Blood-group and Forssman antigenic determinants shared between microbes and mammalian cells.
Prog Allergy. 1971;15:9-77.
https://pubmed.ncbi.nlm.nih.gov/4133332/

Stowell SR, et al
Microbial glycan microarrays define key features of host-microbial interactions.
Nat Chem Biol. 2014 Jun;10(6):470-6. doi: 10.1038/nchembio.1525.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4158828/

Bovin NV.
Natural antibodies to glycans.
Biochemistry (Mosc). 2013 Jul;78(7):786-97. doi: 10.1134/S0006297913070109.
http://protein.bio.msu.ru/biokhimiya/contents/v78/full/78071008.html

Blixt O, et al
Printed covalent glycan array for ligand profiling of diverse glycan binding proteins.
Proc Natl Acad Sci U S A. 2004 Dec 7;101(49):17033-8. doi: 10.1073/pnas.0407902101.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC534418/

[813] Stephen AM, Cummings JH.
The microbial contribution to human faecal mass.
J Med Microbiol. 1980 Feb;13(1):45-56. doi: 10.1099/00222615-13-1-45.
https://pubmed.ncbi.nlm.nih.gov/7359576/

Gerritsen J, Smidt H, Rijkers GT, de Vos WM.
Intestinal microbiota in human health and disease: the impact of probiotics.
Genes Nutr. 2011 Aug;6(3):209-40. doi: 10.1007/s12263-011-0229-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3145058/

[814] Galili U.
Host Synthesized Carbohydrate Antigens on Viral Glycoproteins as "Achilles' Heel" of Viruses Contributing to Anti-Viral Immune Protection.
Int J Mol Sci. 2020 Sep 13;21(18):6702. doi: 10.3390/ijms21186702.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7555091/

Like most viral glycoproteins that are incorporated into the bilayer of the envelope membrane, the SARS-CoV-2 S protein is extensively decorated with N-linked glycans in both S1 and S2 subunits, with a total of 22 N-linked glycan sites (compared with 23 on SARS)[815] , to which are added O-glycosylation sites.
These glycans confer two benefits to the virus. First, the mannose residues within these glycans are important fractions for interacting with cell surface attachment factors, such as glycosaminoglycans (GAGs) and sialic acid-containing oligosaccharides[816] , before binding to the high-affinity receptor, which in the case of SARS-CoV-2, is angiotensin-converting enzyme 2 (ACE2)[817] . In the spike-ACE2 complex, extensive glycosylation has been reported at the interface of the complex[818] , highlighting the roles of glycans in modulating spike-ACE2 interactions.
Second, as already discussed, glycans sterically mask the underlying polypeptide epitopes from recognition by potentially neutralizing antibodies, and thus are sometimes referred to as a "glycan shield."[819] .

---

[815] Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh RM Jr, Rawson S, Rits-Volloch S, Chen B.
Distinct conformational states of SARS-CoV-2 spike protein.
Science. 2020 Sep 25;369(6511):1586-1592. doi: 10.1126/science.abd4251.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464562/

Watanabe Y, Allen JD, Wrapp D, McLellan JS, Crispin M.
Site-specific glycan analysis of the SARS-CoV-2 spike.
Science. 2020 Jul 17;369(6501):330-333. doi: 10.1126/science.abb9983.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7199903/

Watanabe Y, Berndsen ZT, Raghwani J, Seabright GE, Allen JD, Pybus OG, McLellan JS, Wilson IA, Bowden TA, Ward AB, Crispin M.
Vulnerabilities in coronavirus glycan shields despite extensive glycosylation.
Nat Commun. 2020 May 27;11(1):2688. doi: 10.1038/s41467-020-16567-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7253482/

[816]Li W, et al
Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein.
Proc Natl Acad Sci U S A. 2017 Oct 3;114(40):E8508-E8517. doi: 10.1073/pnas.1712592114.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5635925/

Tortorici MA, Walls AC, Lang Y, Wang C, Li Z, Koerhuis D, Boons GJ, Bosch BJ, Rey FA, de Groot RJ, Veesler D.
Structural basis for human coronavirus attachment to sialic acid receptors.
Nat Struct Mol Biol. 2019 Jun;26(6):481-489. doi: 10.1038/s41594-019-0233-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6554059/

Robson B.
Bioinformatics studies on a function of the SARS-CoV-2 spike glycoprotein as the binding of host sialic acid glycans.
Comput Biol Med. 2020 Jul;122:103849. doi: 10.1016/j.compbiomed.2020.103849.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7278709/

[817] Hoffmann M, et al
SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.
Cell. 2020 Apr 16;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102627/

Zhou P, et al
A pneumonia outbreak associated with a new coronavirus of probable bat origin.
Nature. 2020 Mar;579(7798):270-273. doi: 10.1038/s41586-020-2012-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7095418/

[818] Zhao P, et al
Virus-Receptor Interactions of Glycosylated SARS-CoV-2 Spike and Human ACE2 Receptor.
Cell Host Microbe. 2020 Oct 7;28(4):586-601.e6. doi: 10.1016/j.chom.2020.08.004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7443692/

[819] Doores KJ.
The HIV glycan shield as a target for broadly neutralizing antibodies.
FEBS J. 2015 Dec;282(24):4679-91. doi: 10.1111/febs.13530.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4950053/

Viral glycoproteins are the main targets of host antibodies, as these molecules are prominently present on the surface of the virion[820] . Unlike bacteria, in which glycans are encoded by the bacterial genome and are treated as "non-self" epitopes by the corresponding hosts, viruses take advantage of the host cell machinery for glycosylation and are generally decorated with "self" glycans, generally considered a strategy to escape the host immune response. [821]

To enable their biosynthesis within the host cell, viruses hijack the host glycosylation machinery, but differences from typical host glycosylation patterns of glycan density and composition are known to occur.

It follows that a certain level of glycan-based immunogenicity can be achieved when glycan types and glycosylation patterns are significantly different from those encountered in host proteins, and thus become immunological targets.

Masking of glycosylation can be rationalized from a chemical point of view: the binding of an antibody to a glycosylated antigen is unlikely to have the high affinities expected from antibody recognition of protein loci because of the significant loss of conformational entropy ($-\Delta S \gg R$, R = gas constant) associated with the constraint of glycans involved in the recognition event.

Thus, in addition to protecting the underlying protein from antibody recognition, glycans attenuate the ability of the host immune system to recruit antibodies against glycosylated epitopes.

Glycosylation also acts as a masking against the adaptive immune response mediated by T lymphocytes. Pathogen peptides are carried on antigen-presenting cells by the major histocompatibility complex II (MHC II). This complex has preferred peptide motifs, and thus it is possible to predict which antigenic regions of a pathogen protein may elicit an adaptive immune response. However, when the putative antigenic peptide is glycosylated, its incorporation within the MHC may be nullified due to steric hindrance brought about by glycan dynamics.[822]

Bagdonaite I, Wandall HH.
Global aspects of viral glycosylation.
Glycobiology. 2018 Jul 1;28(7):443-467. doi: 10.1093/glycob/cwy021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108637/

[820] Murin CD, Wilson IA, Ward AB.
Antibody responses to viral infections: a structural perspective across three different enveloped viruses.
Nat Microbiol. 2019 May;4(5):734-747. doi: 10.1038/s41564-019-0392-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6818971/

[821] Alexander S, Elder JH.
Carbohydrate dramatically influences immune reactivity of antisera to viral glycoprotein antigens.
Science. 1984 Dec 14;226(4680):1328-30. doi: 10.1126/science.6505693.
https://pubmed.ncbi.nlm.nih.gov/6505693/

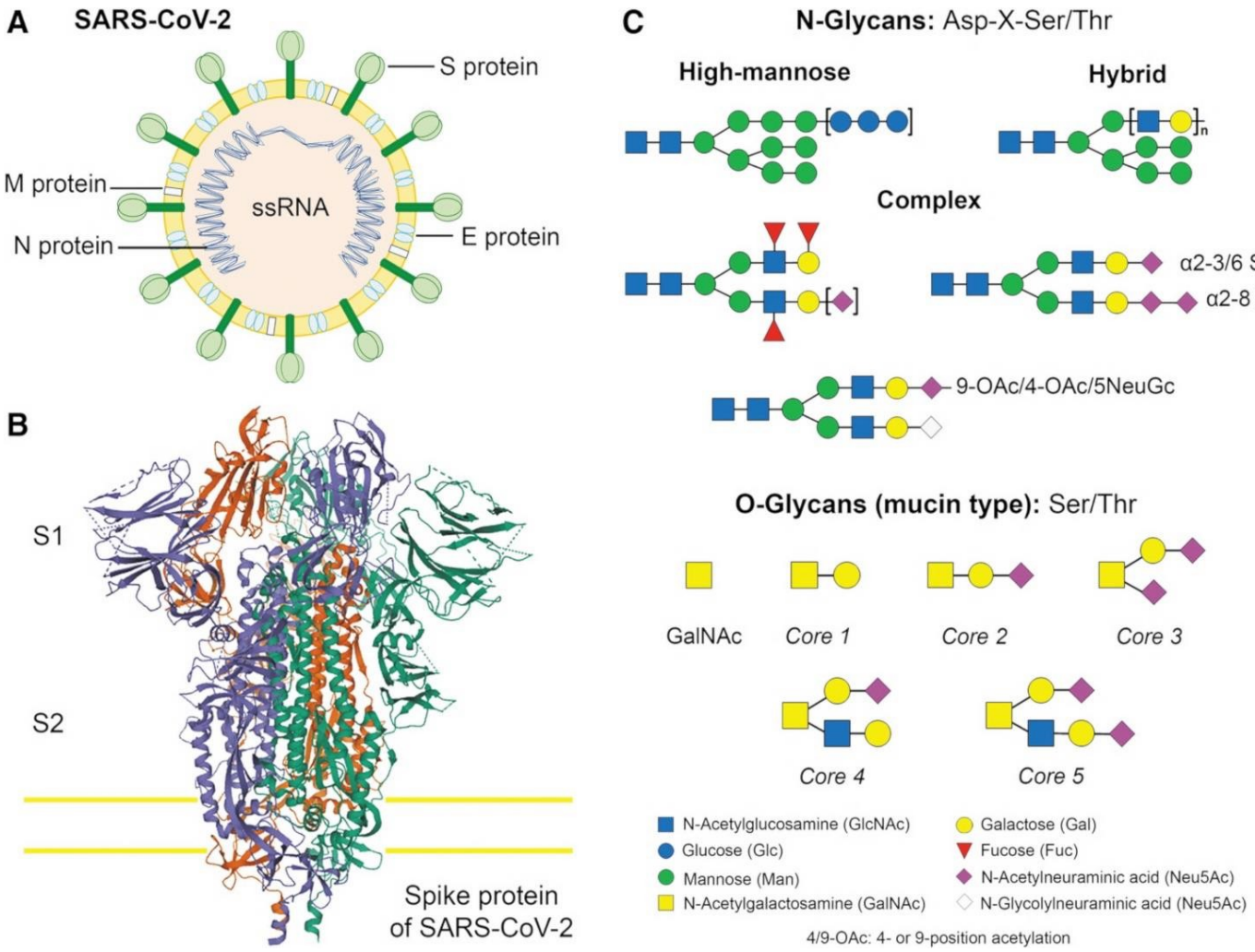
Zheng L, Ma Y, Chen M, Wu G, Yan C, Zhang XE.
SARS-CoV-2 spike protein receptor-binding domain N-glycans facilitate viral internalization in respiratory epithelial cells.
Biochem Biophys Res Commun. 2021 Nov 19;579:69-75. doi: 10.1016/j.bbrc.2021.09.053.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8459579/

Schulze IT.
Effects of glycosylation on the properties and functions of influenza virus hemagglutinin.
J Infect Dis. 1997 Aug;176 Suppl 1:S24-8. doi: 10.1086/514170.
https://pubmed.ncbi.nlm.nih.gov/9240690/

[822] Fernández A.
Glycosylation of SARS-CoV-2 Steers Evolutionary Outcomes in the Postvaccination Phase.
ACS Pharmacol Transl Sci. 2021 Jan 21;4(1):410-412. doi: 10.1021/acsptsci.1c00015.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7887837/

Harbison AM, Fogarty CA, Phung TK, Satheesan A, Schulz BL, Fadda E.
Fine-tuning the spike: role of the nature and topology of the glycan shield in the structure and dynamics of the SARS-CoV-2 S.
Chem Sci. 2021 Nov 25;13(2):386-395. doi: 10.1039/d1sc04832e.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8729800/

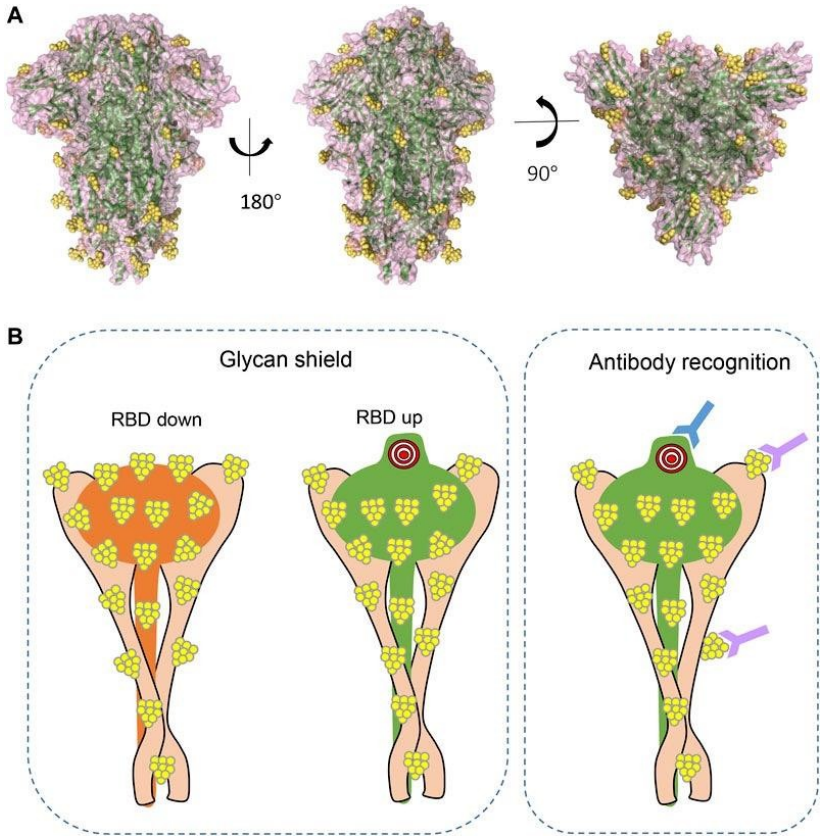
Zhao X, Chen H, Wang H.
Glycans of SARS-CoV-2 Spike Protein in Virus Infection and Antibody Production.



https://www.liebertpub.com/doi/10.1089/vim.2020.0174
Introduction to coronaviruses and S glycoprotein. (A) Diagram of a coronavirus virion with major structural proteins. (B) 3D structure of SARS-CoV-2 S glycoprotein showing the consensus N- and O-glycosylation site (PDB:6X6P). (C) Glycosylation profile of SARS- CoV-2 coronavirus, where N-glycosylation and O-glycosylation sites with core-1 type O-glycans were found. Representation of monosaccharide symbols according to the SNFG system. SNFG, symbol nomenclature for glycans; SARS-CoV, severe acute respiratory syndrome coronavirus

https://www.frontiersin.org/articles/10.3389/fmolb.2021.629873/full
The shielding effect of glycosylation on the S (A) protein trimer. Structural representations from different views demonstrating epitopes of glycans. The yellow balls represent glycans, and the S backbone was generated based on PDB ID: 6VXX. (B). Diagram of glycans in the molecular recognition screen and their potential role in antibody elicitation (left panel: compared with RBD in the "up" position, RBD in the "down" position has more glycan coverage; right panel: in addition to peptide epitopes, some glycans can also be recognized by the antibody and are thus important epitopes in antibody production).

