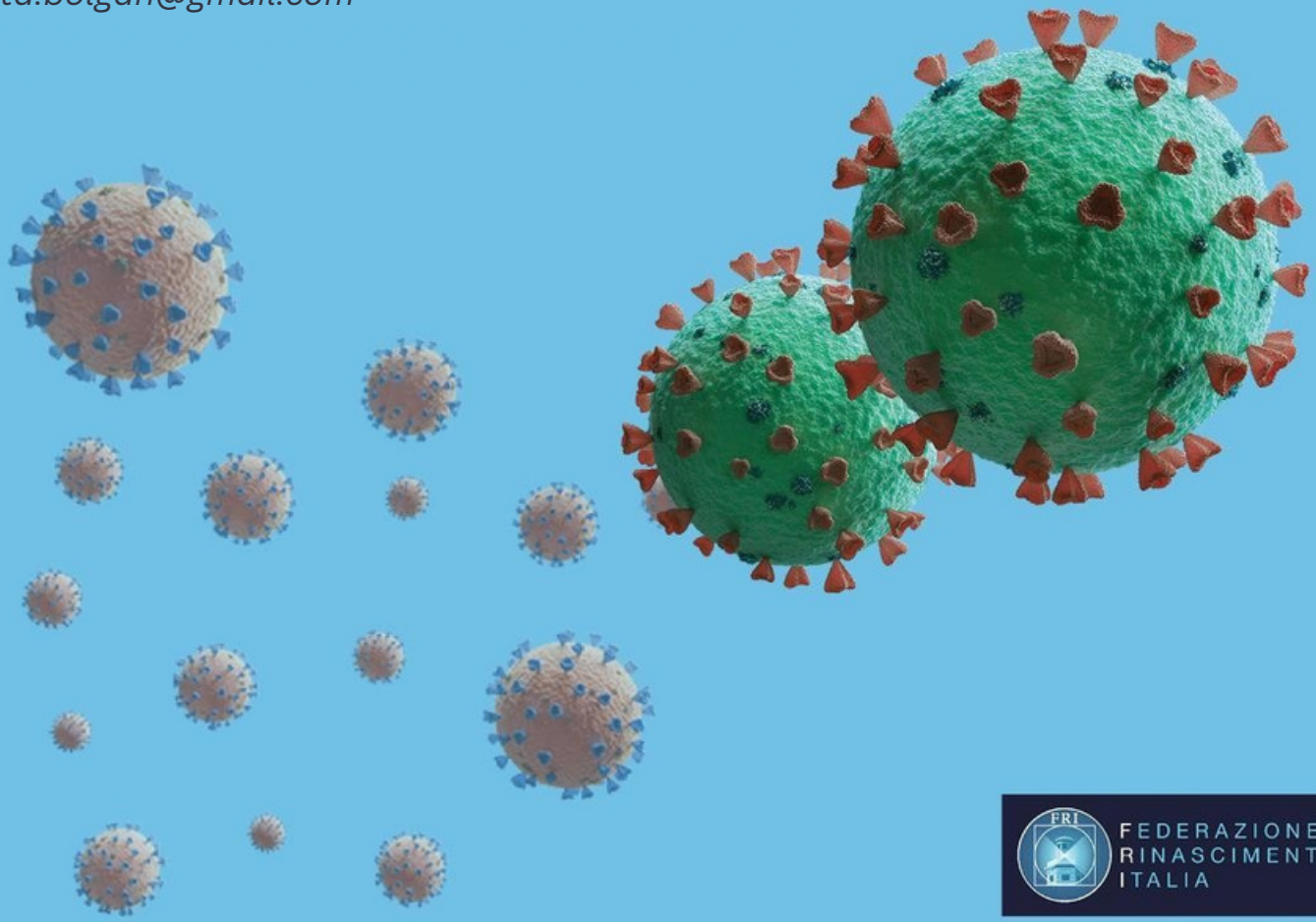
CHAPTER 1

COVID-19 CLINICAL PRESENTATION AND IMMUNOPATHOGENESIS

DR. LORETTA BOLGAN

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

loretta.bolgan@gmail.com



FEDERAZIONE RINASCIMENTO ITALIA

rinascimentoitalia.it

COVID-19

CHAPTER 1 **CLINICAL PRESENTATION AND IMMUNOPATHOGENESIS**

SUMMARY

INTRODUCTION **4**
COVID-19 - situation in the world **4**
COVID-19 - Situation in Italy **5**
CLINICAL PRESENTATION **6**
HISTOPATHOLOGICAL RESULTS OF AUTOPSIES **17**
IMMUNOPATHOGENESIS **21**
Structure of SARS-Cov-2 ..... **22**
IN-DEPTH STUDY **24**
THE IMMUNE SYSTEM 24
Innate Immunity 24
Adaptive Immunity 25
THE INFLAMMATORY RESPONSE TO VIRAL INFECTION 27
Acute inflammation 27
Systemic effects of inflammation 27
Polarization of macrophages 28
The adaptive Th1 and Th2 response 29
Cytokines 33
Cytokines and its receptors in viral infections 35
The cytokine storm 39
IMMUNE RESPONSES INDUCED BY SARS-COV-2 INFECTION **43**
FEVER IN THE RESPONSE TO SARS-COV-2 VIRAL INFECTION AND ITS COMPLICATIONS **46**
CYTOKINE STORM SYMPTOMATOLOGY **52**
Subtypes of cytokine storm syndrome 56
SARS-COV2 IMMUNE EVASION STRATEGIES **60**
Endothelial and vascular cell damage 62
Antibody-dependent enhancement (ADE) and immune complex vasculitis 63
The mechanism of ADE in the induction of COVID-19 66
The function of Fcγ receptors in dendritic cells and macrophages 67
The neonatal Fc receptor (FcRn) 69
The immunopathology of anti-SARS vaccines and the ADE from SARS-Cov-2 75
The ADE from influenza vaccine 79
Intravenous immunoglobulin infusion therapy: the mechanism of action 82
INDIVIDUAL FACTORS **86**
COVID-19 in old age 89
COVID-19 pediatric 90
COVID-19 in pregnancy 92
IMMUNE RESPONSE, AGE, AND GENDER DIFFERENCE **93**
Pediatric age **93**
The hyporesponsiveness to pediatric vaccines 96
Maternal-fetal immunopathology 105

Microbiota and the immune system in pregnancy and the newborn....113
Advanced age....122
Immuno-senescence and inflamm-aging....124
Microbiota and senescence....134
Microbiota and COVID-19....143
Gender difference....145
THE AUTOIMMUNE/INFLAMMATORY SYNDROME....150

INTRODUCTION

COVID-19 - situation in the world [1]

On **Dec. 31, 2019,** the Wuhan Municipal Health Commission (China) reported to the World Health Organization a cluster of pneumonia cases of unknown etiology in Wuhan city, Hubei province of China.

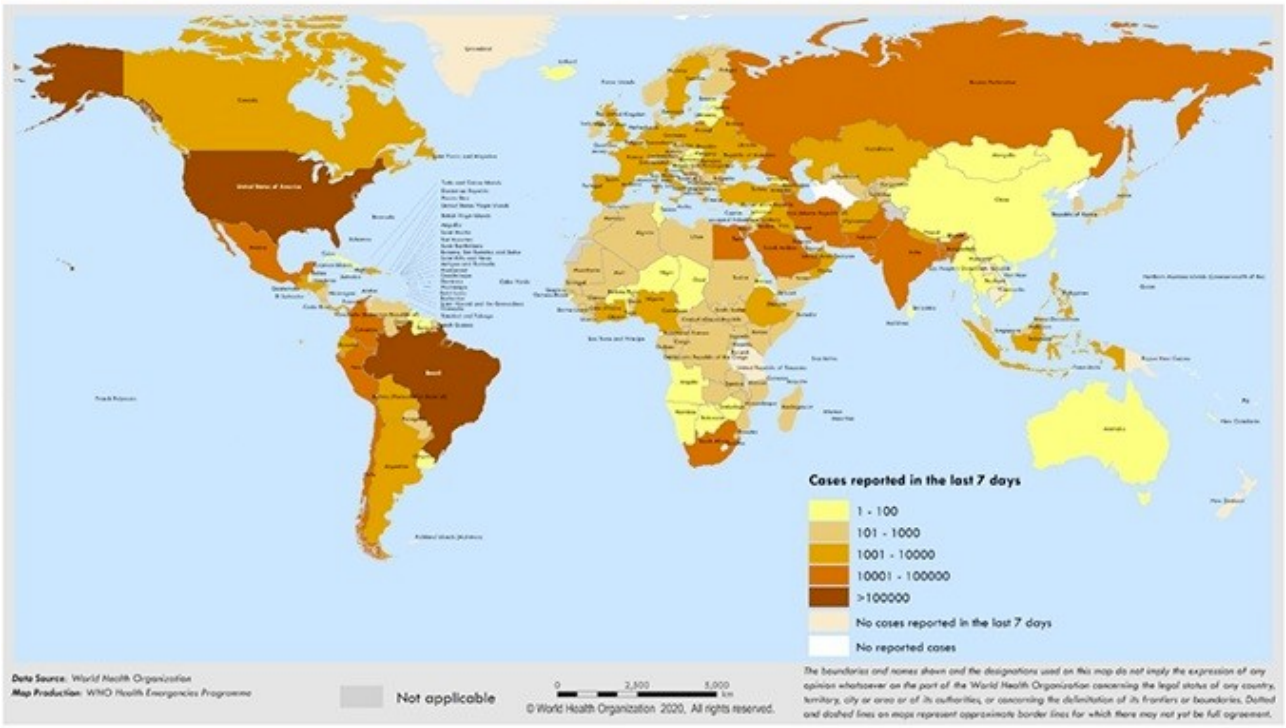
On **January 9, 2020**, the Chinese CDC reported that it had identified a new coronavirus (named by the International Committee on Taxonomy of Viruses SARS-CoV-2: Severe Acute Respiratory Syndrome by Coronavirus 2) as the causative agent of the respiratory disease later named Covid-19 (COronaVIrus Disease 19)

On **January 30**, WHO declared the Coronavirus outbreak in China an **International Public Health Emergency**. WHO elevated the threat for the coronavirus epidemic to "**very high**" level on Feb. 28, 2020.

On **March 11, 2020**, WHO Director-General Tedros Adhanom Ghebreyesus called the spread of Covid-19 no longer an epidemic confined to certain geographical areas, but a **pandemic** spread across the globe.

Surveillance

Figure 1. Number of confirmed COVID-19 cases reported in the last seven days by country, territory or area, 08 June to 14 June**



***See Annex 1 for data, table and figure notes.*

1 https://covid19.who.int/

http://www.salute.gov.it/portale/nuovocoronavirus/dettaglioContenutiNuovoCoronavirus.jsp?lingua=italiano&id=5338&area=nuovoCoronavirus&menu=empty

COVID-19 - Situation in Italy [2]

The first two cases of Coronavirus in Italy, a Chinese tourist couple, were confirmed **Jan. 30** by the Lazzaro Spallanzani Institute in Rome, where they were admitted in isolation from Jan. 29 and declared cured Feb. 26. The first case of secondary transmission occurred in Codogno, a town in Lombardy in the province of Lodi, on **Feb. 18, 2020**.

The Italian government declared a **state of emergency** on **Jan. 31**, allocated the first funds and appointed Civil Protection Chief Angelo Borrelli as extraordinary commissioner for the emergency. A technical-scientific committee to cope with the emergency was established by a decree of the Head of the Civil Protection Department on **February 5, 2020,** which was later expanded by an order dated April 18, 2020.As provided for in Decree Law 18 of 2020, the President of the Council of Ministers by a decree dated **March 18, 2020**, appointed Domenico Arcuri as extraordinary commissioner for the implementation and coordination of the measures needed to contain and combat the Covid-19 epidemiological emergency.

Data **June 14**, 6 p.m. (MINISTRY OF HEALTH) There are 236,989 **total cases** since the start of the pandemic:
26,274 people currently positive176 ,370 cured34 ,345 died.

PCM-DPC dati formiti dal Ministero della Salute

Regione	AGGIORNAMENTO 14/06/2020 ORE 17.00										
	POSITIVI AL nCoV				DIMESSI/ GUARITI	DECEDUTI	CASI TOTALI	INCREMENTO CASI TOTALI (rispetto al giorno precedente)	TAMPONI	CASI TESTATI	Incremento tamponi
	Ricoverati con sintomi	Terapia intensiva	Isolamento domiciliare	Totale attualmente positivi							
Lombardia	2.116	94	13.779	15.989	59.220	16.449	91.658	+244	892.641	530.683	9.336
Piemonte	517	26	2.105	2.648	24.339	4.012	31.059	+30	368.065	234.561	2.842
Emilia Romagna	192	14	1.431	1.637	22.232	4.204	28.073	+17	407.039	241.833	6.137
Veneto	40	1	731	772	16.469	1.978	19.219	+7	812.540	371.726	14.250
Toscana	34	16	449	449	8.569	1.085	10.180	+8	292.101	206.348	2.577
Liguria	85	3	155	243	8.115	1.521	9.879	+4	126.705	68.766	834
Lazio	305	37	980	1.322	5.825	808	7.955	+14	297.615	242.245	2.961
Marche	21	0	605	626	5.139	993	6.758	+4	121.354	73.298	1.046
Campania	53	2	264	319	3.860	430	4.609	+1	240.290	121.618	5.624
Puglia	57	2	359	418	3.565	532	4.515	0	147.249	96.820	1.344
Trento	5	1	60	66	3.917	464	4.447	+1	103.910	55.054	1.772
Sicilia	32	3	802	837	2.341	279	3.457	+1	179.438	150.233	1.119
Friuli V. G.	15	1	87	103	2.850	343	3.269	0	160.824	93.641	1.964
Abruzzo	71	4	436	511	2.312	456	3.279	+4	91.445	61.873	1.105
Bolzano	8	2	85	95	2.227	291	2.613	+3	76.195	36.607	460
Umbria	9	1	10	20	1.339	77	1.436	0	81.976	58.013	821
Sardegna	12	0	21	33	1.198	132	1.363	0	68.769	58.281	690
Valle d'Aosta	7	0	0	7	1.040	144	1.191	0	16.597	12.806	121
Calabria	15	1	28	44	1.021	97	1.162	0	81.981	79.919	726
Molise	0	0	74	74	342	23	439	0	18.845	17.947	350
Basilicata	0	1	10	11	363	27	401	0	35.139	34.349	458
TOTALE	3.594	209	22.471	26.274	176.370	34.345	236.989	+338	4.620.718	2.846.621	56.527

ATTUALMENTE POSITIVI	26.274
TOTALE GUARITI	176.370
TOTALE DECEDUTI	34.345
CASI TOTALI	236.989

2 https://www.epicentro.iss.it/coronavirus/sars-cov-2-sorveglianza-dati

http://www.salute.gov.it/portale/nuovocoronavirus/dettaglioContenutiNuovoCoronavirus.jsp?lingua=italiano&id=5351&area=nuovoCoronavirus&menu=empty

CLINICAL PRESENTATION [3]

Human infection with SARS-CoV-2 leads to a wide range of clinical manifestations ranging from asymptomatic, mild, moderate to severe-fatal. [4]

Some characteristics of deceased patients based on the ISS Report (data June 4, 2020):

Average age [5]

80 years old

Median age

82 years (higher by almost 20 years than that of infected patients whose median age is 62 years)

Sex

men 58.7% women 41.3%

---

[3] Pediatr Allergy Immunol. 2020 May 2. doi: 10.1111/pai.13271.
The first, holistic immunological model of COVID-19: implications for prevention, diagnosis, and public health measures.
Matricardi PM1, Dal Negro RW2, Nisini R3.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/pai.13271

Lancet 2020 Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3.
Clinical Course and Risk Factors for Mortality of Adult Inpatients With COVID-19 in Wuhan, China: A Retrospective Cohort Study
Fei Zhou et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270627/pdf/main.pdf

JAMA 2020 Mar 19;323(16):1612-1614. doi: 10.1001/jama.2020.4326.
Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State.
Matt Arentz 1, Eric Yim 2, Lindy Klaff 2, Sharukh Lokhandwala 2, Francis X Riedo 2, Maria Chong 3, Melissa Lee
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7082763/

N Engl J Med 2020 May 21;382(21):2012-2022. doi: 10.1056/NEJMoa2004500.
Covid-19 in Critically Ill Patients in the Seattle Region - Case Series
Pavan K Bhatraju et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7143164/pdf/NEJMoa2004500.pdf

N Engl J Med 2020 Apr 17;NEJMc2010419. doi: 10.1056/NEJMc2010419.
Clinical Characteristics of Covid-19 in New York City
Parag Goyal et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7182018/pdf/NEJMc2010419.pdf

JAMA 2020 Apr 22;e206775. doi: 10.1001/jama.2020.6775.
Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area
Safiya Richardson et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7177629/

[4]

for consultation
https://www.uptodate.com/contents/coronavirus-disease-2019-covid-19-epidemiology-virology-clinical-features-diagnosis-and-prevention#H3392906512

Internet Book of Critical Care (IBCC) COVID-19
https://emcrit.org/ibcc/covid19/

Coronavirus Disease 2019 (COVID-19) Epidemiology, Pathogenesis, Diagnosis, and Therapeutics
Editors Shailendra K. Saxena 2020 Publisher Springer Singapore DOI 10.1007/978-981-15-4814-7
https://link.springer.com/content/pdf/10.1007%2F978-981-15-4814-7.pdf

Pediatr Allergy Immunol. 2020 May 2. doi: 10.1111/pai.13271. [Epub ahead of print].
The first, holistic immunological model of COVID-19: implications for prevention, diagnosis, and public health measures.
Matricardi PM1, Dal Negro RW2, Nisini R3.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/pai.13271

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance

[5] **Mean**: value of the ratio of the sum of numerical data to the number of data.
Median: central value of numerical data

Prior medical conditions at the time of admission

Patients with 0 pre-existing conditions 4.1%
Patients with 1 pre-existing condition 14.8%
Patients with 2 pre-existing conditions 21.5%
Patients with 3 or more pre-existing conditions 59.7%