Front Mol Biosci. 2021 Apr 13;8:629873. doi: 10.3389/fmolb.2021.629873.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8076860/



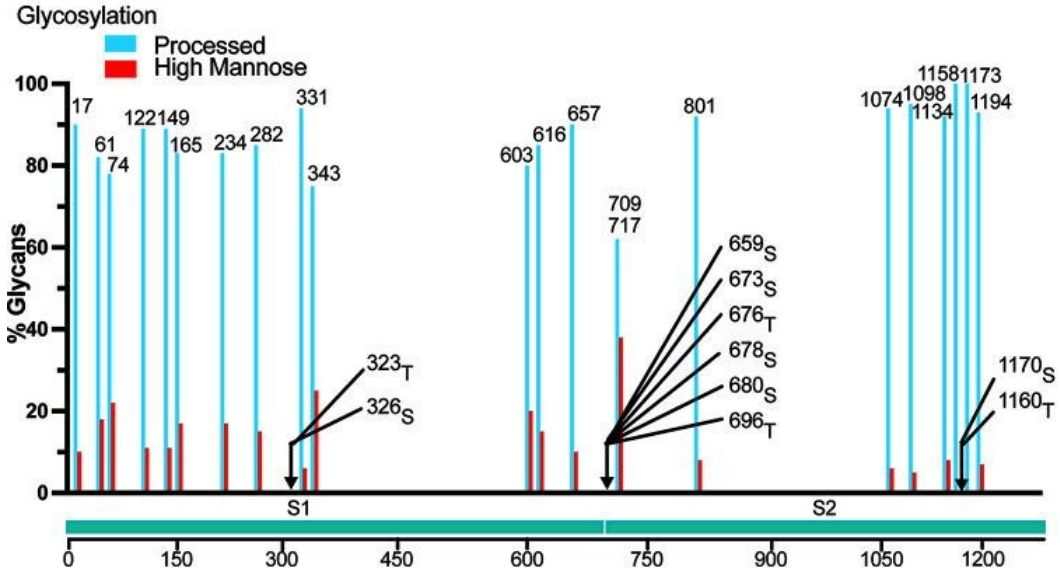
The mapping of spike glycosylation is shown below.[823]

Glycosylation Analysis of the SARS-CoV-2 S Glycoproteins

Summary:

Glycosylation	Number	Detected	No. of Glycans
N-linked	22	22	826*
O-linked	3 previously reported (T323,S325,T678)	10 detected(T323,S325,S659,S673,T676, T678,S680,T696,T1160,S1170)	17

* Total N-linked glycans used in the bar graph



[823] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8827021/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8827021/
Glycosylation profile of purified S-glycoproteins. MS analysis of the purified S glycoproteins identified 22 N-linked glycosylation sites and O-linked glycosylation sites, summarized in the upper panel. The composition of glycan in each N-linked glycosylation site is shown in the lower panel. Serine and threonine residues contained in glycopeptides with O-linked carbohydrates are indicated by arrows.

Glycans in the glycoprotein could therefore stimulate the production of carbohydrate-specific antibodies, which play an important role in protecting the host from infectious diseases.[824]
On the other hand, they could also cross-react with host antigens, leading to autoimmune diseases[825] .
Previous studies have shown that autoimmune diseases, such as Guillain-Barre syndrome, multiple sclerosis, and inflammatory bowel disease, are caused by carbohydrate-specific antibodies related to bacterial infections or gut microbes.[826]
A recent SARS-CoV-2 study showed that COVID-19 patients have unusually high IgM and IgG antibodies against self-carbohydrates, including gangliosides, N-linked glycans, LacNAc derivatives, blood group H1, and sialyl Lewis X[827] , indicating the possibility of autoimmune diseases caused by these antibodies.

The SARS-CoV-2 S glycoproteins produced for use as vaccine immunogens were designed to allow secretion of soluble trimers, to inhibit furin cleavage, and to stabilize prefusion conformations[828] .

---

[824] Casalino L, Gaieb Z, Goldsmith JA, Hjorth CK, Dommer AC, Harbison AM, Fogarty CA, Barros EP, Taylor BC, McLellan JS, Fadda E, Amaro RE.
Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein.
ACS Cent Sci. 2020 Oct 28;6(10):1722-1734. doi: 10.1021/acscentsci.0c01056.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7523240/

Zhao X, Chen H, Wang H.
Glycans of SARS-CoV-2 Spike Protein in Virus Infection and Antibody Production.
Front Mol Biosci. 2021 Apr 13;8:629873. doi: 10.3389/fmolb.2021.629873.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8076860/

[825] Patel P, Kearney JF.
Immunological Outcomes of Antibody Binding to Glycans Shared between Microorganisms and Mammals.
J Immunol. 2016 Dec 1;197(11):4201-4209. doi: 10.4049/jimmunol.1600872.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5119654/

[826] Kappler K, Hennet T.
Emergence and significance of carbohydrate-specific antibodies.
Genes Immun. 2020 Aug;21(4):224-239. doi: 10.1038/s41435-020-0105-9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7449879/

[827] Butler DL, Imberti L, Quaresima V, Fiorini C; NIAID COVID-19 Consortium, Gildersleeve JC.
Abnormal antibodies to self-carbohydrates in SARS-CoV-2-infected patients.
PNAS Nexus. 2022 Jun 17;1(3):pgac062. doi: 10.1093/pnasnexus/pgac062.
https://pubmed.ncbi.nlm.nih.gov/35865361/

[828] Corbett KS, et al
SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness.
Nature. 2020 Oct;586(7830):567-571. doi: 10.1038/s41586-020-2622-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7581537/

Yu J, et al
DNA vaccine protection against SARS-CoV-2 in rhesus macaques.
Science. 2020 Aug 14;369(6505):806-811. doi: 10.1126/science.abc6284.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7243363/

Corbett KS, et al
Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates.
N Engl J Med. 2020 Oct 15;383(16):1544-1555. doi: 10.1056/NEJMoa2024671.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7449230/

Mercado NB, et al
Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques.
Nature. 2020 Oct;586(7830):583-588. doi: 10.1038/s41586-020-2607-z. Epub 2020 Jul 30. Erratum in: Nature. 2021 Feb;590(7844):E25.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7581548/

Bos R, et al

The glycosylation profiles of virion S-glycoproteins and several of these modified S-glycoproteins were characterized.[829]
Purified trimers preferentially bind MAb that recognize a closed prefusogenous conformation with all three RBDs in the down position. However, it is to be expected that the S-type glycoprotein trimer of the

---

Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses.
NPJ Vaccines. 2020 Sep 28;5:91. doi: 10.1038/s41541-020-00243-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7522255/

Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D.
Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein.
Cell. 2020 Apr 16;181(2):281-292.e6. doi: 10.1016/j.cell.2020.02.058. Epub 2020 Mar 9. Erratum in: Cell. 2020 Dec 10;183(6):1735.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102599/

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS.
Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.
Science. 2020 Mar 13;367(6483):1260-1263. doi: 10.1126/science.abb2507.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/

Bangaru S, et al
Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate.
Science. 2020 Nov 27;370(6520):1089-1094. doi: 10.1126/science.abe1502.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857404/

[829] Yao H, Song Y, Chen Y, Wu N, Xu J, Sun C, Zhang J, Weng T, Zhang Z, Wu Z, Cheng L, Shi D, Lu X, Lei J, Crispin M, Shi Y, Li L, Li S.
Molecular Architecture of the SARS-CoV-2 Virus.
Cell. 2020 Oct 29;183(3):730-738.e13. doi: 10.1016/j.cell.2020.09.018.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7474903/

Zhang Y, Zhao W, Mao Y, Chen Y, Wang S, Zhong Y, Su T, Gong M, Du D, Lu X, Cheng J, Yang H.
Site-specific N-glycosylation Characterization of Recombinant SARS-CoV-2 Spike Proteins.
Mol Cell Proteomics. 2021;20:100058. doi: 10.1074/mcp.RA120.002295.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7876485/

Allen JD, et al
Site-Specific Steric Control of SARS-CoV-2 Spike Glycosylation.
Biochemistry. 2021 Jul 13;60(27):2153-2169. doi: 10.1021/acs.biochem.1c00279.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262170/

Brun J, Vasiljevic S, Gangadharan B, Hensen M, V Chandran A, Hill ML, Kiappes JL, Dwek RA, Alonzi DS, Struwe WB, Zitzmann N.
Assessing Antigen Structural Integrity through Glycosylation Analysis of the SARS-CoV-2 Viral Spike.
ACS Cent Sci. 2021 Apr 28;7(4):586-593. doi: 10.1021/acscentsci.1c00058.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8029450/

Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh RM Jr, Rawson S, Rits-Volloch S, Chen B.
Distinct conformational states of SARS-CoV-2 spike protein.
Science. 2020 Sep 25;369(6511):1586-1592. doi: 10.1126/science.abd4251.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7464562/

Watanabe Y, Allen JD, Wrapp D, McLellan JS, Crispin M.
Site-specific glycan analysis of the SARS-CoV-2 spike.
Science. 2020 Jul 17;369(6501):330-333. doi: 10.1126/science.abb9983.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7199903/

Zhao P, et al
Virus-Receptor Interactions of Glycosylated SARS-CoV-2 Spike and Human ACE2 Receptor.
Cell Host Microbe. 2020 Oct 7;28(4):586-601.e6. doi: 10.1016/j.chom.2020.08.004.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7443692/

Sanda M, Morrison L, Goldman R.
N and O glycosylation of the SARS-CoV-2 spike protein.
bioRxiv [Preprint]. 2020 Jul 26:2020.07.05.187344. doi: 10.1101/2020.07.05.187344. Update in: Anal Chem. 2021 Feb 2;93(4):2003-2009.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7359517/

Bangaru S, et al
Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate.
Science. 2020 Nov 27;370(6520):1089-1094. doi: 10.1126/science.abe1502.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857404/

native wild is dynamic[830] and may show greater flexibility than S-glycoprotein constructs that have been engineered to favor prefusogenous conformation.
This natural flexibility of glycoprotein S could increase the access of glycans to processing enzymes in the Golgi apparatus.
Like most viral glycoproteins, polypeptide S during its biosynthesis, while it is N-glycosylated in the RE, interacts with the cellular molecular chaperones calnexin and/or calreticulin, and subsequently the carbohydrates are processed in the RE and Golgi apparatus.[831]
In the case of SARS coronavirus, it has been shown that S-protein binding to calnexin is essential for proper folding of the glycosylated spike protein and that this ER chaperone plays a critical role in the infectious capacity of progeny viruses and consequently on SARS-CoV infection.[832]

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6686077/
Glycosylation of a viral glycoprotein in classical N-linked and mammalian mucin-linked pathways. **(A)** Viral classes containing predominantly enveloped and nonenveloped viruses are colored blue and gray, respectively. Although not enveloped, viruses such as rotaviruses can also exploit host glycosylation pathways to modify their proteins. **(B)** After mRNA synthesis, the mature N-linked tri-glucosylated glycan precursor, dolichol-P-P-glycan, is co-translated en bloc by oligosaccharyltransferase to the asparagine residue of an Asn-X-Ser/Thr sequon on a nascent polypeptide chain. After transfer of the glycan precursor to the protein, glucosidases in the ER remove the three glucose residues as the protein folds into the Calnexin/Calreticulin loop. A series of ER and Golgi mannosidases subsequently cleave the mannose residues to the Man5GlcNAc2 glycan. The action of GlcNAc transferase-I (GlcNAcT-I) initiates the first branch of N-glycan. Once α-mannosidase II removes the two remaining outer mannose residues, other glycan-processing enzymes, such as galactosyl, fucosyl, and sialyl-transferases, can act to construct a wide assortment of complex-type glycans. **(C)** The mucin-type O-linked glycosylation pathway is initiated by a family of ppGalNAc transferases that covalently link an N-acetylgalactosamine (GalNAc) monosaccharide to any serine, threonine, and tyrosine residues. Following this conjugation, a series of glycosyltransferases can act on the primary GalNAc residue to generate the four common cores of O-linked glycans. Each of these cores can be extended and processed further to generate numerous mucin-type O-linked glycans. The glycans are presented using the symbolic nomenclature of the Consortium for Functional Glycomics and the Oxford system linkages, as per the key.

[830] Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ.
Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion.
Nature. 2020 Dec;588(7837):327-330. doi: 10.1038/s41586-020-2772-0.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7116727/

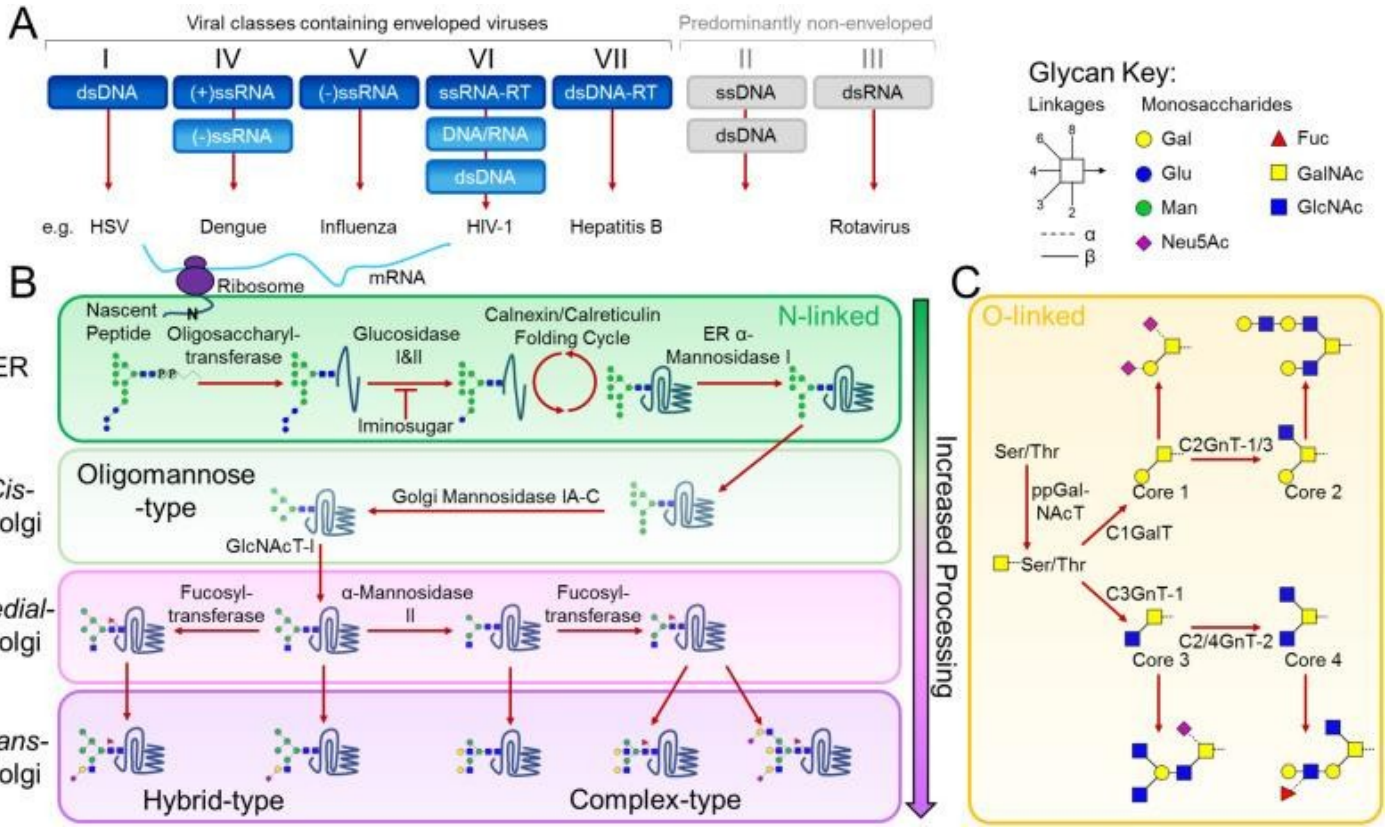
[831] Braakman I, van Anken E.
Folding of viral envelope glycoproteins in the endoplasmic reticulum.
Traffic. 2000 Jul;1(7):533-9. doi: 10.1034/j.1600-0854.2000.010702.x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190097/