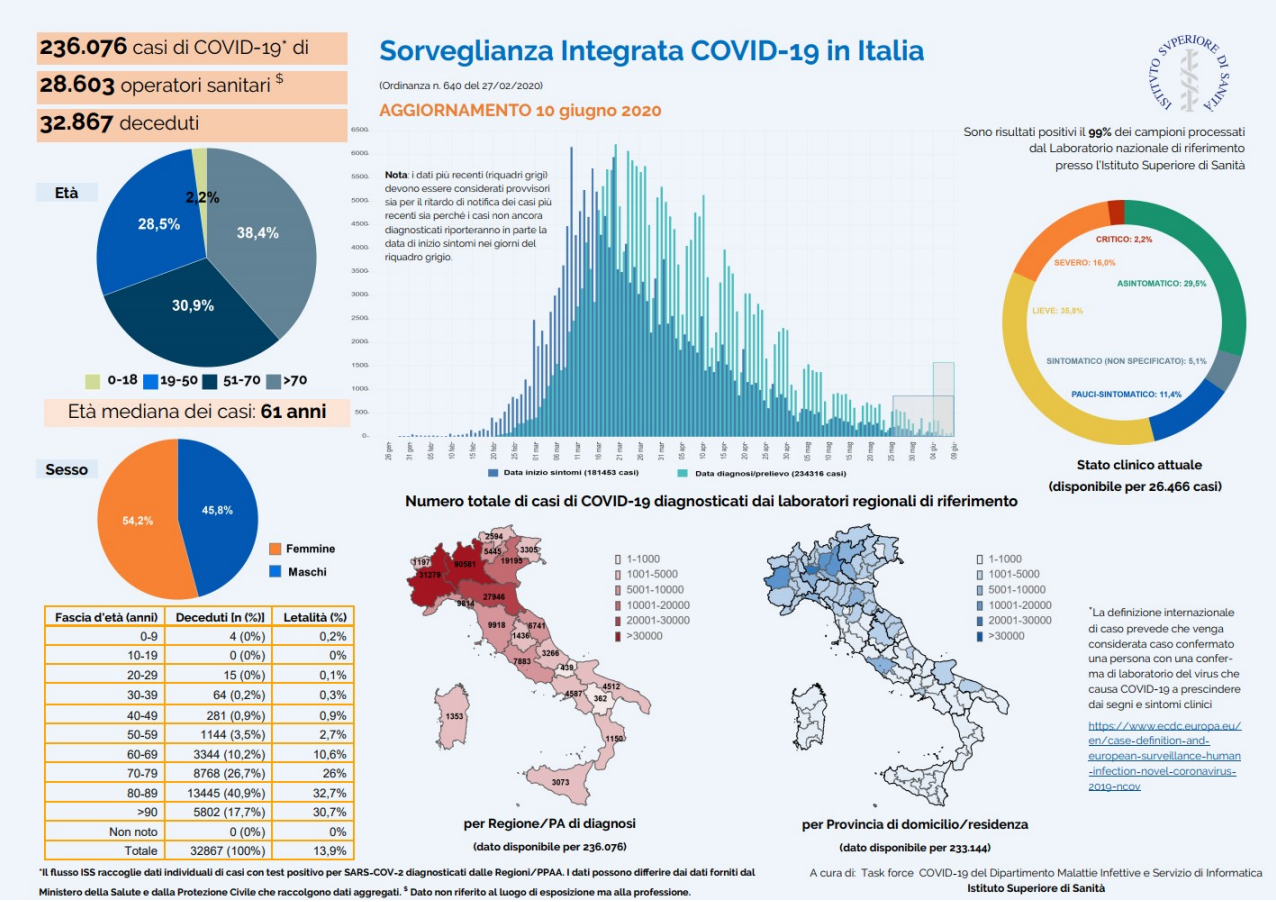
Geographic areas with the highest percentage of fatalities

Lombardy with 49.8 percent
Emilia Romagna with 12.8 percent
Piedmont with 8.5 percent.
Veneto with 6 percent

Symptoms most commonly observed before hospitalization in deceased persons

fever 76%
dyspnea 73%
cough 39%
diarrhea 6%
hemoptysis 1%

Infographic: [6]



https://www.epicentro.iss.it/coronavirus/bollettino/Infografica_10giugno%20ITA.pdf

It should be remembered that the causes of deaths have not been confirmed by autopsy results and virus detection in tissues for most of the deceased, so data on the number of deaths from COVID-19 may not reflect the actual situation. [7]

[6] https://www.epicentro.iss.it/coronavirus/bollettino/Report-COVID-2019_4_giugno.pdf

https://www.epicentro.iss.it/coronavirus/bollettino/Infografica_10giugno%20ITA.pdf

https://www.epicentro.iss.it/coronavirus/sars-cov-2-decessi-italia

[7] DEATHS AND CAUSES OF DEATH: WHAT ISTAT PRODUCES
https://www.istat.it/it/archivio/240401

https://www.epicentro.iss.it/coronavirus/bollettino/Report-COVID-2019_11_giugno.pdf

The following information tables are also given below:

https://www.istat.it/it/files//2020/03/Covid_Indicazioni_scheda_morte.pdf

Dr. Loretta Bolgan Rev_3 15.06.2020

Caratteristiche dei pazienti deceduti positivi all'infezione da SARS-CoV-2 in Italia

Dati al 11 giugno 2020

1. Campione

L'analisi si basa su un campione di 32.938 pazienti deceduti e positivi all'infezione da SARS-CoV-2 in Italia.

Tabella 1. Distribuzione geografica dei decessi

Regione	N.	%
Lombardia	16349	49,6
Emilia Romagna	4192	12,7
Piemonte	2846	8,6
Veneto	1964	6,0
Liguria	1547	4,7
Toscana	1084	3,3
Marche	940	2,9
Lazio	772	2,3
Puglia	530	1,6
Trento	468	1,4
Abruzzo	453	1,4
Campania	365	1,1
Friuli Venezia Giulia	341	1,0
Sicilia	295	0,9
Bolzano	293	0,9
Valle d'Aosta	144	0,4
Sardegna	131	0,4
Calabria	96	0,3
Umbria	76	0,2
Basilicata	29	0,1
Molise	23	0,1

2. Dati demografici

L'età media dei pazienti deceduti e positivi a SARS-CoV-2 è 80 anni (mediana 82, range 0-100, Range InterQuartile - IQR 74-88). Le donne sono 13.692 (41,6%). La figura 1 mostra che l'età mediana dei pazienti deceduti positivi a SARS-CoV-2 è più alta di 20 anni rispetto a quella dei pazienti che hanno contratto l'infezione (età mediane: pazienti deceduti 82 anni – pazienti con infezione 62 anni). La figura 2 mostra il numero dei decessi per fascia di età. Le donne decedute dopo aver contratto infezione da SARS-CoV-2 hanno un'età più alta rispetto agli uomini (età mediane: donne 85 – uomini 79).

Figura 1. Età mediana dei deceduti e diagnosticati positivi all'infezione da SARS-CoV-2

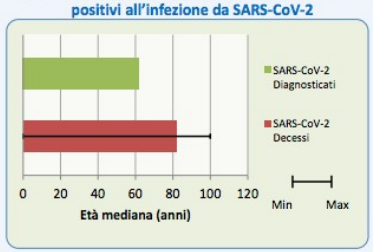
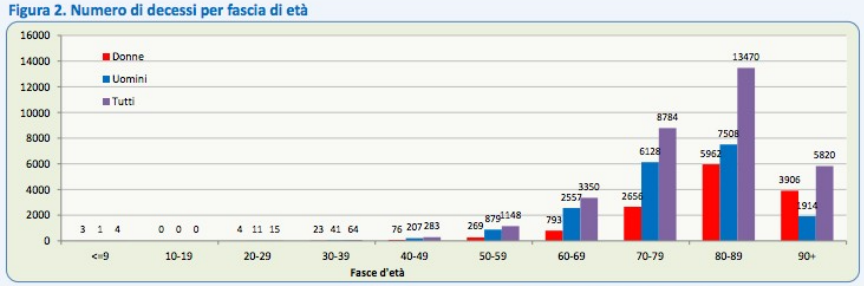


Figura 2. Numero di decessi per fascia di età



3. Patologie preesistenti

La tabella 2 presenta le più comuni patologie croniche preesistenti (diagnosticate prima di contrarre l'infezione da SARS-CoV-2) nei pazienti deceduti. Questo dato è stato ottenuto da 3438 deceduti per i quali è stato possibile analizzare le cartelle cliniche. Il numero medio di patologie osservate in questa popolazione è di 3,3 (mediana 3, Deviazione Standard 1,9). Complessivamente, 144 pazienti (4,2% del campione) presentavano 0 patologie, 505 (14,7%) presentavano 1 patologia, 738 (21,5%) presentavano 2 patologie e 2051 (59,7%) presentavano 3 o più patologie. Prima del ricovero in ospedale, il 23% dei pazienti deceduti SARS-CoV-2 positivi seguiva una terapia con ACE-inibitori e il 16% una terapia con Sartani (bloccanti del recettore per l'angiotensina). Nelle donne (n=1137) il numero medio di patologie osservate è di 3,3 (mediana 3, Deviazione Standard 1,9); negli uomini (n=2301) il numero medio di patologie osservate è di 3,2 (mediana 3, Deviazione Standard 2,0).

Tabella 2. Patologie preesistenti osservate più frequentemente

Patologie	Donne		Uomini		Totale	
	N.	%	N.	%	N.	%
Cardiopatia ischemica	236	20,8	721	31,3	957	27,8
Fibrillazione atriale	260	22,9	498	21,6	758	22,0
Scompenso cardiaco	209	17,8	330	14,1	539	15,7
Ictus	118	10,4	231	10,0	349	10,2
Ipertensione arteriosa	774	68,1	1531	66,5	2305	67,0
Diabete mellito-Tipo 2	322	28,3	718	31,2	1040	30,3
Demenza	266	23,4	296	12,9	562	16,3
BPCO	143	12,6	433	18,8	576	16,8
Cancro attivo negli ultimi 5 anni	185	16,3	366	15,9	551	16,0
Epatopatia cronica	37	3,3	111	4,8	148	4,3
Insufficienza renale cronica	200	17,6	488	21,2	688	20,0
Dialisi	19	1,7	48	2,1	67	1,9
Insufficienza respiratoria	61	5,4	119	5,2	180	5,2
HIV	0	0,0	7	0,3	7	0,2
Malattie autoimmuni	67	5,9	70	3,0	137	4,0
Obesità	127	11,2	250	10,9	377	11,0
Numero di patologie	**N.**	**%**	**N.**	**%**	**N.**	**%**
0 patologie	33	2,9	111	4,8	144	4,2
1 patologia	161	14,2	344	15,0	505	14,7
2 patologie	250	22,0	488	21,2	738	21,5
3 o più patologie	693	60,9	1358	59,0	2051	59,7

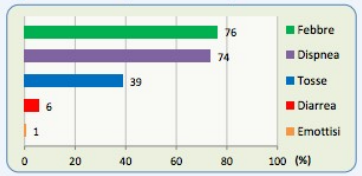
4. Diagnosi di ricovero

Nel 92,4% delle diagnosi di ricovero erano menzionate condizioni (per esempio polmonite, insufficienza respiratoria) o sintomi (per esempio, febbre, dispnea, tosse) compatibili con COVID-19. In 241 casi (7,6% dei casi) la diagnosi di ricovero non era da correlarsi all'infezione. In 38 casi la diagnosi di ricovero riguardava esclusivamente patologie neoplastiche, in 88 casi patologie cardiovascolari (per esempio infarto miocardico acuto, scompenso cardiaco, ictus), in 31 casi patologie gastrointestinali (per esempio colecistite, perforazione intestinale, occlusione intestinale, cirrosi), in 84 casi altre patologie.

5. Sintomi

La figura 3 mostra i sintomi più comunemente osservati prima del ricovero nei pazienti deceduti positivi all'infezione da SARS-CoV-2. Febbre dispnea e tosse rappresentano i sintomi più comuni. Meno frequenti sono diarrea e emottisi. Il 5,7% delle persone non presentava alcun sintomo al momento del ricovero.

Figura 3. Sintomi più comuni nei pazienti deceduti



6. Complicanze

L'insufficienza respiratoria è stata la complicanza più comunemente osservata in questo campione (96,9% dei casi), seguita da danno renale acuto (22,1%), sovrainfezione (13,0%) e danno miocardico acuto (11,0%).

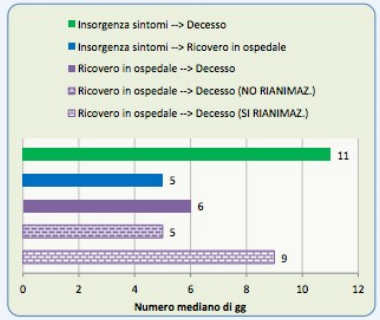
7. Terapie

La terapia antibiotica è stata comunemente utilizzata nel corso del ricovero (86% dei casi), meno usata quella antivirale (60%), più raramente la terapia steroidea (38%). Il comune utilizzo di terapia antibiotica può essere spiegato dalla presenza di sovrainfezioni o è compatibile con inizio terapia empirica in pazienti con polmonite, in attesa di conferma laboratoristica di COVID-19. In 793 casi (23,4%) sono state utilizzate tutte e tre le terapie. Al 3,9% dei pazienti deceduti positivi all'infezione da SARS-CoV-2 è stato somministrato Tocilizumab.

8. Tempi

La figura 4 mostra i tempi mediani (in giorni) che trascorrono dall'insorgenza dei sintomi al decesso (11 giorni), dall'insorgenza dei sintomi al ricovero in ospedale (5 giorni) e dal ricovero in ospedale al decesso (6 giorni). Il tempo intercorso dal ricovero in ospedale al decesso è di 4 giorni più lungo in coloro che sono stati trasferiti in rianimazione rispetto a quelli che non sono stati trasferiti (9 giorni contro 5 giorni).

Figura 4. Tempi mediani di ricovero (in giorni) nei pazienti deceduti positivi all'infezione da SARS-CoV-2



9. Decessi di età inferiore ai 50 anni

All'11 giugno sono 366 dei 32.938 (1,1%) pazienti deceduti SARS-CoV-2 positivi di età inferiore ai 50 anni. In particolare, 83 di questi avevano meno di 40 anni (53 uomini e 30 donne con età compresa tra i 0 e i 39 anni. Di 7 pazienti di età inferiore ai 40 anni non sono disponibili informazioni cliniche; degli altri pazienti, 62 presentavano gravi patologie preesistenti (patologie cardiovascolari, renali, psichiatriche, diabete, obesità) e 14 non avevano diagnosticate patologie di rilievo.

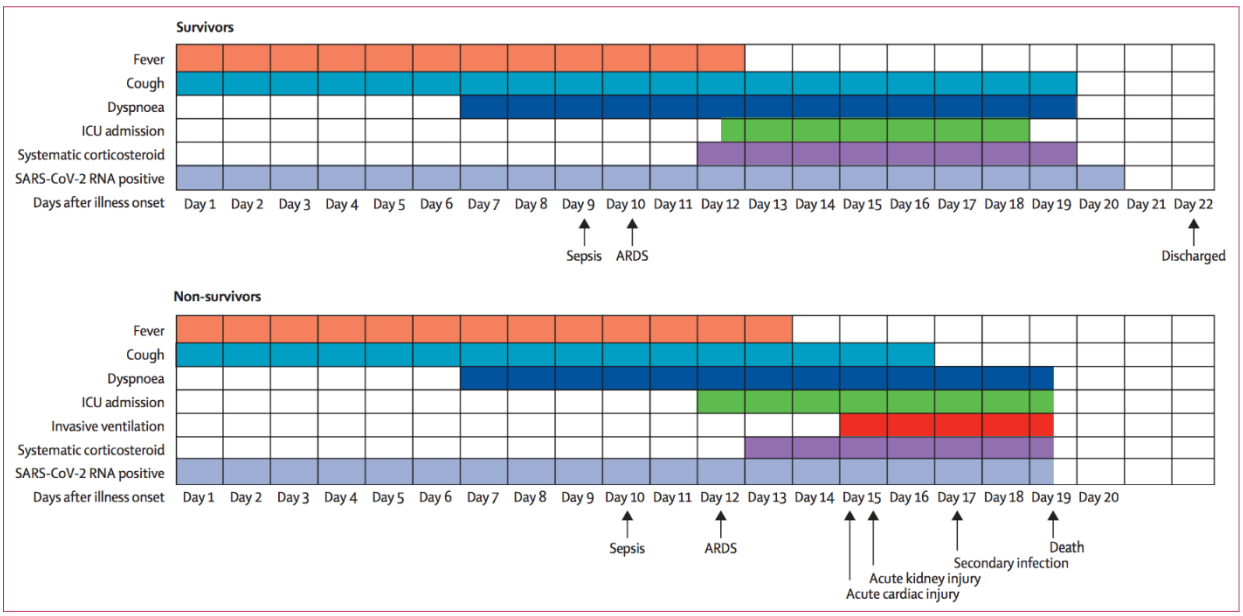
Questo report è stato prodotto dai membri del Gruppo della Sorveglianza COVID-19
Luigi Palmieri, Xanthi Andrianou, Pierfrancesco Barbariol, Antonino Bella, Stefania Bellino, Eva Benelli, Luigi Bertinato, Stefano Boros, Gianfranco Brambilla, Giovanni Calcagnini, Marco Canevelli, Maria Rita Castrucci, Federica Censi, Alessandra Ciervo, Elisa Colaizzo, Fortunato D'Ancona, Martina Del Manso, Chiara Donfrancesco, Massimo Fabiani, Francesco Facchiano, Antonietta Filia, Marco Floridia, Fabio Galati, Marina Giuliano, Tiziana Grisetti, Yllka Kodra; Martin Langer, Ilaria Lega, Cinzia Lo Noce, Pietro Maiozzi, Fiorella Malchiodi Albedi, Valerio Manno, Margherita Martini, Alberto Mateo Urdiales, Eugenio Mattei, Claudia Meduri, Paola Meli, Giada Minelli, Manuela Nebuloni, Lorenza Nisticò, Marino Nonis, Graziano Onder, Lucia Palmisano, Nicola Petrosillo, Patrizio Pezzotti, Flavia Pricci, Ornella Punzo, Vincenzo Puro, Valeria Raparelli, Giovanni Rezza, Flavia Riccardo, Maria Cristina Rota, Paolo Salerno, Debora Serra, Andrea Siddu, Paola Stefanelli, Manuela Tamburo De Bella, Dorina Tiple, Brigid Unim, Luana Vaianella, Nicola Vanacore, Monica Vichi, Emanuele Rocco Villani, Amerigo Zona, Silvio Brusaferro.