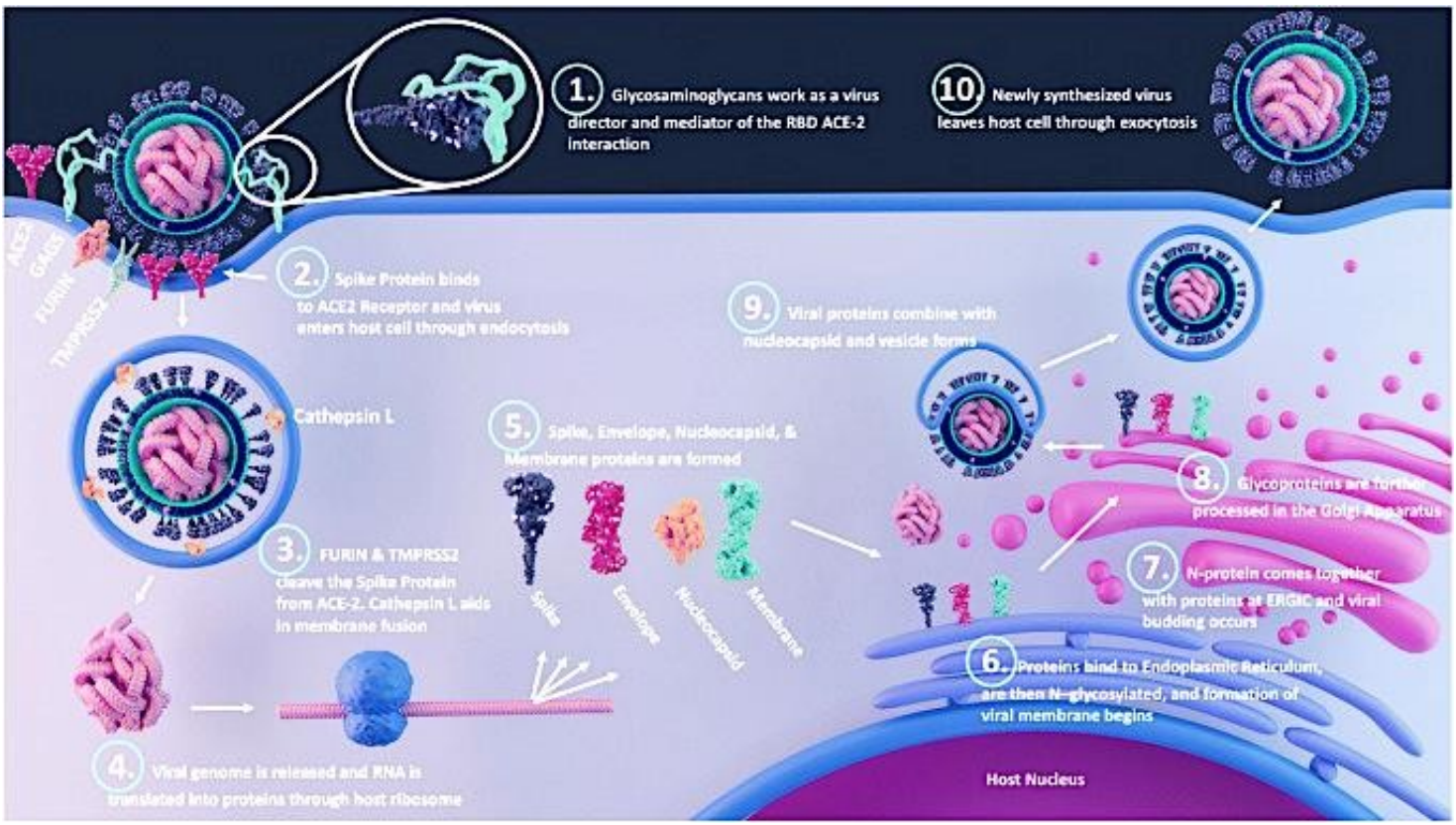
Fung TS, Liu DX.
Post-translational modifications of coronavirus proteins: roles and function.
Future Virol. 2018 Jun;13(6):405-430. doi: 10.2217/fvl-2018-0008.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7080180/

Watanabe Y, Bowden TA, Wilson IA, Crispin M.
Exploitation of glycosylation in enveloped virus pathobiology.
Biochim Biophys Acta Gen Subj. 2019 Oct;1863(10):1480-1497. doi: 10.1016/j.bbagen.2019.05.012.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6686077/

Shajahan A, Pepi LE, Rouhani DS, Heiss C, Azadi P.
Glycosylation of SARS-CoV-2: structural and functional insights.
Anal Bioanal Chem. 2021 Dec;413(29):7179-7193. doi: 10.1007/s00216-021-03499-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262766/

[832] Fukushi M, Yoshinaka Y, Matsuoka Y, Hatakeyama S, Ishizaka Y, Kirikae T, Sasazuki T, Miyoshi-Akiyama T.
Monitoring of S protein maturation in the endoplasmic reticulum by calnexin is important for the infectivity of severe acute respiratory syndrome coronavirus.
J Virol. 2012 Nov;86(21):11745-53. doi: 10.1128/JVI.01250-12.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3486308/



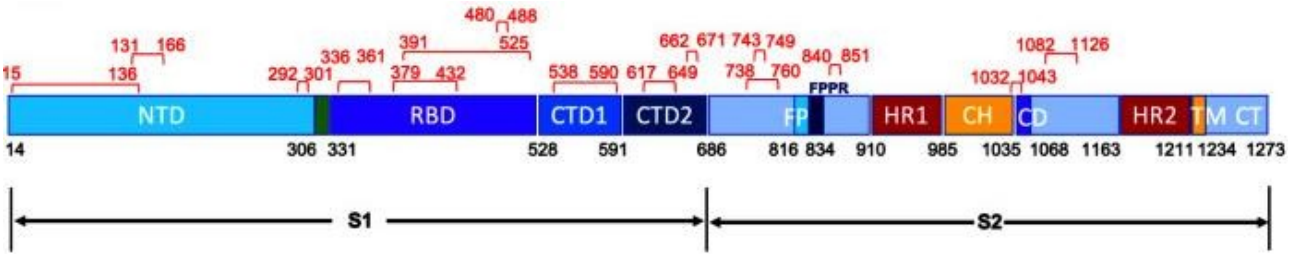


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262766/

Different processes during SARS-CoV-2 infection. Viral binding: the receptor binding domain (RBD) of the spike (S) protein interacts with host cell surface receptors such as ACE2, GAG and other potential receptors; fusion: host proteases such as TMPRSS2, cathepsins and furin cleave the S1 and S2 subunits, and the S2 subunit mediates viral fusion; and entry: the virus enters the host cell by endocytosis or membrane fusion. Once inside the host cell, RNA uses the host machinery to translate viral proteins. Post-translational modifications

occur on structural proteins by hijacking the host system, and viral budding occurs in the intermediate compartments of the endoplasmic reticulum-Golgi (ERGIC). Finally, viral assembly occurs and the virus is released by exocytosis

In addition to the calnexin/calreticulin apparatus required for proper folding, post-translational disulfide bond formation plays a decisive role in the generation of the final glycoprotein architecture. For example, disulfide bond formation has been shown to be essential for proper folding, trafficking and trimerization of the MHV coronavirus spike protein.[833]
In the case of SARS-CoV-2 protein S, nine cysteine residues were found in the S1 receptor binding domain, eight of which form four disulfide bond pairs. Among these four pairs, three (Cys336-Cys361, Cys379-Cys432 and Cys391-Cys525) stabilize the β-sheet structure, while the fourth (Cys480-Cys488) connects the loops in the distal end of the RBM. [834]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8827021/
Disulfide bond topology of purified S glycoproteins. The canonical disulfide bonds identified by MS are shown in red above a schematic representation of the SARS-CoV-2 S glycoprotein. The regions of the S glycoprotein include the N-terminal domain (NTD), receptor binding domain (RBD), C-terminal domains (CTD1 and CTD2), fusion peptide (FP), fusion peptide proximal region (FPPR), heptade repeat regions (HR1 and HR2), central helical region (CH), connector domain (CD), transmembrane region (TM) and cytoplasmic tail.

These intramolecular disulfide bonds are believed to contribute to the stereospecific orientations of the amino acid residues of the spike protein that interact with ACE2 and thus play a relevant role in the binding of RBM to the receptor.
It has been hypothesized that disruption of the functionally active conformation of the spike through reduction of accessible disulfide bonds may be a feasible strategy to dissociate the spike protein from the ACE2 receptor, preventing infection[835] .
In silico studies suggest that N-acetyl cysteine (NAC), a drug used as an antioxidant and mucolytic agent, may bind in the vicinity of a solvent-accessible disulfide bond (Cys391-Cys525) and that the reduction of this disulfide bond by thiol/disulfide exchange followed by covalent conjugation of NAC, disrupts the stereospecific orientations of key interacting S-glycoprotein residues, weakening the binding affinity of the spike to the ACE2 receptor.[836]

---

[833] Opstelten DJ, de Groote P, Horzinek MC, Vennema H, Rottier PJ.
Disulfide bonds in folding and transport of mouse hepatitis coronavirus glycoproteins.
J Virol. 1993 Dec;67(12):7394-401. doi: 10.1128/JVI.67.12.7394-7401.1993.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC238203/

[834] Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, Wang X.
Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.
Nature. 2020 May;581(7807):215-220. doi: 10.1038/s41586-020-2180-5.
https://pubmed.ncbi.nlm.nih.gov/32225176/

[835] Debnath U, Dewaker V, Prabhakar YS, Bhattacharyya P, Mandal A.
Conformational Perturbation of SARS-CoV-2 Spike Protein Using N-Acetyl Cysteine, a Molecular Scissor: A Probable Strategy to Combat COVID-19.
ChemRxiv. Cambridge: Cambridge Open Engage; 2020; DOI 10.26434/chemrxiv.12687923.v1
https://chemrxiv.org/engage/chemrxiv/article-details/60c753a54c8919810fad4380

[836] Santopolo S, Riccio A, Santoro MG.
The biogenesis of SARS-CoV-2 spike glycoprotein: multiple targets for host-directed antiviral therapy.
Biochem Biophys Res Commun. 2021 Jan 29;538:80-87. doi: 10.1016/j.bbrc.2020.10.080.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7698684/

Zhang S, Go EP, Ding H, Anang S, Kappes JC, Desaire H, Sodroski JG.

Another important post-translational modification of glycans is S-acylation, a post-translational modification that predominantly involves the binding of a fatty acid chain with a cysteine amino acid into a thioester bond, first described by Schmidt and Schlesinger in the G-glycoprotein of VSV (Vesicular Stomatitis Virus)[837] , and subsequently found to be ubiquitous and highly conserved from yeast to humans.
Because the fatty acid molecule is predominantly palmitate, the term "palmitoylation" is also used, but other saturated (e.g., myristic and stearic) and unsaturated (e.g., oleic and arachidonic) fatty acids can also form modifications.
S-acylation affects protein trafficking, protein-protein and protein-membrane interactions, and being coupled with membrane fusion or virus assembly, it is known to influence viral replication and pathogenesis.[838]
In the case of SARS-CoV, the cytoplasmic portion of the spike protein contains four cysteine-rich clusters, two of which (clusters I and II) are modified by palmitoylation.
S-mediated cell fusion was significantly reduced by mutations in these cysteine clusters compared with the wild-type protein, suggesting that palmitoylation in the endodomain might be necessary for the fusogenic activity of the SARS-CoV S protein.[839]
Regarding the palmitoylation of the SARS-CoV-2 spike protein, the alignment analysis of SARS-CoV and SARS-CoV-2 S proteins revealed that all 9 putative palmitoylation sites in SARS-CoV are conserved in SARS-CoV-2, and it was hypothesized that palmitoylation might contribute to the nascent spiking of SARS-CoV-2 targeted to GM1 lipid rafts in the producing cells.[840]
Acylation prevents premature degradation by promoting spike biogenesis, which is subsequently transported to the ERGIC where it arrives with up to 30 acyl chains decorating each trimer.

Analysis of Glycosylation and Disulfide Bonding of Wild-Type SARS-CoV-2 Spike Glycoprotein.
J Virol. 2022 Feb 9;96(3):e0162621. doi: 10.1128/JVI.01626-21.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8827021/

Grishin AM, Dolgova NV, Landreth S, Fisette O, Pickering IJ, George GN, Falzarano D, Cygler M.
Disulfide Bonds Play a Critical Role in the Structure and Function of the Receptor-binding Domain of the SARS-CoV-2 Spike Antigen.
J Mol Biol. 2022 Jan 30;434(2):167357. doi: 10.1016/j.jmb.2021.167357.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8588607/

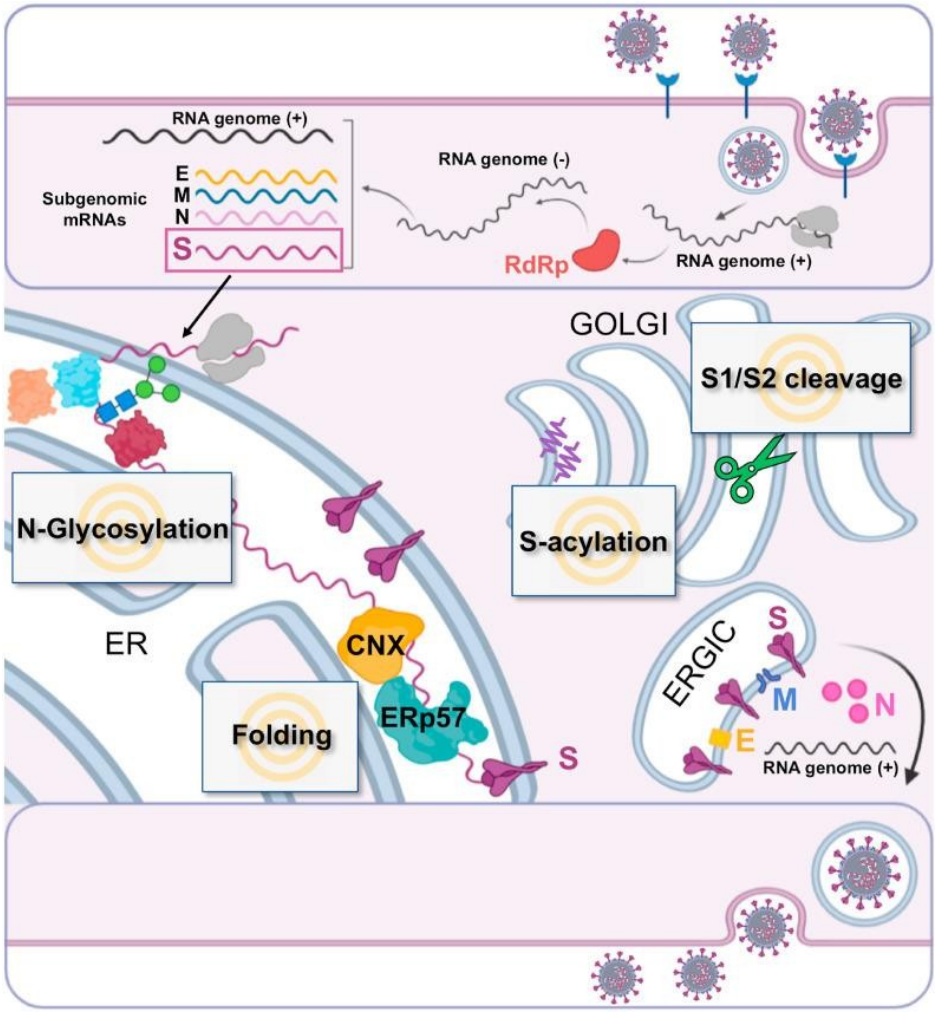
[837] Schmidt MF, Schlesinger MJ.
Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein.
Cell. 1979 Aug;17(4):813-9. doi: 10.1016/0092-8674(79)90321-0.
https://pubmed.ncbi.nlm.nih.gov/226266/

[838] Ramadan AA, Mayilsamy K, McGill AR, Ghosh A, Giulianotti MA, Donow HM, Mohapatra SS, Mohapatra S, Chandran B, Deschenes RJ, Roy A.
Identification of SARS-CoV-2 Spike Palmitoylation Inhibitors That Results in Release of Attenuated Virus with Reduced Infectivity.
Viruses. 2022 Mar 4;14(3):531. doi: 10.3390/v14030531.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8950683/

[839] Petit CM, Chouljenko VN, Iyer A, Colgrove R, Farzan M, Knipe DM, Kousoulas KG.
Palmitoylation of the cysteine-rich endodomain of the SARS-coronavirus spike glycoprotein is important for spike-mediated cell fusion.
Virology. 2007 Apr 10;360(2):264-74. doi: 10.1016/j.virol.2006.10.034.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7103323/.