Table 1. **Clinical Characteristics of the Study Patients, According to Disease Severity and the Presence or Absence of the Primary Composite End Point.***

Characteristic	All Patients (N=1099)	Disease Severity		Presence of Primary Composite End Point†	
		Nonsevere (N=926)	Severe (N=173)	Yes (N=67)	No (N=1032)
Age					
Median (IQR) — yr	47.0 (35.0–58.0)	45.0 (34.0–57.0)	52.0 (40.0–65.0)	63.0 (53.0–71.0)	46.0 (35.0–57.0)
Distribution — no./total no. (%)					
0–14 yr	9/1011 (0.9)	8/848 (0.9)	1/163 (0.6)	0	9/946 (1.0)
15–49 yr	557/1011 (55.1)	490/848 (57.8)	67/163 (41.1)	12/65 (18.5)	545/946 (57.6)
50–64 yr	292/1011 (28.9)	241/848 (28.4)	51/163 (31.3)	21/65 (32.3)	271/946 (28.6)
≥65 yr	153/1011 (15.1)	109/848 (12.9)	44/163 (27.0)	32/65 (49.2)	121/946 (12.8)
Female sex — no./total no. (%)	459/1096 (41.9)	386/923 (41.8)	73/173 (42.2)	22/67 (32.8)	437/1029 (42.5)
Smoking history — no./total no. (%)					
Never smoked	927/1085 (85.4)	793/913 (86.9)	134/172 (77.9)	44/66 (66.7)	883/1019 (86.7)
Former smoker	21/1085 (1.9)	12/913 (1.3)	9/172 (5.2)	5/66 (7.6)	16/1019 (1.6)
Current smoker	137/1085 (12.6)	108/913 (11.8)	29/172 (16.9)	17/66 (25.8)	120/1019 (11.8)
Exposure to source of transmission within past 14 days — no./total no.					
Living in Wuhan	483/1099 (43.9)	400/926 (43.2)	83/173 (48.0)	39/67 (58.2)	444/1032 (43.0)
Contact with wildlife	13/687 (1.9)	10/559 (1.8)	3/128 (2.3)	1/41 (2.4)	12/646 (1.9)
Recently visited Wuhan‡	193/616 (31.3)	166/526 (31.6)	27/90 (30.0)	10/28 (35.7)	183/588 (31.1)
Had contact with Wuhan residents‡	442/611 (72.3)	376/522 (72.0)	66/89 (74.2)	19/28 (67.9)	423/583 (72.6)
Median incubation period (IQR) — days§	4.0 (2.0–7.0)	4.0 (2.8–7.0)	4.0 (2.0–7.0)	4.0 (1.0–7.5)	4.0 (2.0–7.0)
Fever on admission					
Patients — no./total no. (%)	473/1081 (43.8)	391/910 (43.0)	82/171 (48.0)	24/66 (36.4)	449/1015 (44.2)
Median temperature (IQR) — °C	37.3 (36.7–38.0)	37.3 (36.7–38.0)	37.4 (36.7–38.1)	36.8 (36.3–37.8)	37.3 (36.7–38.0)
Distribution of temperature — no./total no. (%)					
<37.5°C	608/1081 (56.2)	519/910 (57.0)	89/171 (52.0)	42/66 (63.6)	566/1015 (55.8)
37.5–38.0°C	238/1081 (22.0)	201/910 (22.1)	37/171 (21.6)	10/66 (15.2)	228/1015 (22.5)
38.1–39.0°C	197/1081 (18.2)	160/910 (17.6)	37/171 (21.6)	11/66 (16.7)	186/1015 (18.3)
>39.0°C	38/1081 (3.5)	30/910 (3.3)	8/171 (4.7)	3/66 (4.5)	35/1015 (3.4)
Fever during hospitalization					
Patients — no./total no. (%)	975/1099 (88.7)	816/926 (88.1)	159/173 (91.9)	59/67 (88.1)	916/1032 (88.8)
Median highest temperature (IQR) — °C	38.3 (37.8–38.9)	38.3 (37.8–38.9)	38.5 (38.0–39.0)	38.5 (38.0–39.0)	38.3 (37.8–38.9)
<37.5°C	92/926 (9.9)	79/774 (10.2)	13/152 (8.6)	3/54 (5.6)	89/872 (10.2)
37.5–38.0°C	286/926 (30.9)	251/774 (32.4)	35/152 (23.0)	20/54 (37.0)	266/872 (30.5)
38.1–39.0°C	434/926 (46.9)	356/774 (46.0)	78/152 (51.3)	21/54 (38.9)	413/872 (47.4)
>39.0°C	114/926 (12.3)	88/774 (11.4)	26/152 (17.1)	10/54 (18.5)	104/872 (11.9)
Symptoms — no. (%)					
Conjunctival congestion	9 (0.8)	5 (0.5)	4 (2.3)	0	9 (0.9)
Nasal congestion	53 (4.8)	47 (5.1)	6 (3.5)	2 (3.0)	51 (4.9)
Headache	150 (13.6)	124 (13.4)	26 (15.0)	8 (11.9)	142 (13.8)
Cough	745 (67.8)	623 (67.3)	122 (70.5)	46 (68.7)	699 (67.7)
Sore throat	153 (13.9)	130 (14.0)	23 (13.3)	6 (9.0)	147 (14.2)
Sputum production	370 (33.7)	309 (33.4)	61 (35.3)	20 (29.9)	350 (33.9)
Fatigue	419 (38.1)	350 (37.8)	69 (39.9)	22 (32.8)	397 (38.5)
Hemoptysis	10 (0.9)	6 (0.6)	4 (2.3)	2 (3.0)	8 (0.8)
Shortness of breath	205 (18.7)	140 (15.1)	65 (37.6)	36 (53.7)	169 (16.4)
Nausea or vomiting	55 (5.0)	43 (4.6)	12 (6.9)	3 (4.5)	52 (5.0)
Diarrhea	42 (3.8)	32 (3.5)	10 (5.8)	4 (6.0)	38 (3.7)
Myalgia or arthralgia	164 (14.9)	134 (14.5)	30 (17.3)	6 (9.0)	158 (15.3)
Chills	126 (11.5)	100 (10.8)	26 (15.0)	8 (11.9)	118 (11.4)
Signs of infection — no. (%)					
Throat congestion	19 (1.7)	17 (1.8)	2 (1.2)	0	19 (1.8)
Tonsil swelling	23 (2.1)	17 (1.8)	6 (3.5)	1 (1.5)	22 (2.1)
Enlargement of lymph nodes	2 (0.2)	1 (0.1)	1 (0.6)	1 (1.5)	1 (0.1)
Rash	2 (0.2)	0	2 (1.2)	0	2 (0.2)
Coexisting disorder — no. (%)					
Any	261 (23.7)	194 (21.0)	67 (38.7)	39 (58.2)	222 (21.5)
Chronic obstructive pulmonary disease	12 (1.1)	6 (0.6)	6 (3.5)	7 (10.4)	5 (0.5)
Diabetes	81 (7.4)	53 (5.7)	28 (16.2)	18 (26.9)	63 (6.1)
Hypertension	165 (15.0)	124 (13.4)	41 (23.7)	24 (35.8)	141 (13.7)
Coronary heart disease	27 (2.5)	17 (1.8)	10 (5.8)	6 (9.0)	21 (2.0)
Cerebrovascular disease	15 (1.4)	11 (1.2)	4 (2.3)	4 (6.0)	11 (1.1)
Hepatitis B infection¶	23 (2.1)	22 (2.4)	1 (0.6)	1 (1.5)	22 (2.1)
Cancer‖	10 (0.9)	7 (0.8)	3 (1.7)	1 (1.5)	9 (0.9)
Chronic renal disease	8 (0.7)	5 (0.5)	3 (1.7)	2 (3.0)	6 (0.6)
Immunodeficiency	2 (0.2)	2 (0.2)	0	0	2 (0.2)

* The denominators of patients who were included in the analysis are provided if they differed from the overall numbers in the group. Percentages may not total 100 because of rounding. Covid-19 denotes coronavirus disease 2019, and IQR interquartile range.
† The primary composite end point was admission to an intensive care unit, the use of mechanical ventilation, or death.
‡ These patients were not residents of Wuhan.
§ Data regarding the incubation period were missing for 808 patients (73.5%).
¶ The presence of hepatitis B infection was defined as a positive result on testing for hepatitis B surface antigen with or without elevated levels of alanine or aspartate aminotransferase.
‖ Included in this category is any type of cancer.

Table taken from: https://www.nejm.org/doi/full/10.1056/NEJMoa2002032[8].
Clinical characteristics of study patients, based on disease severity and the presence or absence of the primary composite end-point



Taken from https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30566-3.pdf[9]
Clinical courses of major symptoms, outcomes and duration of viral spread from disease onset in patients hospitalized with COVID-19
The figure shows the median duration of symptoms and the occurrence of complications and outcomes. ICU = intensive care unit. SARS-CoV-2 = severe acute respiratory syndrome due to coronavirus 2. ARDS = acute respiratory distress syndrome. COVID-19 = coronavirus disease 2019.