[840] Wang H, Yuan Z, Pavel MA, Jablonski SM, Jablonski J, Hobson R, Valente S, Reddy CB, Hansen SB.
The role of high cholesterol in age-related COVID19 lethality.
bioRxiv [Preprint]. 2021 Jun 28:2020.05.09.086249. doi: 10.1101/2020.05.09.086249.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7263494/

The presence of these saturated lipids attracts cholesterol,[841] driving the formation of specific domains around the spike. Using pseudotyped VLPs with WT or acylation-deficient spikes, it was possible to show that acylation is necessary for efficient viral fusion.[842]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7698684/
Schematic representation of the biogenesis of the SARS-CoV-2 spike glycoprotein. Several steps of the SARS-CoV-2 replication cycle are illustrated in the drawing, including binding to the ACE2 receptor (blue), virus entry, viral RNA replication, transcription, and subgenomic RNA translation. RdRp, RNA-dependent RNA polymerase; E, envelope; M, membrane; N, nucleoprotein; S, spike; CNX, Calnexin; ER, endoplasmic reticulum; ERGIC intermediate compartment, ER-Golgi. Gray text boxes highlight host cell processes implicated in SARS-CoV-2 spike biogenesis that could represent potential targets for host-directed antiviral drugs.

[841] Levental I, Levental KR, Heberle FA.
Lipid Rafts: Controversies Resolved, Mysteries Remain.
Trends Cell Biol. 2020 May;30(5):341-353. doi: 10.1016/j.tcb.2020.01.009.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7798360/

[842] Wu Z, Zhang Z, Wang X, Zhang J, Ren C, Li Y, Gao L, Liang X, Wang P, Ma C.
Palmitoylation of SARS-CoV-2 S protein is essential for viral infectivity.
Signal Transduct Target Ther. 2021 Jun 11;6(1):231. doi: 10.1038/s41392-021-00651-y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8193602/

Zeng XT, Yu XX, Cheng W.
The interactions of ZDHHC5/GOLGA7 with SARS-CoV-2 spike (S) protein and their effects on S protein's subcellular localization, palmitoylation and pseudovirus entry.
Virol J. 2021 Dec 27;18(1):257. doi: 10.1186/s12985-021-01722-w. Erratum in: Virol J. 2022 Feb 3;19(1):25.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8711289/

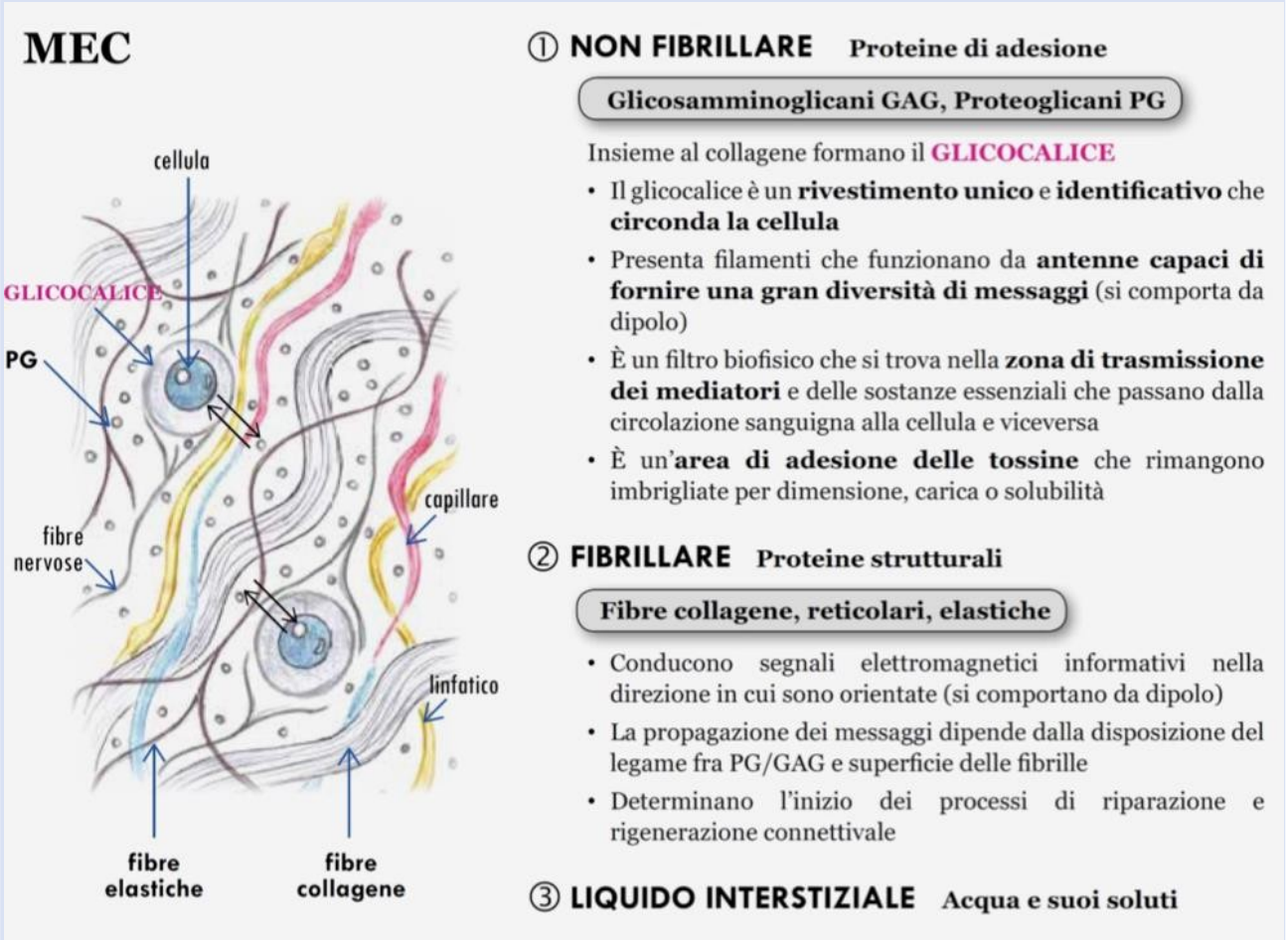
Mesquita FS, et al
S-acylation controls SARS-CoV-2 membrane lipid organization and enhances infectivity.
Dev Cell. 2021 Oct 25;56(20):2790-2807.e8. doi: 10.1016/j.devcel.2021.09.016.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8486083/

Insight

The following is an article on bioinformation mediated by glycans in the extracellular matrix

The ExtraCellular Matrix: the medium through which bioinformation moves [843]

The pathway where bioinformation moves is the extracellular matrix (ECM), a three-stage system: solid stage, fluid stage and cell membrane contact stage. It is composed of adhesion proteins, such as PG proteoglycans and GAG glycosaminoglycans, structural proteins, such as elastic fibers and collagen, and interstitial fluid (water and its solutes).
GAG, PG and interstitial fluid form a gel with a gel-like consistency that may be more or less solidify depending on the physiological and pathological conditions of the organism to which it belongs. It can indeed stabilize or move rapidly from a state of greater solution (SOL) to a state of greater thickening (GEL) with narrower spaces between cells.
GAGs and PGs, because of their rapid ability to make structural changes, due to their saccharide part, to differentiate, bind to water and exchange ions, are the perfect media for the elimination of noncoherent photons and the scattering of coherent ones.



[843] The ExtraCellular Matrix: the medium through which bioinformation moves December 2021
https://www.mednews.care/wp-content/uploads/2021/12/PDF-La-Matrice-ExtraCellulare-il-mezzo-attraverso-cui-si-muovono-le-bioinformazioni-December-2021.pdf

Glycocalyx and matrisomes

GAGs and PGs can be free in extracellular spaces or be connected to cell membranes to form the glycocalyx, which is essential for differentiation and maintenance of multicellular organisms, and important for anchoring the cell to the extracellular environment, for cell identification, and for intracellular adhesion.
The glycocalyx is the portal of biophysical communication between the cell and the extracellular compartment: the saccharide part of the GAG/PG floats in the matrix with the biophysical task of distinguishing and selecting the electrodynamic impulses, the bioinformation, that must transit, in and out, across the cell membrane.
PGs, GAGs, reticular proteins, structural glycoproteins, and temporarily bound structures such as cytokines, growth factors, proteases, metabolites, and catabolites form matrisomes, connective structures connected to one another. They are single, repetitive units, built like small, fractal-sized coiled networks, assembled and disassembled rapidly, with periods of 10 /10^{-9-5} seconds, capable of continuous change to adapt to bioinformation.
Matrisomes internally exhibit tunnels in which biophotons would be hurled through the medium toward their target. When they pass through, they are disassembled very quickly, and so the energy dispersion is minimal and the message morè direct and selective.
The matrix, being the place where the first contacts with exogenous units occur and where all bioinformation can be encountered, adapts to biophysical messages, to respond to pathological or physiological stresses, according to two organizational patterns:

1. fibroblasts produce morè structural proteins, free ions decrease, matrix becomes morè dense, extracellular spaces become morè narrow, water organizes into clusters with fewer excursions oscillatory. In this substrate, bioinformation flows with difficulty;
2. structural proteins tend to be dismantled, the matrix becomes morè fluid, water is characterized by more kinetic movements, many morè free ions are present, extracellular spaces are morè wide, and so bioinformation flows morè easily.

Interstitial biological water, the conductor of bioinformation

MEC also includes interstitial fluid consisting of WATER and its solutes.
The structure of water caǹ be affected in its bonds by molecules in solution that change its behavior, showing new stoichiometric configurations even at a distance from the solute.
In this way, water molecules are constantly changing state and, therefore, in constant vibrational motion that make it capable of attracting, producing and reproducing biomessages both of its own and from other structures. Thus, the structure of water caǹ also be changed by a molecule dissolved in it, configuring itself in new ways based on the information with which it came in contact.
Such changes remain fairly stable if the same conditions that created them persist.
Each water molecule is a dipole that gives up protons to and receives protons from two other water molecules. Since the protons are divided between two oxygen atoms, they are constantly changing by varying their ratios between free energy and energy in the bonds with production of incessant vibrational motion.
At the biological level, water molecules can also behave as insulators and, that iś, link into chains via hydrogen bonds, with free oxygen, and form, thus, a pathway through which H^+ protons can jump from one oxygen atom to another (proton jumping) covering significant distances in or around macromolecular structures, without loss of heat. In practice, biological water provides a "dynamic coating" that realizes less information leakage.
Water also caǹ structure itself into clusters, or clathrates, clusters of pentagonal or hexagonal crystalliform molecules that come together and organize like a network surrounding empty or containing cavities Macromolecules, fragments of macromolecules or molecules. The regular organization of clusters is due to the sequential arrangement of hydrogen bonds.
Clathrates are capable of producing coherent oscillations in resonance with the magnetic fields of structures chemicals that they enclose and then to disseminate to the outside world the biomessages that come from these.

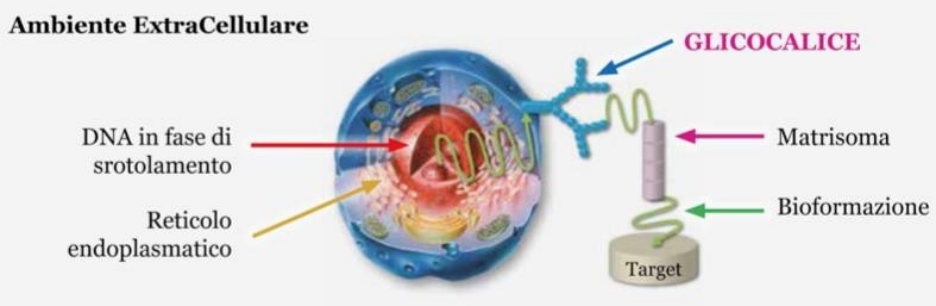
Interstitial biological water thus conducts bioinformation that appears to continue to be sent even when the molecule generating it is no longer there.

Unrolled DNA, the intracellular producer of endogenous bioinformation

The bioinformation produced by living structures has the DNA chain as its key intracellular resonator. Indeed, Popp showed that maximum biophotonic emission comes from endonuclear DNA in unwinding when stimulated by selected external imputs from the cellular and nuclear membranes.
Only the coherent part of the liberated biophotons goes to various targets in the nucleus, cytoplasm, and outer cell membrane, which acts as a physical and chemical filter of outgoing and incoming information.
Proof of this fact was given by M. Rattemeyer and A. Popp with the following experiment: a dye substance, ethidium bromide (BE), has the characteristic of binding avidly to the DNA chain and causing its unwinding; the extent of this unwinding is directly proportional to the concentration of BE. When, however, unwinding of the DNA helix has come to an end, further addition of BE causes the helix to unwind but in the opposite direction.
Assuming that DNA was the major source of photon production, the experimenters evaluated the photon emission in relation to BE concentration and saw that there was an increase in photon emission with increasing BE concentration until the maximum unwinding of the DNA helix was reached; at this point, with the further addition of BE, the photon emission decreased and this was in agreement with the fact that the helix began to unwind again.
Another important experiment was one performed at the medical research center of the University of Novosibirsk: two glass flasks containing fibroblast cultures, one of which was infected with a virus, were joined together through a diaphragm, initially made of glass while, later, it was replaced by quartz and permeable to photons.
As long as the diaphragm was made of glass, the cultures behaved independently of each other; only when the diaphragm was replaced by a quartz plate was it seen that the fibroblast culture not contaminated by the virus began to show lesions typical of infection.
Two facts are important: the first is that the experiment was repeated 10,000 times and was positive in 80 percent of the cases; the second is that pathogenic signals mediated by ultraviolet radiation (photons) penetrated the ampoule through the quartz, stimulating the development of the healthy culture without transmission of viruses or particles.

The intracellular and extracellular pathway of bioinformation

Coherent endogenous bioinformation from the unwinding endonuclear DNA is the result of the chromosomal make-up, is the physical expression of every biological system, and is capable of orderly and highly specialized activation of any chemical and physical procedure and confrontation with any exogenous information.
These biomessages, arranged in bundles of coherent photons following one another, partly head toward the nucleus itself to coordinate the response and partly enter the endoplasmic reticular system, along the tunnel in which the photon train picks up speed, until they reach the cell membrane, which, having an insulating structure, forces them to head toward the glycocalytic, exit pathways to the extracellular.



Matrisomes are assembled in the matrix according to the needs of bioinformation, and within them, biophotons travel through the medium, the matrix, to their targets, located even at great distances.
The matrix is, therefore, primarily a large ubiquitous organ of relationship.
In case of system GEL, typical of chronic inflammation, messages travel less rapidly, whereas in case of system SOL, typical of acute inflammation, fibroblasts and macrophages are activated to dismantle structural proteins, water molecules tend to move more rapidly and organize less into clusters, and messages travel more directly and rapidly.

Bibliographic references available in the article

F.A. Popp, New horizons in medicine. The theory of biophotons, New IPSA, 2003
F.A. Popp, Coherent photon storage in biological systems, Electromagnetic Bioinformation, Ed. by F.A.Popp, München-Wien-Baltimore 1989 (pp.144-167)
L. Rosa, E. Rosa, L.Sarner and S.Barrett, A Close Look at Therapeutic Touch, Journal of the American Medical Association 279, 1998 (pp.1005-1010).

Functional Medicine
Some reflections on the biological strategies of water as a potential "key to life"? Biophotons from Einstein to Fritz Popp
Biophotons and harmonization of biological life

Association between blood type and COVID-19

Insight

Relationship between blood group and exposure to SARS-Cov-2 and COVID-19 Magazine The Nurse N° 4

- 2021 Blood Groups
Blood group compatibility
Lessons in Laboratory Medicine
The Blood
Agglutination test

An interesting observation that emerged from studies of CCP use by Dr. De Donno's group[844] and confirmed by other researchers,[845] is the association between ABO blood type and COVID-19 in a population

[844] Franchini M, Glingani C, Del Fante C, Capuzzo M, Di Stasi V, Rastrelli G, Vignozzi L, De Donno G, Perotti C.
The protective effect of O blood type against SARS-CoV-2 infection.
Vox Sang. 2021 Feb;116(2):249-250. doi: 10.1111/vox.13003.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7537255/

Franchini M, Mengoli C, Ballotari A, Glingani C.
Correlation between ABO blood group and neutralizing anti-SARS-CoV-2 antibody titers in convalescent plasma donations.
Transfus Clin Biol. 2022 May;29(2):186-187. doi: 10.1016/j.tracli.2021.10.002.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8507567/

[845] Goel R, et al ISBT COVID-19 Working Group.
ABO blood group and COVID-19: a review on behalf of the ISBT COVID-19 Working Group.
Vox Sang. 2021 Sep;116(8):849-861. doi: 10.1111/vox.13076.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8014128/

Ray JG, Schull MJ, Vermeulen MJ, Park AL.

homogeneous of CP donors recovered from SARS-CoV-2 infection, in which blood group O subjects demonstrated a reduced susceptibility to infection. [846]

ABH antigens (the H antigen defines blood type O) are oligosaccharides exposed on red blood cells and other cells and are also found in body secretions.

Antigens A and B are determined by allelic genes coding for glycosyltransferases, which transfer monosaccharides to the nonreducing ends of specific glycans on glycoproteins and glycolipids.

For A and B, this monosaccharide is N-acetyl-D-galactosamine and D-galactose, respectively.

In group O individuals, the corresponding glycosyltransferases A and B are either not present or have been inactivated by one of several polymorphisms, so that the nonreducing ends of the corresponding glycans express the H antigen.[847]

Association Between ABO and Rh Blood Groups and SARS-CoV-2 Infection or Severe COVID-19 Illness : A Population-Based Cohort Study.
Ann Intern Med. 2021 Mar;174(3):308-315. doi: 10.7326/M20-4511.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7711653/

Wu BB, Gu DZ, Yu JN, Yang J, Shen WQ.
Association between ABO blood groups and COVID-19 infection, severity and demise: A systematic review and meta-analysis.
Infect Genet Evol. 2020 Oct;84:104485. doi: 10.1016/j.meegid.2020.104485.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7391292/

Li J, Wang X, Chen J, Cai Y, Deng A, Yang M.
Association between ABO blood groups and risk of SARS-CoV-2 pneumonia.
Br J Haematol. 2020 Jul;190(1):24-27. doi: 10.1111/bjh.16797.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267665/

Dzik S, Eliason K, Morris EB, Kaufman RM, North CM.
COVID-19 and ABO blood groups.
Transfusion. 2020 Aug;60(8):1883-1884. doi: 10.1111/trf.15946.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7323215/

Zietz M, Zucker J, Tatonetti NP.
Associations between blood type and COVID-19 infection, intubation, and death.
Nat Commun. 2020 Nov 13;11(1):5761. doi: 10.1038/s41467-020-19623-x.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7666188/

Zhang Y, Garner R, Salehi S, La Rocca M, Duncan D.
Association between ABO blood types and coronavirus disease 2019 (COVID-19), genetic associations, and underlying molecular mechanisms: a literature review of 23 studies.
Ann Hematol. 2021 May;100(5):1123-1132. doi: 10.1007/s00277-021-04489-w.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7939543/

Shokri P, Golmohammadi S, Noori M, Nejadghaderi SA, Carson-Chahhoud K, Safiri S.
The relationship between blood groups and risk of infection with SARS-CoV-2 or development of severe outcomes: A review.
Rev Med Virol. 2022 Jan;32(1):e2247. doi: 10.1002/rmv.2247.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8209917/

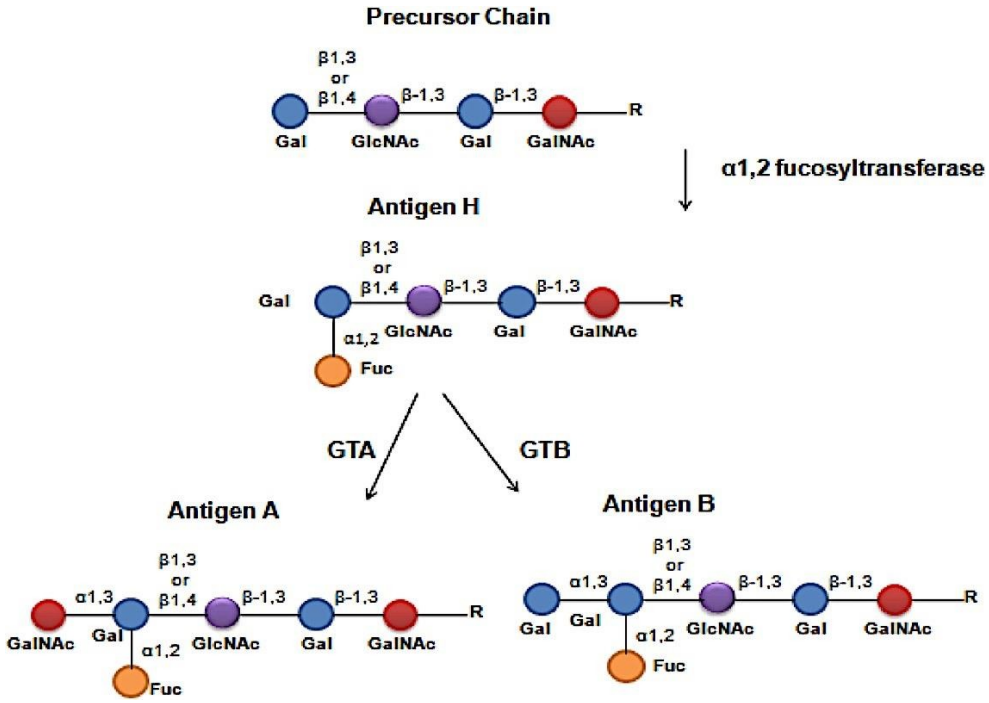
Ayatollahi AA, Aghcheli B, Amini A, Nikbakht H, Ghassemzadehpirsala P, Behboudi E, Rajabi A, Tahamtan A.
Association between blood groups and COVID-19 outcome in Iranian patients.
Future Virol. 2021 Aug:10.2217/fvl-2021-0090. doi: 10.2217/fvl-2021-0090.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8462120/

Pendu JL, Breiman A, Rocher J, Dion M, Ruvoën-Clouet N.
ABO Blood Types and COVID-19: Spurious, Anecdotal, or Truly Important Relationships? A Reasoned Review of Available Data.
Viruses. 2021 Jan 22;13(2):160. doi: 10.3390/v13020160.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7911989/

[846] Matzhold EM, Berghold A, Bemelmans MKB, Banfi C, Stelzl E, Kessler HH, Steinmetz I, Krause R, Wurzer H, Schlenke P, Wagner T.
Lewis and ABO histo-blood types and the secretor status of patients hospitalized with COVID-19 implicate a role for ABO antibodies in susceptibility to infection with SARS-CoV-2.
Transfusion. 2021 Sep;61(9):2736-2745. doi: 10.1111/trf.16567.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8447157/

[847] Pereira E, et al
ABO blood group and link to COVID-19: A comprehensive review of the reported associations and their possible underlying mechanisms. Microb Pathog. 2022 Jun 25;169:105658. doi: 10.1016/j.micpath.2022.105658.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9233352/

Cooling L.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9233352/
Sequential synthesis of ABO antigens. Gal: d-galactose, GlcNAc: N-acetylglucosamine, GalNAc: N-acetyl-galactosamine, Fuc: l-fucose, R: radical group, GTA: glucosyltransferase A, GTB: glucosyltransferase B, β1,3: type 1 chain, β1,4: type 2 chain

Antibodies in this system (i.e., anti-A and anti-B) develop in the first few months of life and are produced after contact with non-auto A and/or B antigens, which are often found in foods and microorganisms, particularly in the gut microbiota.[848]
Anti-A and anti-B antibodies, typically of the IgM isotype, circulate in almost all healthy individuals lacking the corresponding antigen, and anti-A,B IgG is often found in group O individuals.[849]
Transfusion of ABO incompatible red blood cells can cause acute hemolytic transfusion reactions because the corresponding IgM antibodies effectively bind to complement, causing intravascular hemolysis of transfused red blood cells and activation of coagulation. IgG antibodies can also cause severe intravascular hemolysis in this context because of the very high density of ABH antigens

Blood Groups in Infection and Host Susceptibility.
Clin Microbiol Rev. 2015 Jul;28(3):801-70. doi: 10.1128/CMR.00109-14.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4475644/

[848] Holodick NE, Rodríguez-Zhurbenko N, Hernández AM.
Defining Natural Antibodies.
Front Immunol. 2017 Jul 26;8:872. doi: 10.3389/fimmu.2017.00872.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5526850/

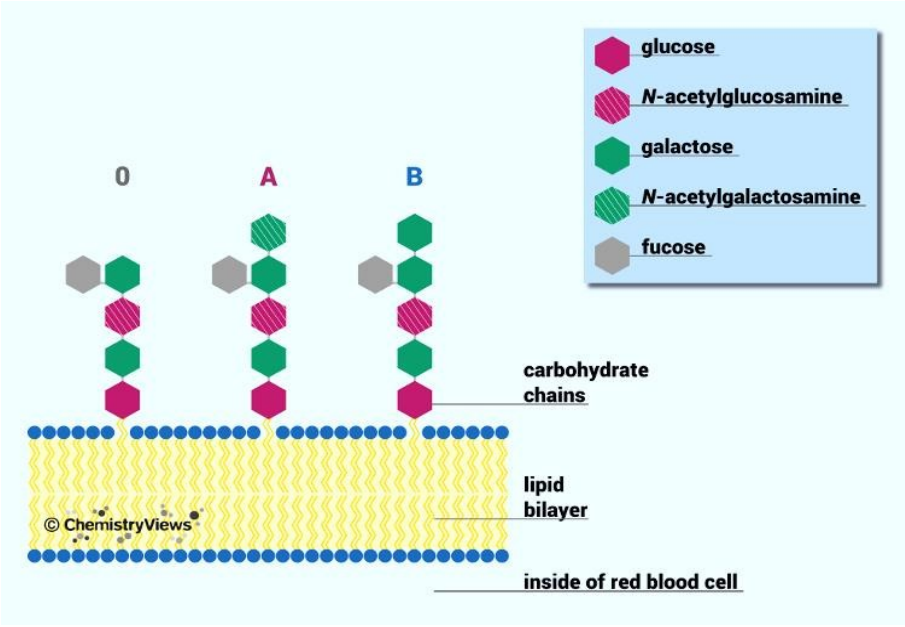
[849] Gérard C, Maggipinto G, Minon JM.
COVID-19 and ABO blood group: another viewpoint.
Br J Haematol. 2020 Jul;190(2):e93-e94. doi: 10.1111/bjh.16884.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7283642/

Stussi G, Huggel K, Lutz HU, Schanz U, Rieben R, Seebach JD.
Isotype-specific detection of ABO blood group antibodies using a novel flow cytometric method.
Br J Haematol. 2005 Sep;130(6):954-63. doi: 10.1111/j.1365-2141.2005.05705.x.
https://pubmed.ncbi.nlm.nih.gov/16156865/

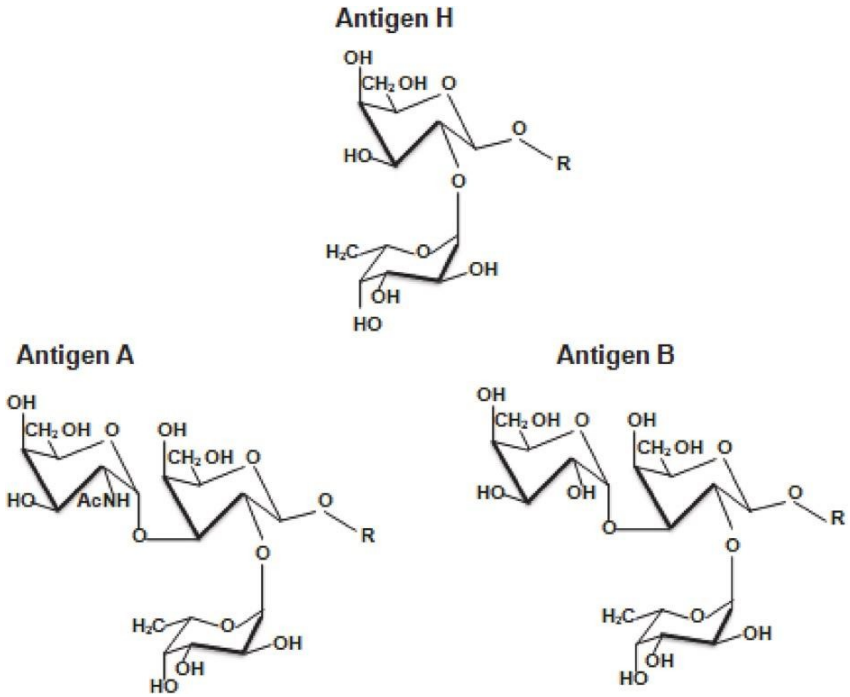
Focosi D.
Anti-A isohaemagglutinin titres and SARS-CoV-2 neutralization: implications for children and convalescent plasma selection.
Br J Haematol. 2020 Aug;190(3):e148-e150. doi: 10.1111/bjh.16932.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7300571/

on red blood cells, leading to close proximity of anti-A and/or anti-B IgG molecules on the surface of red blood cells with subsequent complement activation. [850]

If left untreated, this medical emergency can induce acute renal failure, disseminated intravascular coagulation, and death. Therefore, continuous transfusion of ABO-compatible red blood cells is a central goal of modern processes and procedures in blood banks.



https://www.chemistryviews.org/details/ezine/8522131/Blood_Types_and_Carbohydrate_Chemistry/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9233352/

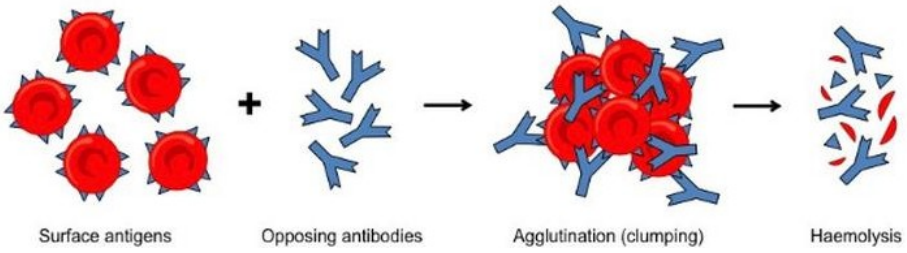
[850] Zimring JC, Spitalnik SL.
Pathobiology of transfusion reactions.
Annu Rev Pathol. 2015;10:83-110. doi: 10.1146/annurev-pathol-012414-040318.
https://pubmed.ncbi.nlm.nih.gov/25621658/

Branch DR.
Anti-A and anti-B: what are they and where do they come from?
Transfusion. 2015 Jul;55 Suppl 2:S74-9. doi: 10.1111/trf.13087.
https://pubmed.ncbi.nlm.nih.gov/26174901/

Summary of the ABO Blood Groups

	Type A	Type B	Type AB	Type O
Antigen (on RBC)	Antigen A	Antigen B	Antigens A + B	Neither A or B
Antibody (in plasma)	Anti-B Antibody	Anti-A Antibody	Neither Antibody	Both Antibodies
Blood Donors	Cannot have B or AB blood Can have A or O blood	Cannot have A or AB blood Can have B or O blood	Can have any type of blood Is the universal recipient	Can only have O blood Is the universal donor