For an in-depth discussion of the clinical presentation of COVID-19, we suggest reading the paper prepared by **Dr. Samuele Ceruti** (Hospital Physician - Intensive Care Medicine Service)

https://drive.google.com/file/d/1HP5AGTB-HIbdL-T7ob33BDl8giAJPIY0/view

[8] N Engl J Med. 2020 Apr 30;382(18):1708-1720. doi: 10.1056/NEJMoa2002032.
Clinical Characteristics of Coronavirus Disease 2019 in China.
Guan WJ et al

[9] Lancet 2020 Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3.
Clinical Course and Risk Factors for Mortality of Adult Inpatients With COVID-19 in Wuhan, China: A Retrospective Cohort Study
Fei Zhou et al

The criticality of autopsies

Without going into detail about the validity of the epidemiological data presented by ISS on COVID-19, as they will be discussed in the chapter on SARS-Cov-2 transmission and infection, it should be emphasized for the purposes of the review that will be presented in this chapter that, despite the impressive scientific work worldwide to study the genetic characteristics of the virus the mechanism of the disease and its therapies, there is **still a lack of essential clinical and laboratory data** to be able to establish the true number of those who have died from COVID-19 and those infected with SARS- Cov-2, just as it is not yet possible to define the true impact of lockdown on the containment of the epidemic.
In particular, it is well known that **autopsy results have been very poor** and that only recently (late April-May 2020, when more than 25,000 people had already died in Italy [10]) were the first results of post-mortem histopathological analysis of RT-PCR-positive patients for SARS-Cov-2 [11] . **These results showed a more complex clinical picture** than that of respiratory distress syndrome, believed to be the main cause of deaths, complicated by disseminated thromboembolism [12].
This allowed clinicians to quickly change the adopted therapy with a major positive impact on the mortality of the most critically ill patients. [13]

---

[10] https://covid19.healthdata.org/italy

[11] J Clin Med. 2020 Apr 26;9(5). pii: E1259. doi: 10.3390/jcm9051259.
COVID-19 Deaths: Are We Sure It Is Pneumonia? Please, Autopsy, Autopsy, Autopsy!
Pomara C1.2, Li Volti G3, Hat F4.5.
https://www.mdpi.com/2077-0383/9/5/1259/htm

[12] Ann Intern Med. 2020 May 6. doi: 10.7326/M20-2003.
Autopsy Findings and Venous Thromboembolism in Patients With COVID-19: A Prospective Cohort Study.
Wichmann D1 et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7240772/pdf/aim-olf-M202003.pdf

Am J Respir Crit Care Med. 2020 May 15;201(10):1299-1300. doi: 10.1164/rccm.202003-0817LE.
COVID-19 Does Not Lead to a "Typical" Acute Respiratory Distress Syndrome.
Gattinoni L1, Coppola S2, Cressoni M3, Busana M1, Rossi S4, Chiumello D2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7233352/pdf/rccm.202003-0817LE.pdf

J Thromb Thrombolysis. 2020;1-14. doi:10.1007/s11239-020-02134-3
COVID-19 update: Covid-19-associated coagulopathy.
Becker RC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7225095/

Acad Radiol. 2020;27(6):900. doi:10.1016/j.acra.2020.04.010
COVID-19 Pulmonary Involvement: Is Really an Interstitial Pneumonia?
Boraschi P.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7158787/pdf/main.pdf

Wichmann D, Sperhake JP, Lütgehetmann M, et al.
Autopsy Findings and Venous Thromboembolism in Patients With COVID-19
Ann Intern Med. 2020;M20-2003. doi:10.7326/M20-2003
https://www.acpjournals.org/doi/10.7326/M20-2003

https://www.medicinenet.com/endotracheal_intubation/article.htm

https://www.medpagetoday.com/infectiousdisease/covid19/86535

[13] JAMA. 2020 Apr 6. doi: 10.1001/jama.2020.5394. [Epub ahead of print].
Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy.
Grasselli G et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7136855/

Ann Intensive Care. 2020 Mar 30;10(1):37. doi: 10.1186/s13613-020-00653-z.
The experience of high-fow nasal cannula in hospitalized patients with 2019 novel coronavirus-infected pneumonia in two hospitals of Chongqing, China
Wang K1, Zhao W2, Li J3, Shu W4, Duan J5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7104710/pdf/13613_2020_Article_653.pdf

Monaldi Arch Chest Dis. 2020 May 5;90(2). doi: 10.4081/monaldi.2020.1342.
Management of COVID-19: the risks associated with treatment are clear, but the benefits remain uncertain.
Zareifopoulos N1, Lagadinou M2, Karela A3, Platanaki C4, Karantzogiannis G5, Velissaris D6.
https://www.monaldi-archives.org/index.php/macd/article/download/1342/1021

The study of Covid-19 is also complicated by the fact that to date, only research on nonhuman primate animal models carried out with the aim of assessing the causal link between the presence of the virus and the occurrence of COVID-19 is available, by exposure to the virus and histopathological analysis of damaged tissues [14].

In those studies, however, while confirming the contagiousness of SARS-Cov-2 and its ability to induce COVID-19-like pathology, there was no evidence of disseminated thromboembolism, evidence that makes comparison with the potentially fatal complication of the disease in humans more difficult.

We do not yet have biochemical indices that could be used as biomarkers of SARS- CoV-2 infection correlated with the functions of the affected organs, and therefore **quantifying the actual number of deaths caused only by SARS-Cov-2 and not in the presence of SARS-Cov-2 is still under discussion**. [15]

Kock's and Evans' postulates can be considered the essential criteria to be used to establish the cause-and-effect relationship linking a microorganism to a disease. [16]

[14] Science. 2020 Apr 17. pii: eabb7314. doi: 10.1126/science.abb7314.
Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. Rockx B1 et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164679/pdf/abb7314.pdf

bioRxiv 2020.04.08.031807; doi: https://doi.org/10.1101/2020.04.08.031807
Comparison of SARS-CoV-2 infections among 3 species of non-human primates
Shuaiyao Lu et al
https://www.biorxiv.org/content/10.1101/2020.04.08.031807v1.full.pdf

[15] https://www.primocanale.it/notizie/polemica-sui-numeri-del-coronavirus-bonsignore-non-ci-sono-pi-morti-per-altre-patologie--218746.html

http://www.deplazio.net/images/stories/SISMG/SISMG_COVID19.pdf

https://www.epicentro.iss.it/coronavirus/pdf/Rapporto_Istat_ISS.pdf

COVID-19 pandemic? The tests
By Fabio Franchi1 (Version 1, April 3, 2020,)
http://www.sspp.it/wp-content/uploads/2020/04/COVID-colpevole-senza-regolare-processo-ff_.pdf

[16] http://www.quadernodiepidemiologia.it/epi/cause/pos_hk.htm
http://www.quadernodiepidemiologia.it/epi/cause/pos_eva.htm

To date, the conditions of virus isolation, its contagiousness, and the causal link with COVID-19 are still being discussed and investigated.

For further study, reading **Dr. Fabio Franchi**'s paper **Covid-19: Coronavirus guilty without due process is** suggested

https://www.dietrolospecchio.it/covid-19-coronavirus-colpevole-senza-regolare-processo

The delay in conducting autopsies has been related to the recommendation of the Italian authorities in charge of pandemic management to limit their performance [17], a procedure instead provided for the confirmation of COVID-19 deaths by WHO in its guideline "*Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases*" (tab.1,2) [18], and that this did not allow for immediate clarification of the causes of the deaths and consequently the causal link between the presence of the virus and the disease, the correct therapeutic approaches and containment of the spread of the infection, with very heavy repercussions on both patients and the entire population. [19]

Although this ministerial circular certainly had a negative impact on the urgent information to be acquired about the disease, however, it should be considered that the recommendations contained therein are precautionary in nature and were, and still are, intended to protect health care workers assigned to conduct autopsies from infection.

[17] Virchows Arch 2020 Apr 29;1-3. doi: 10.1007/s00428-020-02828-2.
The Autopsy Debate During the COVID-19 Emergency: The Italian Experience
Anna Sapino 1 2, Fabio Facchetti 3, Emanuela Bonoldi 4, Andrea Gianatti 5, Mattia Barbareschi 6,
Italian Society of Pathological Anatomy and Cytology - SIAPEC
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190281/pdf/428_2020_Article_2828.pdf

https://www.affaritaliani.it/blog/cose-nostre/covid-19-le-autopsie-non-vanno-fatte-ordine-del-ministero-della-salute-671347.html

https://www.affaritaliani.it/static/upl2020/covi/0001/covid-19--circolare-del-ministero-della-salutepdf2.pdf

[18] Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases. Interim guidance March 2, 2020
https://apps.who.int/iris/bitstream/handle/10665/331329/WHO-COVID-19-laboratory-2020.4-eng.pdf?sequence=1&isAllowed=y

[19] https://www.affaritaliani.it/static/upl2020/comu/comunicato-stampa--2-.pdf

Indeed, Sars-Cov-2 is classified as a **class 3 infectious agent** and as such currently requires a very stringent **biosafety level 3**, [20] for which most hospital and research facilities are neither equipped nor licensed.

The Biohazard

Definitions

Legislative Decree 626/94 gives the following definitions:

• Biological agent: "any microorganism even if genetically modified, cell culture and human endoparasite that could cause infection, allergy or intoxication."

• Microorganism: "any microbiological entity, cellular or otherwise, capable of reproducing or transferring genetic material."

• Cell culture "the result of in vitro growth of cells derived from multicellular organisms." Genetically modified microorganism (GMM): "organism whose genetic material has been modified in the laboratory in a way that does not occur in nature by natural crossing and/or recombination."

Classification of biological agents

The assessment of risk from biological agents must begin with a proper identification of the microorganisms to which the worker may be exposed. The risk of developing an infection following exposure to a biological agent is, in fact, related to the following variables:

• infectivity, ability of a microorganism to penetrate and multiply in the host;
• Pathogenicity, ability to produce disease following infection;
• transmissibility, ability of a microorganism to be transmitted from an infected subject to a susceptible subject;
• neutralizability, availability of effective prophylactic measures, to prevent the disease, or therapeutic measures for treatment;
• Virulence, the set of characteristics of infectivity and pathogenicity.