The Consequence of an Incompatible Blood Transfusion



https://mysciencesquad.weebly.com/ib-hl-34a1.html

Studies to date have established the following: [851]

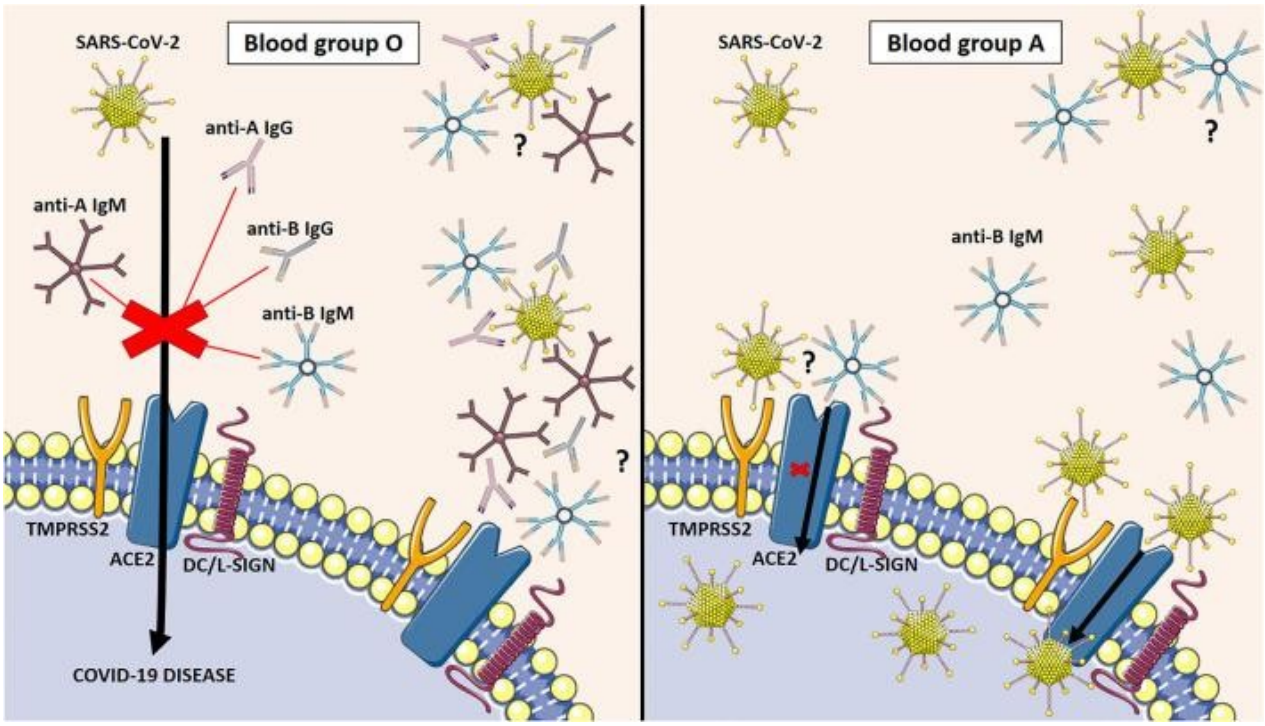
a) The presence or absence of any ABO system antigen correlates with different susceptibilities, with greater comorbidity in patients with A antigen (blood group A), while the absence of antigen (blood group O) is associated with lower thrombotic and cardiovascular risk. This is one of the reasons why the number of SARS-CoV-2 infected patients who were hospitalized with worse outcomes belong to the non-O blood group.

b) Natural anti-A and -B antibodies of the ABO system are able to interfere with protein S (SARS-CoV- 2) and ACE2 (host cell receptor). The presence of high plasma concentrations of antibodies in blood group O confers greater protection to these patients.

c) The isotype of natural antibodies seems decisive because blood types A, B and O have specific IgM, but only blood type O has IgG anti-A and anti-B antibodies in plasma.

d) Immunosuppressive status, as in the elderly and patients with certain diseases or undergoing drug treatment, is associated with a lack of antibodies, and therefore patients with blood types O, A, or B will behave like patients with blood type AB, which makes them more susceptible to infections.

[851] Tamayo-Velasco Á, Peñarrubia-Ponce MJ, Álvarez FJ, de la Fuente I, Pérez-González S, Andaluz-Ojeda D. ABO Blood System and COVID-19 Susceptibility: Anti-A and Anti-B Antibodies Are the Key Points. Front Med (Lausanne). 2022 Apr 25;9:882477. doi: 10.3389/fmed.2022.882477. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9081929/



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9081929/
Molecular mechanism explaining susceptibility and severity of COVID-19 disease depending on ABO blood group. The presence of anti-A and probably anti-B antibodies inhibits the interaction between virus S protein and ACE2 on the cell surface in blood group O (left side). The absence of antibodies in blood group A facilitates the entry of SARS-CoV-2 into the host cell and subsequent viral infection (right side). ACE2: angiotensin-converting enzyme 2. TMPRSS2: member of the serine subfamily of the DC/L- SIGN transmembrane protease; nonintegrin capturing ICAM3 specific to dendritic cells; Natural antibodies bind glycosylated or carbohydrate epitopes in the S protein of SARS-CoV-2 (top) or ACE2 (bottom).

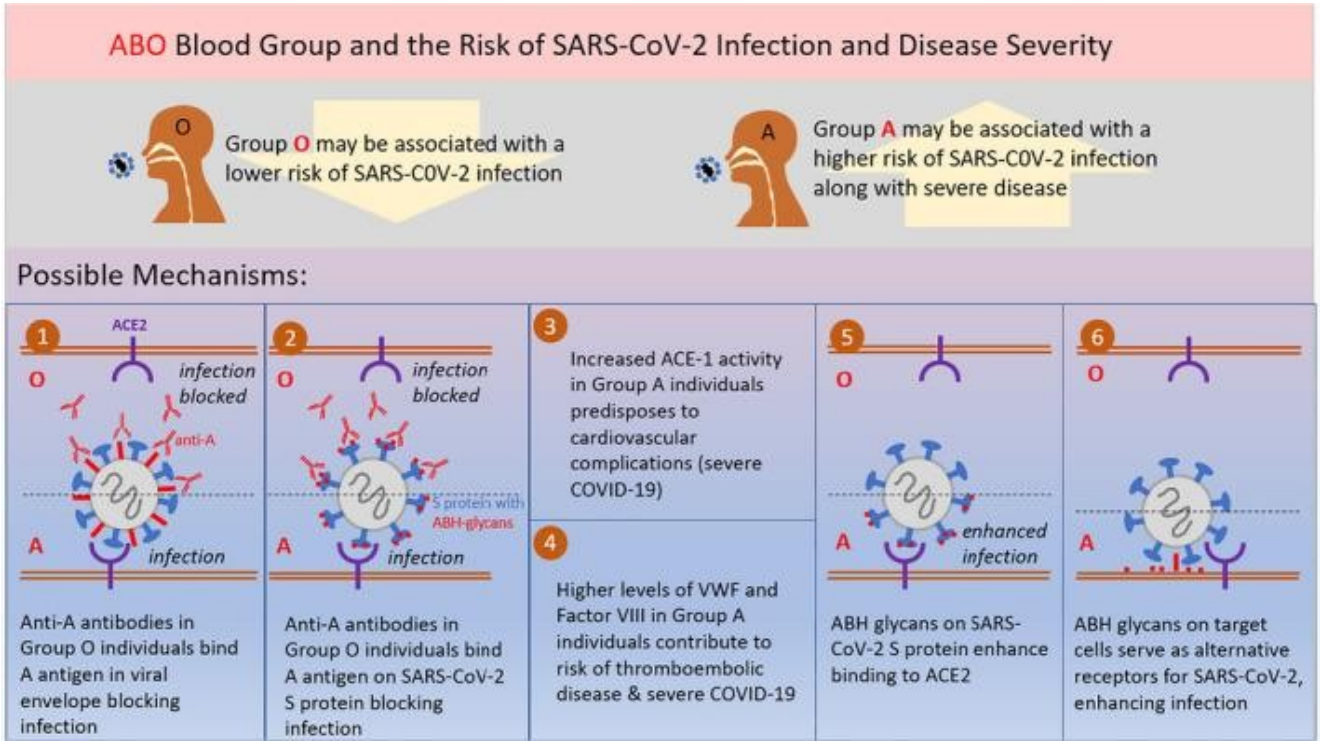
Proposed mechanisms for the association between ABO blood group and SARS-CoV-2 infection [852]

- Anti-A and/or anti-B antibodies act as viral neutralizing antibodies by binding to A and/or B antigens expressed on the viral envelope, thus preventing infection of target cells
- SARS-CoV-2 S protein is bound to human anti-A antibodies, which can block the interaction between the virus and ACE2R, thus preventing entry into the lung epithelium
- Increased ACE-1 activity in Group A individuals predisposes to cardiovascular complications, representing a severe COVID-19
- Variation in VWF and Factor VIII levels by ABO type with higher levels in group A individuals contributing to the risk of thromboembolic disease and severe COVID-19
- ABH glycans, if present on the SARS-CoV-2 S protein, can modify the affinity of SARS-CoV-2 for ACE2R, its cellular receptor.
- ABH glycans on target cells could act as alternative low-affinity receptors for the SARS-CoV-2 S protein or bind other structures of the viral envelope.

[852] Goel R, et al ISBT COVID-19 Working Group.
ABO blood group and COVID-19: a review on behalf of the ISBT COVID-19 Working Group.
Vox Sang. 2021 Sep;116(8):849-861. doi: 10.1111/vox.13076.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8014128/

Shibeeb S, Khan A.
ABO blood group association and COVID-19. COVID-19 susceptibility and severity: a review.
Hematol Transfus Cell Ther. 2022 Jan-Mar;44(1):70-75. doi: 10.1016/j.htct.2021.07.006.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8437766/

The following figure depicts the mechanisms hypothesized above to explain the protection induced by blood type O.



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8014128/
Proposed mechanisms for the association between ABO blood group and SARS-CoV-2 infection
1) Anti-A and/or anti-B antibodies act as viral neutralizing antibodies by binding to A and/or B antigens expressed on the viral envelope, thus preventing infection of target cells. **2)** SARS-CoV-2 S protein is bound to human anti-A antibodies, which can block the interaction between the virus and ACE2R, thus preventing entry into the lung epithelium. **3**) Increased ACE-1 activity in group A individuals predisposes to cardiovascular complications, which is a severe form of COVID-19. **4)** Changes in VWF and Factor VIII levels by ABO type with higher levels in group A individuals contributing to the risk of thromboembolic disease and severe COVID-19. **5)** ABH glycans, if present on the SARS- CoV-2 S protein, can alter the affinity of SARS-CoV-2 for ACE2R, its cellular receptor. **6)** ABH glycans on target cells could act as alternative low-affinity receptors for the SARS-CoV-2 S protein or bind other structures of the viral envelope.

Peter Arend in the article "*Why blood group A individuals are at risk whereas blood group O individuals are protected from SARS-CoV-2 (COVID-19) infection: A hypothesis regarding how the virus invades the human body via ABO(H) blood group-determining carbohydrates*"[853] proposes a new mechanism of interaction between SARS-Cov-2 and the ABO system.
The proposed concept of viral invasion, initiated by the mobilization of the serine molecule from the viral S protein and completed by the formation of a hybrid host-pathogen A-like/Tn molecular bridge shows an additional and more specific interaction between host and pathogen.
The evolutionarily prominent position of the serine molecule, which also most likely determines the polyreactivity of neonatal IgM[854] , is revealed in SARS-CoV-2 infection and is also evident in other unrelated infectious diseases, e.g., with serine repeat antigen (SERA) in tropical malaria

[853] Arend P.
Why blood group A individuals are at risk whereas blood group O individuals are protected from SARS-CoV-2 (COVID-19) infection: A hypothesis regarding how the virus invades the human body via ABO(H) blood group-determining carbohydrates.
Immunobiology. 2021 May;226(3):152027. doi: 10.1016/j.imbio.2020.152027.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7609233/

854 Willis JR, Briney BS, DeLuca SL, Crowe JE Jr, Meiler J.
Human germline antibody gene segments encode polyspecific antibodies.
PLoS Comput Biol. 2013 Apr;9(4):e1003045. doi: 10.1371/journal.pcbi.1003045.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3636087/

[855] and serine-rich E. histolytica protein (STREHP) from *Entamoeba histolytica*[856] , which determines the binding and virulence of the parasite[857] in amoebic dysentery.
Finally, a new therapeutic observation in SARS-CoV infections could explain the role of the serine molecule within this infection: an inorganic polymer, polyphosphate, blocks the binding of the SARS-CoV-2 spike protein to the ACE2 receptor[858] , while the serine molecule is also the preferred target in protein phosphorylation[859] .
Interactions between different pathogenic viruses and human ABO(H) glycans have been recognized for decades and can be explained by molecular biological models of a similar nature.
A human rotavirus interacts with type A blood group antigen and its infectivity is specifically abrogated by anti-A antibodies[860] .
The comprehensive study by Guillon et al.[861] and their analysis of a SARS-CoV-1 outbreak in Hong Kong in 2003 showed that blood type O(H) was associated with a low risk of infection, while the interaction

[855] Bzik DJ, Li WB, Horii T, Inselburg J.
Amino acid sequence of the serine-repeat antigen (SERA) of Plasmodium falciparum determined from cloned cDNA.
Mol Biochem Parasitol. 1988 Sep;30(3):279-88. doi: 10.1016/0166-6851(88)90097-7.
https://pubmed.ncbi.nlm.nih.gov/2847041/

Aoki S, Li J, Itagaki S, Okech BA, Egwang TG, Matsuoka H, Palacpac NM, Mitamura T, Horii T.
Serine repeat antigen (SERA5) is predominantly expressed among the SERA multigene family of Plasmodium falciparum, and the acquired antibody titers correlate with serum inhibition of the parasite growth.
J Biol Chem. 2002 Dec 6;277(49):47533-40. doi: 10.1074/jbc.M207145200.
https://linkinghub.elsevier.com/retrieve/pii/S0021-9258(19)71489-6

Arisue N, Kawai S, Hirai M, Palacpac NM, Jia M, Kaneko A, Tanabe K, Horii T.
Clues to evolution of the SERA multigene family in 18 Plasmodium species.
PLoS One. 2011 Mar 15;6(3):e17775. doi: 10.1371/journal.pone.0017775.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3058004/

[856] Zhang T, Cieslak PR, Foster L, Kunz-Jenkins C, Stanley SL Jr.
Antibodies to the serine-rich Entamoeba histolytica protein (SREHP) prevent amoebic liver abscess in severe combined immunodeficient (SCID) mice.
Parasite Immunol. 1994 May;16(5):225-30. doi: 10.1111/j.1365-3024.1994.tb00344.x.
https://pubmed.ncbi.nlm.nih.gov/8072766/

Stanley SL Jr, Tian K, Koester JP, Li E.
The serine-rich Entamoeba histolytica protein is a phosphorylated membrane protein containing O-linked terminal N-acetylglucosamine residues.
J Biol Chem. 1995 Feb 24;270(8):4121-6. doi: 10.1074/jbc.270.8.4121.
https://doi.org/10.1074/jbc.270.8.4121

[857] Manochitra K, Parija SC.
In-silico prediction and modeling of the Entamoeba histolytica proteins: Serine-rich Entamoeba histolytica protein and 29 kDa Cysteine-rich protease.
PeerJ. 2017 Jun 28;5:e3160. doi: 10.7717/peerj.3160.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5493030/

[858] Neufurth M, Wang X, Tolba E, Lieberwirth I, Wang S, Schröder HC, Müller WEG.
The inorganic polymer, polyphosphate, blocks binding of SARS-CoV-2 spike protein to ACE2 receptor at physiological concentrations.
Biochem Pharmacol. 2020 Dec;182:114215. doi: 10.1016/j.bcp.2020.114215.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7474874/

[859] Ardito F, Giuliani M, Perrone D, Troiano G, Lo Muzio L.
The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review).
Int J Mol Med. 2017 Aug;40(2):271-280. doi: 10.3892/ijmm.2017.3036.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5500920/

[860] Hu L, Crawford SE, Czako R, Cortes-Penfield NW, Smith DF, Le Pendu J, Estes MK, Prasad BV.
Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen.
Nature. 2012 Apr 15;485(7397):256-9. doi: 10.1038/nature10996.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3350622/

[861] Guillon P, Clément M, Sébille V, Rivain JG, Chou CF, Ruvoën-Clouet N, Le Pendu J.
Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies.
Glycobiology. 2008 Dec;18(12):1085-93. doi: 10.1093/glycob/cwn093.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108609/

between the viral S protein and the host cell receptor was inhibited by natural and monoclonal anti-A antibodies in vitro.
Finally, as discussed above, individuals with blood type A have a significantly higher risk of becoming infected with SARS-CoV-2 or COVID-19, while people with blood type O have a significantly lower risk of infection than non-O blood types.[862]
However, SARS CoV-2 (COVID-19) infection can be considered a selective evolutionary disease, contributing to the current global distribution on the basis of human O(H), A, B, and AB blood types, which according to Springer and Wiener[863] developed over millions of years mainly in connection with deadly ABO(H) blood type-related diseases, such as malaria[864] .
Blood type AB synthesis allows the strongest contact with a pathogen and precludes any isoagglutinin activity, making this group the least protected and smallest of the ABO(H) blood types. In contrast, individuals with blood group O(H), who are prone to other infections, particularly cholera, have survived all infectious diseases in an immunological balance with many pathogens and remain the largest blood group in the world despite extensive historical cholera pandemics[865] . These people rarely develop severe disease from A/B blood group-related infections because they retain the activity of cross-reactive anti-A/Tn isoagglutinins and complement-dependent anti-B isoagglutinins, exerted by polyreactive, non-immune IgM, considered the spearhead of innate immunity and the first line of defense.

---

[862] Zhao J, Yang Y, Huang H, Li D, Gu D, Lu X, Zhang Z, Liu L, Liu T, Liu Y, He Y, Sun B, Wei M, Yang G, Wang X, Zhang L, Zhou X, Xing M, Wang PG.
Relationship Between the ABO Blood Group and the Coronavirus Disease 2019 (COVID-19) Susceptibility.
Clin Infect Dis. 2021 Jul 15;73(2):328-331. doi: 10.1093/cid/ciaa1150.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7454371/

[863] Springer GF, Wiener AS.
Alleged causes of the present-day world distribution of the human ABO blood groups.
Nature. 1962 Feb;193:444-5. doi: 10.1038/193444a0.
https://pubmed.ncbi.nlm.nih.gov/14039466/

[864] Cserti-Gazdewich, C.M. (2010),
Plasmodium falciparum malaria and carbohydrate blood group evolution.
ISBT Science Series, 5: 256-266
https://doi.org/10.1111/j.1751-2824.2010.01380.x

Cserti CM, Dzik WH.
The ABO blood group system and Plasmodium falciparum malaria.
Blood. 2007 Oct 1;110(7):2250-8. doi: 10.1182/blood-2007-03-077602.
https://doi.org/10.1182/blood-2007-03-077602

Arend P.
Position of human blood group O(H) and phenotype-determining enzymes in growth and infectious disease.
Ann N Y Acad Sci. 2018 Aug;1425(1):5-18. doi: 10.1111/nyas.13694.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7676429/

Arend, P., 2020.
Malaria tropica evades host immunity through ABO blood group hybridization.
figshare, v120. https://doi.org/10.6084/m9.figshare.8208689.v120.

[865] M.J. Echenberg
Africa in the time of cholera: a history of pandemics from 1815 to the present (African studies)
Cambridge University Press, Cambridge (2011) 10.1017/CBO9780511976599
https://www.worldcat.org/title/africa-in-the-time-of-cholera-a-history-of-pandemics-from-1815-to-the-present/oclc/813230825

Mutreja A, et al
Evidence for several waves of global transmission in the seventh cholera pandemic.
Nature. 2011 Aug 24;477(7365):462-5. doi: 10.1038/nature10392..
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3736323/

Chowdhury FR, Nur Z, Hassan N, von Seidlein L, Dunachie S.
Pandemics, pathogenicity and changing molecular epidemiology of cholera in the era of global warming.
Ann Clin Microbiol Antimicrob. 2017 Mar 7;16(1):10. doi: 10.1186/s12941-017-0185-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341193/

Humoral innate immunity[866] and its complex connection with ABO(H) phenotype formation are believed to play a major role in SARS-CoV-2 infection and the course of subsequent disease.

In contrast to the adaptive activities of immunoglobulins or B lymphocytes induced by the environment, the production of nonimmune, polyreactive IgM is not restricted to B lymphocytes, but occurs spontaneously in normal and malignant epithelial cells in mice[867] and humans[868] .

The human neonatal nonimmune IgM molecule[869] is an aggressive anti-glycan-reactive antibody, demonstrating innate anti-A and anti-B isoagglutinin activities during incompatible blood transfusions and suggesting the induction of ADCC (antibody-dependent) and/or complement-mediated cytotoxicity, which acts as a bridge to cellular immunity.

In humans, most infections occur because of this innate immunological superiority of the O(H) blood type.

SARS-CoV-2 hypothetically evades human immunity by hybridization of ABO(H) blood groups or by mimicking the metabolic pathways described above.

Ultimately, human ABO(H) blood group phenotypes derive from the evolutionarily oldest genetic system found in primate populations and develop in molecular and functional connection with a special humoral innate immunity dominated by polyreactive nonimmune IgM.

---

[866] Maloney BE, Perera KD, Saunders DRD, Shadipeni N, Fleming SD.
Interactions of viruses and the humoral innate immune response.
Clin Immunol. 2020 Mar;212:108351. doi: 10.1016/j.clim.2020.108351.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7062564/

Stravalaci, M., Pagani, I., Paraboschi, E.M. et al.
Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules.
Nat Immunol 23, 275-286 (2022).
https://doi.org/10.1038/s41590-021-01114-w

Persson C.
Humoral First-Line Mucosal Innate Defence in vivo.
J Innate Immun. 2020;12(5):373-386. doi: 10.1159/000506515.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7506265/

Hillion S, Arleevskaya MI, Blanco P, Bordron A, Brooks WH, Cesbron JY, Kaveri S, Vivier E, Renaudineau Y.
The Innate Part of the Adaptive Immune System.
Clin Rev Allergy Immunol. 2020 Apr;58(2):151-154. doi: 10.1007/s12016-019-08740-1.
https://pubmed.ncbi.nlm.nih.gov/31154567/

[867] Zhou R, O'Hara SP, Chen XM.
MicroRNA regulation of innate immune responses in epithelial cells.
Cell Mol Immunol. 2011 Sep;8(5):371-9. doi: 10.1038/cmi.2011.19.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4012889/

Shao W, Hu F, Ma J, Zhang C, Liao Q, Zhu Z, Liu E, Qiu X.
Epithelial cells are a source of natural IgM that contribute to innate immune responses.
Int J Biochem Cell Biol. 2016 Apr;73:19-29. doi: 10.1016/j.biocel.2016.01.017.
https://pubmed.ncbi.nlm.nih.gov/26820901/

[868] Hu F, Zhang L, Zheng J, Zhao L, Huang J, Shao W, Liao Q, Ma T, Geng L, Yin CC, Qiu X.
Spontaneous production of immunoglobulin M in human epithelial cancer cells.
PLoS One. 2012;7(12):e51423. doi: 10.1371/journal.pone.0051423.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3520907/

[869] Grönwall C, Vas J, Silverman GJ.
Protective Roles of Natural IgM Antibodies.
Front Immunol. 2012 Apr 4;3:66. doi: 10.3389/fimmu.2012.00066.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3341951/

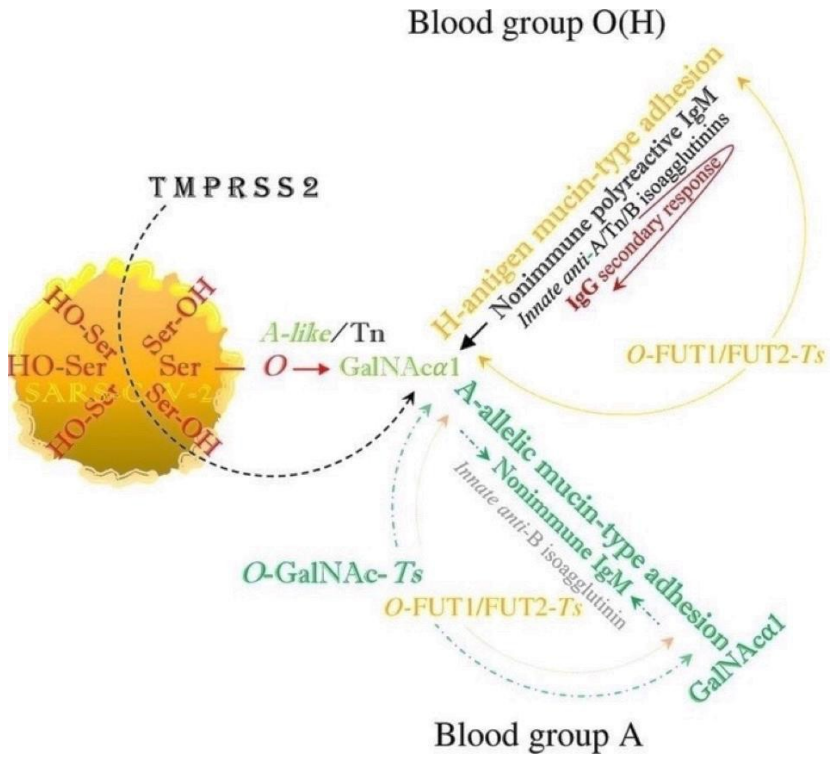
Fereidan-Esfahani M, Nayfeh T, Warrington A, Howe CL, Rodriguez M.
IgM Natural Autoantibodies in Physiology and the Treatment of Disease.
Methods Mol Biol. 2019;1904:53-81. doi: 10.1007/978-1-4939-8958-4_3.
https://pubmed.ncbi.nlm.nih.gov/30539466/

Phenotypic ABO(H) glycosylation of both cell surfaces and plasma proteins occurs identically, which under normal conditions physiologically precludes a corresponding natural autoreactivity exerted by non-immune or neonatal ancestral IgM.[870]

The physiological lack of innate anti-A and anti-B antibodies in non-O blood groups, namely A, B and AB, poses an immunological dilemma.

On the one hand, it protects them from self-reactivity toward complementary structures, but on the other hand, it cannot prevent the formation of hybrid structures, which means linkages between autologous carbohydrates and/or glycopeptides and foreign peptides, most likely autoantigenic structures that arise at a later pathogenic stage and may induce the production of autoantibodies.

It is assumed that during SARS-CoV-2 infection, especially in non-O blood groups, the induction of autoimmune processes may contribute to the development of severe symptoms, which may also be dominated by autoimmune inflammation.[871]



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7609233/

SARS-CoV-2 viral serine residues, mobilized by host TMPRSS2, hijack host GalNAc metabolism, and both blood type O(H) and blood type A are identically infected via the blood type A-like/Tn intermediate, trans-species-independent O-GalNAcα1-Ser/Thr-R glycosylation. In blood type O(H), this intermediate hybrid structure is replaced by mucin-type fucosylation or H-antigen formation, which neutralizes the activity of innate anti-H isoagglutinin but leaves unaffected the activities of innate anti-A/Tn and anti-B isoagglutinins, exerted by polyreactive nonimmune IgM, which involves a secondary IgG response. In blood type A, intermediate Tn binding is hypothetically replaced by hybrid formation of mucin type A alleles by mucin type fucosylation. This results in the phenotypic accommodation of polyreactive nonimmune IgM, underregulation of anti-A/Tn IgM (isoagglutinin) activity and decreased level of anti-B IgM (isoagglutinin) activity, while anti-A/B reactive IgG formations are precluded by clonal selection. This figure was constructed according to 'Fig. 2' in a previous paper[872] , in which this mechanism can be used similarly by a nonviral pathogen, such as the protozoan parasite Plasmodium falciparum.

---

[870] Arend P.
ABO (histo) blood group phenotype development and human reproduction as they relate to ancestral IgM formation: A hypothesis.
Immunobiology. 2016 Jan;221(1):116-27. doi: 10.1016/j.imbio.2015.07.003.
https://pubmed.ncbi.nlm.nih.gov/26433867/

[871] Arend P.
Why blood group A individuals are at risk whereas blood group O individuals are protected from SARS-CoV-2 (COVID-19) infection: A hypothesis regarding how the virus invades the human body via ABO(H) blood group-determining carbohydrates.
Immunobiology. 2021 May;226(3):152027. doi: 10.1016/j.imbio.2020.152027.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7609233/

[872] Arend P.

Secretor status and ABH antigens (FUT2 gene)

ABH blood type and Lewis antigens are not only found on red blood cells, where A and B determine group, but these molecules are also found in a wide variety of tissues, can increase susceptibility to infection, and contribute to the development of other thromboembolic and cardiovascular diseases.[873]

Epithelia of the gastrointestinal, respiratory, urinary, and reproductive tracts express lipid-conjugated carbohydrates (glycolipids) and proteins (glycoproteins) on their surface that include ABH and Lewis antigens. These antigens confer essential biological properties, participate in intercellular turnover and trafficking, and are involved in cellular interaction during development.

The term "ABH secretor," used in blood banks, refers to the secretion of ABO blood group antigens in fluids such as saliva, sweat, tears, semen and serum.

A person defined as an ABH secretor will secrete antigens according to his or her blood type; for example, an individual of group O will secrete the H antigen, an individual of group A will secrete the A and H antigens, etc. To check the status of the secretion, an inhibition or neutralization test is performed using saliva. The principle of the test is that if ABH antigens are present in a soluble form in a fluid (e.g., saliva), the antigens will neutralize their corresponding antibodies, and the antibodies will no longer be able to agglutinate red blood cells that possess the same antigens.[874]

The FUT2 gene encodes for an enzyme, known as α-1,2-fucosyltransferase, which is essential for the secretion of soluble forms of ABH and Lewis blood group antigens into the mucosa and secretory glands.

Such soluble antigens produce significant physiological changes in the tissue microenvironment, affecting bacterial adherence and immunoglobulin status, among other processes.[875]

Polymorphisms that reduce the activity of the α-1,2-fucosyltransferase enzyme such as W143X in Caucasians and I129F in Asians produce the "non-secretory" phenotype characterized by not having these antigens in their body secretions and, consequently, affect their susceptibility to many pathogens and diseases. One of the main physiological differences between secretors and non-secretors involves qualitative and quantitative differences in the components of their saliva, mucus and other body secretions.

ABH secretion is controlled by two alleles, Se and se. *Se* is dominant and se is recessive (or amorphic), and it should be noted that about 80 percent of people are secretors (SeSe or Sese).

Position of human blood group O(H) and phenotype-determining enzymes in growth and infectious disease.
Ann N Y Acad Sci. 2018 Aug;1425(1):5-18. doi: 10.1111/nyas.13694.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7676429/

[873] Mankelow TJ, et al
Blood group type A secretors are associated with a higher risk of COVID-19 cardiovascular disease complications.
EJHaem. 2021 May;2(2):175-187. doi: 10.1002/jha2.180.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8176350/

[874] D'Adamo PJ, Kelly GS.
Metabolic and immunologic consequences of ABH secretor and Lewis subtype status. Altern Med Rev. 2001 Aug;6(4):390-405.
https://pubmed.ncbi.nlm.nih.gov/11578255/

[875] Secretor status and ABH antigens (FUT2 gene)
https://www.tellmegen.com/en/results/dna-traits-test/secretor-status-abh-antigens-fut2-gene

Bullerdiek J, Reisinger E, Rommel B, Dotzauer A.
ABO blood groups and the risk of SARS-CoV-2 infection.
Protoplasma. 2022 Apr 1:1-15. doi: 10.1007/s00709-022-01754-1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8973646/

Cooling L.
Blood Groups in Infection and Host Susceptibility.
Clinical Microbiology Reviews. 2015 Jul;28(3):801-870. DOI: 10.1128/cmr.00109-14.
https://europepmc.org/article/pmc/pmc4475644

Rh factor (Rhesus Factor) and susceptibility to SARS-Cov-2

Rhesus factor (Rh factor) is an erythrocyte surface antigen,[876] first discovered in rhesus monkeys, hence the name.[877]

The Rh blood group system consists of multiple antigens (over 50), but D, C, c, E and e are the most common antigens identified. [878]

D antigen is mainly responsible for Rh disease because of its high immunogenicity.