On the basis of all these factors, a classification of biological agents into 4 risk groups was proposed, which Legislative Decree 626/94, with the exclusion of Group 1, reported in Article 75 and Annex XI.

• **Group 1** microorganisms are generally nonpathogenic and have little likelihood of causing disease in humans, so they pose a low risk both individually and collectively; all agents not reported in groups 2, 3 and 4 are considered in this group by exclusion; generally these microorganisms constitute the *normal microbial flora.*

• **Group 2** microorganisms can cause disease following exposure, so they pose a moderate individual risk. Effective prophylactic and therapeutic measures are usually available for these, so they pose a limited risk to the community. Microorganisms in this group include both agents that are transmitted by the fecal-oral route, such as *Vibrio cholerae, Salmonella, and hepatitis A virus*, and agents with aerogenic transmission (these are eliminated by coughing, phonation, sneezing, and enter the body by the respiratory route), such as *influenza viruses and measles virus.*

[20] Anthony F. Henwood
Coronavirus disinfection in histopathology,
Journal of Histotechnology, (2020) DOI: 10.1080/01478885.2020.1734718
https://www.siapec.it/public/uploads/eventi/Histopathology%20disinfection.pdf

Barbareschi M, Ascoli V, Bonoldi E, et al.
Biosafety in surgical pathology in the era of SARS-Cov2 pandemic. A statement of the Italian Society of Surgical Pathology and Cytology.
Pathologica 10.32074/1591-951X-14-20 [published online ahead of print, 2020 Apr 1] https://www.pathologica.it/article/view/103/141

https://www.microbiologiaitalia.it/didattica/livelli-di-biosicurezza/

http://www.salute.gov.it/portale/temi/documenti/usmaf/formazione2018/Classificazione_degli_agenti_infettivi_in_relazione_alla_sicurezza_biologica.pdf

https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html

https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847_2

• **Group 3** microorganisms can cause serious illness and pose a high individual risk; these can spread in the community, but effective prophylactic and therapeutic measures are usually available so they pose a low collective risk. Members of this group include microorganisms that recognize multiple routes of infection (respiratory, digestive, and direct contact), e.g., *Salmonella typhi, Hepatitis B Virus, AIDS Virus.*

• **Group 4** microorganisms cause serious diseases and pose a high individual risk, these spread easily and rapidly in the community, no prophylactic and therapeutic measures are available so they pose a high collective risk. An example is given by the *Ebola Virus.*

In risk assessment, however, one must consider not only the type of microorganism to which one may be exposed but also the manner in which the biological agent is handled, that is, the procedures adopted, and the quantities to which the worker is exposed during the work activity.

http://www.aslcn2.it/media/2013/07/Informazioni-testo-unico-rischio-biologico.pdf

CORRISPONDENZA TRA I GRUPPI DI RISCHIO E I LIVELLI DI BIOSICUREZZA DEI LABORATORI

Gruppo	Livello di Biosicurezza	Tipo di Laboratorio	Pratiche	Attrezzature
1	Base Livello 1	Insegnamento di base, ricerca	Buona pratica di laboratorio	Nessuna, banco da lavoro
2	Base Livello 2	Diagnostica di base, ricerca	Buona pratica di laboratorio più Dispositivi di protezione Individuali (DPI) e segnale di pericolo	Banco da lavoro più Cappe di sicurezza per le procedure che producono aerosol
3	Contenimento Livello 3	Diagnostica specialistica, ricerca	Come Livello 2 più DPI speciali, accesso controllato, ventilazione senza ricircolo	Cappe di sicurezza per tutte le procedure
4	Massimo contenimento Livello 4	Patogeni pericolosi	Come Livello 3 più ingresso autorizzato, doccia di decontaminazione, adeguato sistema di smaltimento dei materiali monouso come rifiuti	Cappe di sicurezza di classe III (glove-box) o Tute pressurizzate con Cappe di classe II, più autoclave passante e sistema di ventilazione con filtri assoluti

WHO Laboratory biosafety manual, 2004, III edition

This major limitation is compounded by an inexplicable **delay in performing genetic analysis of the virus by sequencing** on a large number of samples, which would have made it possible to define the accuracy of the test in RT-PCR, the dynamics of virus evolution (mutation rate), and, if performed on autopsy tissues (a study not yet carried out to date!), to define the characteristics of the disease in a much more informative way.

Thus, the impact on mortality due to, on the one hand, the limited data on autopsies and the impossibility of performing them after the fact due to the provision to proceed with cremation of most of the deceased, and, on the other hand, the change in drug therapy as a result of improved diagnosis, still remains to be investigated and quantified.

Were the pathological features of COVID-19 already known?

Although a new name (COVID-19) has been coined for the disease caused by SARS-Cov-2, it was actually already known from the characteristics of the diseases associated with SARS-Cov-1 infection and human coronaviruses [21] that the mechanism leading to the even fatal complication is an **autoimmune/inflammatory reaction** with the clinical features of ***cytokine (or cytokine release) storm syndrome*** **and in its subtypes** (macrophage activation syndrome - MAS [22] and hemophagocytic lymphohistiocytosis - HLS [23]) .

In this pathology, **the excessive inflammatory stimulus due to the cytokine storm leads to multi-organ damage** (lungs, intestines, brain, heart, kidneys, ect.), which is the final stage of the pathological process induced by the immune system itself against the structures of the body. [24] Further investigation of the immunopathology of COVID-19 is the subject of this chapter.

---

[21] Semin Immunopathol. 2017 Jul;39(5):529-539. doi: 10.1007/s00281-017-0629-x
Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology.
Channappanavar R1, Perlman S2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7079893/pdf/281_2017_Article_629.pdf.

Respirology. 2006 Nov;11(6):715-22.
Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome.
Chien JY1, Hsueh PR, Cheng WC, Yu CJ, Yang PC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7192207/pdf/RESP-11-715.pdf

Aging (Albany NY). 2020 May 2;12. doi: 10.18632/aging.103101.
Immune environment modulation in pneumonia patients caused by coronavirus: SARS-CoV, MERS-CoV and SARS-CoV-2.
Yao Z1, Zheng Z1, Wu K1, Junhua Z1.
https://paperchase-aging.s3-us-west-1.amazonaws.com/pdf/vgbdsA4AtLiNjLZgc.pdf

[22] Autoimmun Rev. 2020 Apr 3:102537. doi: 10.1016/j.autrev.2020.102537.
The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease.
McGonagle D1, Sharif K2, O'Regan A3, Bridgewood C4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195002/pdf/main.pdf

[23] Lancet. 2020 Mar 28;395(10229):1033-1034. doi: 10.1016/S0140-6736(20)30628-0.
COVID-19: consider cytokine storm syndromes and immunosuppression.
Mehta P1, McAuley DF2, Brown M3, Sanchez E4, Tattersall RS5, Manson JJ6; HLH Across Speciality Collaboration, UK.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270045/pdf/main.pdf

[24] RMD Open 2020 May;6(1):e001295. doi: 10.1136/rmdopen-2020-001295.
Storm, Typhoon, Cyclone or Hurricane in Patients With COVID-19? Beware of the Same Storm That Has a Different Origin
Alessia Alunno 1, Francesco Carubbi 2, Javier Rodríguez-Carrio
https://rmdopen.bmj.com/content/rmdopen/6/1/e001295.full.pdf

Immunity 2020 May 19;52(5):731-733. doi: 10.1016/j.immuni.2020.04.003.
COVID-19: A New Virus, but a Familiar Receptor and Cytokine Release Syndrome
Toshio Hirano 1, Masaaki Murakami
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7175868/pdf/main.pdf

J Immunother Cancer. 2018 Jun 15;6(1):56. doi: 10.1186/s40425-018-0343-9.
Cytokine release syndrome.
Shimabukuro-Vornhagen A et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6003181/pdf/40425_2018_Article_343.pdf

Nat Rev Immunol. 2020 Apr 6. doi: 10.1038/s41577-020-0305-6.
In the eye of the COVID-19 cytokine storm.
Vaninov N1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7132547/pdf/41577_2020_Article_305.pdf

Arthritis Rheumatol. 2020 Apr 15. doi: 10.1002/art.41285.
On the alert for cytokine storm: immunopathology in COVID-19.
Henderson LA1 et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262347/pdf/ART-9999-na.pdf

Microbiol Mol Biol Rev. 2012 Mar;76(1):16-32. doi: 10.1128/MMBR.05015-11.
Into the eye of the cytokine storm.
Tisoncik JR1, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294426/pdf/zmr16.pdf

Science. 2020 Apr 17. pii: eabb8925. doi: 10.1126/science.abb8925.
Cytokine release syndrome in severe COVID-19.

HISTOPATHOLOGICAL FINDINGS OF AUTOPSIES [25]

Moore BJB1, June CH1.
https://science.sciencemag.org/content/368/6490/473/tab-pdf.

J Thromb Haemost. 2020 Apr 15. doi: 10.1111/jth.14844.
Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19.
Dolhnikoff M et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262093/pdf/JTH-9999-na.pdf

J Med Virol. 2020 Apr 16. doi: 10.1002/jmv.25900.
High IL-6/IFN-γ ratio could be associated with severe disease in COVID-19 patients.
Lagunas-Rangel FA1, Chávez-Valencia V2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262117/pdf/JMV-9999-na.pdf.