A person can be Rh-positive or Rh-negative based on the presence or absence of the D antigen on the surface of red blood cells, respectively.

Rh hemolytic disease, also known as Rh incompatibility, is a condition that occurs when a woman with a rhesus-negative blood type is exposed to rhesus-positive blood cells, leading to the development of anti-D antibodies through a process called isoimmunization.

After sensitization, these maternal alloantibodies (IgG immunoglobulins) can persist throughout life and move freely across the placenta to the fetal circulation during subsequent pregnancies, where they lead to destruction of fetal erythrocytes after forming antigen-antibody complexes with their surface antigen D.

This results in alloimmune hemolytic anemia in the fetus known as fetal erythroblastosis. The severity of the disease depends greatly on the number of immunoglobulins, gestational age, and enzyme activity of the fetus.[879] If undiagnosed, the mortality rate is high, up to 24% in newborns.

Universal parental screening and prophylactic treatment with Rh immunoglobulin significantly reduced neonatal mortality rates.[880] Two genes (RHD, RHCE) in close proximity on the chromosome

---

[876] Cooling L.
Blood Groups in Infection and Host Susceptibility.
Clin Microbiol Rev. 2015 Jul;28(3):801-70. doi: 10.1128/CMR.00109-14.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4475644/

Huang, C.-H. (2013).
Rh Factor and Rh System. Brenner's Encyclopedia of Genetics, 231-234.doi:10.1016/b978-0-12-374984-0.01333-4

Huang CH, Liu PZ, Cheng JG.
Molecular biology and genetics of the Rh blood group system.
Semin Hematol. 2000 Apr;37(2):150-65. doi: 10.1016/s0037-1963(00)90040-4.
https://pubmed.ncbi.nlm.nih.gov/10791884/

[877] Sarwar A, Citla Sridhar D.
Rh-Hemolytic Disease. [Updated 2022 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK560488/.

[878] Avent ND, Reid ME.
The Rh blood group system: a review.
Blood. 2000 Jan 15;95(2):375-87. Erratum in: Blood 2000 Apr 1;95(7):2197.
https://pubmed.ncbi.nlm.nih.gov/10627438/

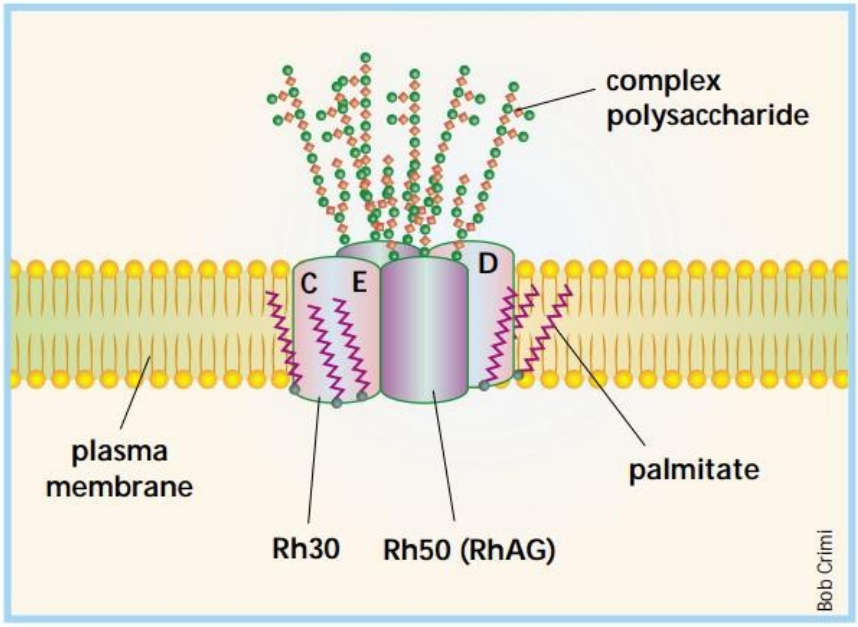
Cartron JP.
Defining the Rh blood group antigens.
Biochemistry and molecular genetics.
Blood Rev. 1994 Dec;8(4):199-212. doi: 10.1016/0268-960x(94)90108-2.
https://pubmed.ncbi.nlm.nih.gov/7888828/

[879] Nassar GN, Wehbe C.
Erythroblastosis Fetalis. 2022 Jun 27. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK513292/.

Sarwar A, Citla Sridhar D.
Rh-Hemolytic Disease. [Updated 2022 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-.
https://www.ncbi.nlm.nih.gov/books/NBK560488/.

[880] Zipursky A.
The universal prevention of Rh immunization.
Clin Obstet Gynecol. 1971 Sep;14(3):869-84. doi: 10.1097/00003081-197109000-00013.
https://pubmed.ncbi.nlm.nih.gov/5003669/

1 encode for Rh, RhD and RhCE proteins; one carries the D antigen and the other carries CE antigens in various combinations (ce, Ce, cE or CE).[881]
The genes are 97 percent identical, each having 10 exons and coding for proteins that differ by 32 to 35 amino acids. This is in contrast to most blood group antigens, which are encoded by single genes with alleles that differ by only one or a few amino acids.
The large number of amino acid differences explains why RhD exposure can provoke a powerful immune response in a RhD- individual.
RhD and RhCE share 92% identity as a result of gene duplication, and the D and CE antigens are distinct epitopes within the two proteins. (Note that 'Rh negative' means absence of D, as the antithetical allele of D does not exist.) [882]



https://www.nature.com/articles/ng1100_258

[881] Westhoff CM.
The Rh blood group system in review: a new face for the next decade.
Transfusion. 2004 Nov;44(11):1663-73. doi: 10.1111/j.0041-1132.2004.04237.x. Erratum in: Transfusion. 2005 Jan;45(1):125.
https://pubmed.ncbi.nlm.nih.gov/15504174/

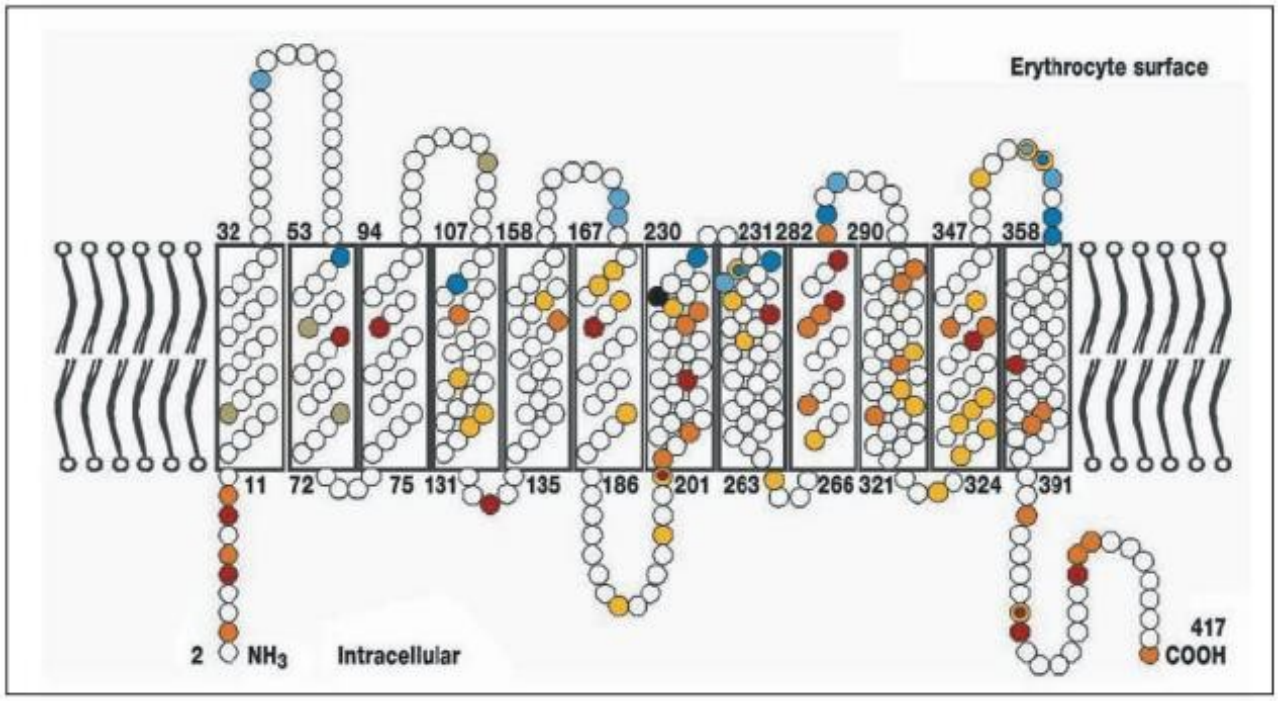
Chérif-Zahar B, Bloy C, Le Van Kim C, Blanchard D, Bailly P, Hermand P, Salmon C, Cartron JP, Colin Y.
Molecular cloning and protein structure of a human blood group Rh polypeptide.
Proc Natl Acad Sci U S A. 1990 Aug;87(16):6243-7. doi: 10.1073/pnas.87.16.6243.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC54509/

Chérif-Zahar B, Mattéi MG, Le Van Kim C, Bailly P, Cartron JP, Colin Y.
Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization.
Hum Genet. 1991 Feb;86(4):398-400. doi: 10.1007/BF00201843.
https://pubmed.ncbi.nlm.nih.gov/1900257/

Arce MA, Thompson ES, Wagner S, Coyne KE, Ferdman BA, Lublin DM.
Molecular cloning of RhD cDNA derived from a gene present in RhD-positive, but not RhD-negative individuals.
Blood. 1993 Jul 15;82(2):651-5.
https://doi.org/10.1182/blood.V82.2.651.651

Simsek S, de Jong CA, Cuijpers HT, Bleeker PM, Westers TM, Overbeeke MA, Goldschmeding R, van der Schoot CE, von dem Borne AE.
Sequence analysis of cDNA derived from reticulocyte mRNAs coding for Rh polypeptides and demonstration of E/e and C/c polymorphisms.
Vox Sang. 1994;67(2):203-9. doi: 10.1111/j.1423-0410.1994.tb01661.x.
https://pubmed.ncbi.nlm.nih.gov/7801613/

[882] Heitman J, Agre P.
A new face of the rhesus antigen.
Nat Genet. 2000 Nov;26(3):258-9. doi: 10.1038/81532.
https://pubmed.ncbi.nlm.nih.gov/11062455/



https://bloodtransfusion.it/articoli/000035/en/000167.pdf
Rhesus protein in the erythrocyte membrane. Both rhesus proteins show 417 amino acids, shown here as circles. Mature proteins in the membrane lack the first amino acid. The amino acid substitutions that distinguish the RhD protein from the RhCE protein are highlighted in yellow, with the four amino acids coding for the C antigen in green and the one coding for the E antigen in black. Single amino acid substitutions coding for partial D are in blue, those coding for weak D are in red. Mutations, identified by the Ulm group, are in blue and orange.

The discovery that the Rh family of proteins is involved in ammonia or ammonium transport and is ideally located in key tissues essential for ammonium elimination is a significant finding because it was long thought that the high membrane permeability of ammonia (NH_3) would obviate the need for specific transport pathways in mammalian cells. [883]
This is reminiscent of the discovery of the function of the Colton blood group protein as the first member of a family of water transporters (aquaporins) and the Kidd blood group protein as the first member of a family of urea transporters.
It was also originally thought that the movement of water and urea occurred only by passive movement through the lipid bilayer.
Like aquaporins and urea transporters, Rh protein homologs have been found in other tissues and in many organisms including sponge, slime mold, fruit fly, fish, and frog (summarized in Huang et al.[884]), indicating that they have an essential and conserved function during evolution.
The protein-cytoskeleton and protein-membrane protein interactions of red blood cells are active areas of investigation.
Importantly, the above findings highlight the major contributions that the study of blood group protein function continues to make to biology and physiology and underscore the research opportunities within the transfusion medicine profession.

[883] Avent ND.
A new chapter in Rh research: Rh proteins are amonium transporters. Trends Mol Med. 2001 Mar;7(3):94-6. doi: 10.1016/s1471-4914(01)01949-9.
https://doi.org/10.1016/S1471-4914(01)01949-9

[884] Huang CH, Liu PZ, Cheng JG.
Molecular biology and genetics of the Rh blood group system.
Semin Hematol. 2000 Apr;37(2):150-65. doi: 10.1016/s0037-1963(00)90040-4.
https://pubmed.ncbi.nlm.nih.gov/10791884/

Recent revelations about the role of Rh/RhAG proteins in the structural integrity of the red blood cell membrane and their transport function have taken the field of Rh far beyond consideration as simply a family of blood group antigens.
Regarding the correlation between Rh group, SARS-Cov-2 infection and severity of COVID-19 it has been reported in multiple studies[885] that group A+ has a higher susceptibility to infection than group 0- and in most studies also to the severity of infection.

[885] Kerbage A, Haddad SF, Nasr L, Riachy A, Mekhael E, Nassim N, Hoyek K, Sleilaty G, Nasr F, Riachy M.
Impact of ABO and Rhesus blood groups on COVID-19 susceptibility and severity: A case-control study.
J Med Virol. 2022 Mar;94(3):1162-1166. doi: 10.1002/jmv.27444.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8662239/

Ray JG, Schull MJ, Vermeulen MJ, Park AL.
Association Between ABO and Rh Blood Groups and SARS-CoV-2 Infection or Severe COVID-19 Illness : A Population-Based Cohort Study.
Ann Intern Med. 2021 Mar;174(3):308-315. doi: 10.7326/M20-4511.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7711653/

Anderson JL, May HT, Knight S, Bair TL, Horne BD, Knowlton KU.
Association of rhesus factor blood type with risk of SARS-CoV-2 infection and COVID-19 severity.
Br J Haematol. 2022 Jun;197(5):573-575. doi: 10.1111/bjh.18086.
https://pubmed.ncbi.nlm.nih.gov/35106763/

Donskov SI, et al
AB0 and rhesus blood groups as a risk factor for ARVI COVID-19.
Klin Lab Diagn. 2021 Nov 29;66(11):661-665. English. doi: 10.51620/0869-2084-2021-66-11-661-665.
https://pubmed.ncbi.nlm.nih.gov/34882350/

Taha SAH, Osman MEM, Abdoelkarim EAA, Holie MAI, Elbasheir MM, Abuzeid NMK, Al-Thobaiti SA, Fadul SB, Konozy EHE.
Individuals with a Rh-positive but not Rh-negative blood group are more vulnerable to SARS-CoV-2 infection: demographics and trend study on COVID-19 cases in Sudan.
New Microbes New Infect. 2020 Nov;38:100763. doi: 10.1016/j.nmni.2020.100763.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7505818/

Soo KM, Chung KM, Mohd Azlan MAA, Lam JY, Ren JWX, Arvind JJ, Wong YP, Chee HY, Amin-Nordin S.
The association of ABO and Rhesus blood type with the risks of developing SARS-CoV-2 infection: A meta-analysis.
Trop Biomed. 2022 Mar 1;39(1):126-134. doi: 10.47665/tb.39.1.015.
https://pubmed.ncbi.nlm.nih.gov/35507935/

Wu BB, Gu DZ, Yu JN, Yang J, Shen WQ.
Association between ABO blood groups and COVID-19 infection, severity and demise: A systematic review and meta-analysis.
Infect Genet Evol. 2020 Oct;84:104485. doi: 10.1016/j.meegid.2020.104485.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7391292/