Cell Death Differ. 2020 Mar 23. doi: 10.1038/s41418-020-0530-3.
COVID-19 infection: the perspectives on immune responses.
Shi Y1,2, Wang Y3, Shao C4, Huang J4, Gan J4, Huang X4, Bucci E5,6, Piacentini M7, Ippolito G7, Melino G8,9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7091918/pdf/41418_2020_Article_530.pdf

Clin Infect Dis. 2020 Mar 12. pii: ciaa248. doi: 10.1093/cid/ciaa248.
Dysregulation of immune response in patients with COVID-19 in Wuhan, China.
Qin C1, Zhou L1, Hu Z1, Zhang S1, Yang S1, Tao Y2, Xie C3, Ma K4, Shang K1, Wang W1, Tian DS1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108125/pdf/ciaa248.pdf

Autoimmun Rev. 2020 Apr 3:102537. doi: 10.1016/j.autrev.2020.102537.
The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease.
McGonagle D1, Sharif K2, O'Regan A3, Bridgewood C4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195002/pdf/main.pdf

Internet Book of Critical Care (IBCC) COVID-19
https://emcrit.org/ibcc/covid19/

[25] Arch Pathol Lab Med. 2020 May 8. doi: 10.5858/arpa.2020-0165-SA.
SARS-CoV-2 Pandemic: Review of the Literature and Proposal for Safe Autopsy Practice.
Aquila I1, Sacco MA1, Abenavoli L1, Malara N1, Arena V1, Grassi S1, Ausania F1, Boccuto L1, Ricci C1,2, Gratteri S1,2, Oliva A1,2, Ricci P1.
https://www.archivesofpathology.org/doi/pdf/10.5858/arpa.2020-0165-SA

Zhonghua Bing Li Xue Za Zhi. 2020 Mar 15;49(0):E009. doi: 10.3760/cma.j.cn112151-20200312-00193.
[A pathological report of three COVID-19 cases by minimally invasive autopsies].
[Article in Chinese; Abstract available in Chinese from the publisher]
Yao XH1 et al.

Virchows Arch. 2020 Apr 29. doi: 10.1007/s00428-020-02828-2.
The autopsy debate during the COVID-19 emergency: the Italian experience.
Sapino A1,2, Facchetti F3, Bonoldi E4, Gianatti A5, Barbareschi M6; Italian Society of Pathological Anatomy and Cytology - SIAPEC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7190281/pdf/428_2020_Article_2828.pdf

ISS COVID-19 Report No. 6/2020 - Procedure for performing diagnostic findings in deceased patients with SARS-CoV-2 infection. March 23, 2020 version. https://www.iss.it/documents/20126/0/Rapporto+COVID-19+n.+6_2020+Autopsies+v27+March.pdf/c4b363a1-a246-c36c-d007-ae24ed7e648b?t=1585307031219. Accessed April 8, 2020

Respiratory infection by COVID-19 DOCUMENT ON AUTOPSY AND DIAGNOSTIC RESCURRENCE produced by COMLAS AND SIAPEC-IAP https://www.siapec.it/public/uploads/archiviodocumenti/PRD%20COVID-19-9%20rev001%20010420.pdf. Accessed April 8, 2020

Pathologica. 2020 Mar 26. doi: 10.32074/1591-951X-13-20.
Management of the corpse with suspect, probable or confirmed COVID-19 respiratory infection - Italian interim recommendations for personnel potentially exposed to material from corpses, including body fluids, in morgue structures, during autopsy practice
Fineschi V et al ; Scientific Society of Hospital Legal Medicine of the National Health System (COMLAS), Crivelli F20, Bonoldi E21, Facchetti F22, Nebuloni M23, Sapino A24,25; Italian Society of Anatomical Pathology and Cytology (SIAPEC).
https://www.pathologica.it/article/view/101/132

Ann Intern Med. 2020 May 6. doi: 10.7326/M20-2003
Autopsy Findings and Venous Thromboembolism in Patients With COVID-19: A Prospective Cohort Study.
Wichmann D et al https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7240772/pdf/aim-olf-M202003.pdf

J Clin Pathol. 2020 May;73(5):239-242. doi: 10.1136/jclinpath-2020-206522. Epub 2020 Mar 20.
Autopsy in suspected COVID-19 cases.
Hanley B1, Lucas SB2, Youd E3, Swift B4, Osborn M5.
https://jcp.bmj.com/content/jclinpath/73/5/239.full.pdf

Despite the declining frequency of postmortem examinations, **autopsy remains the gold standard for determining the cause of death**. Establishing the pathophysiology of death is not only limited to forensic considerations but can also provide useful clinical and epidemiological insights. Selective approaches to postmortem diagnosis, such as limited postmortem sampling on complete autopsy, can provide valuable insights for the management of appropriate control measures.

It is therefore imperative to perform as accurate autopsies as possible on patients who have died with suspected or confirmed COVID-19 infection, particularly in the presence of numerous co-morbidities. Only by working with a complete set of histological specimens obtained through autopsy can the exact causes of death be ascertained, clinical management be optimized, and clinicians be helped to report effective treatment in a timely manner to reduce mortality.[26]

In addition, the autopsy study of the transmission route and colonization of the virus in various tissues is of great relevance in understanding whether the containment and management actions of the epidemic through physical and social distancing and the use of protective devices were really effective in preventing the spread of infection.

Ann Intern Med. 2020 May 14. doi: 10.7326/M20-2566.
Pulmonary Arterial Thrombosis in COVID-19 With Fatal Outcome: Results From a Prospective, Single-Center, Clinicopathologic Case Series.
Lax SF1, Skok K2, Zechner P2, Kessler HH3, Kaufmann N2, Koelblinger C2, Vander K4, Bargfrieder U2, Trauner M5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7249507/pdf/aim-olf-M202566.pdf

J Thromb Haemost. 2020 Apr 15. doi: 10.1111/jth.14844.
Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19.
Dolhnikoff M et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262093/pdf/JTH-9999-na.pdf

Nature. 2020 May 7. doi: 10.1038/d41586-020-01355-z.
Autopsy slowdown hinders quest to determine how coronavirus kills.
Ledford H.
https://www.nature.com/articles/d41586-020-01355-z

Lancet. 2020 May 2;395(10234):1417-1418. doi: 10.1016/S0140-6736(20)30937-5.
Endothelial cell infection and endotheliitis in COVID-19.
Varga Z1, Flammer AJ2, Steiger P3, Haberecker M1, Andermatt R3, Zinkernagel AS4, Mehra MR5, Schuepbach RA3, Ruschitzka F6, Moch H1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7172722/pdf/main.pdf

J Thromb Haemost. 2020 Apr 15. doi: 10.1111/jth.14844.
Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19.
Dolhnikoff M et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262093/pdf/JTH-9999-na.pdf

Histopathology. 2020 May 4. doi: 10.1111/his.14134.
Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction
Menter T et al
https://onlinelibrary.wiley.com/doi/epdf/10.1111/his.14134

Am J Forensic Med Pathol. 2020 Apr 24. doi: 10.1097/PAF.000000000567.
COVID-19: POSTMORTEM DIAGNOSTIC AND BIOSAFETY CONSIDERATIONS.
Lacy JM et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7202125/pdf/paf-publish-ahead-of-print-10.1097.paf.0000000000000567.pdf

Lisa M Barton, et al
COVID-19 Autopsies, Oklahoma, USA,
American Journal of Clinical Pathology, Volume 153, Issue 6, June 2020, Pages 725-733, https://doi.org/10.1093/ajcp/aqaa062
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7184436/pdf/aqaa062.pdf

[26] J Clin Med. 2020 Apr 26;9(5). pii: E1259. doi: 10.3390/jcm9051259.
COVID-19 Deaths: Are We Sure It Is Pneumonia? Please, Autopsy, Autopsy, Autopsy!
Pomara C1.2, Li Volti G3, Hat F4.5.
https://www.mdpi.com/2077-0383/9/5/1259/htm
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7287760/pdf/jcm-09-01259.pdf

In biopsy or autopsy studies, **lung pathology** [27] in COVID-19 patients in both early and advanced stages was manifested by **diffuse alveolar damage** with the formation of hyaline membranes, mononuclear cells and macrophages infiltrating the airspaces, and diffuse thickening of the alveolar wall.

Viral particles were observed by electron microscopy in type 2 bronchial and alveolar epithelial cells. In addition, **atrophy of the spleen**, **necrosis of the hilar lymph node**, **focal hemorrhage in the kidney**, **enlargement of the liver** with inflammatory cell infiltration, **edema, and diffuse degeneration of neurons in the brain were** present in some patients.

SARS-CoV-2 virus particles have been isolated from respiratory specimens as well as stool and urine samples from COVID-19 patients, suggesting that multiple organ dysfunction in patients with severe COVID-19 is at least partly caused by direct attack by the virus. [28]

To answer the still open questions about the mechanism of induction of COVID-19, an important contribution comes from the autopsy results of SARS-Cov [29] cases: the results obtained from patients who died during the SARS epidemic in 2003 confirm that, as with COVID-19, the pathological changes of SARS can be summarized in four aspects:

- lung lesions,
- Damage to immune organs,
- Systemic vasculitis,
- systemic toxicity reactions

Lung lesions mainly involve the alveoli and are mainly composed of desquamative pulmonary alveolitis and bronchitis, as no significant changes in width have been observed in most alveolar walls or interlobular septa.
Other lesions include formation of hyaline membranes, massive exudation of inflammatory cells in the alveoli, irregular hemorrhage and focal necrosis, and organization of exudates in the alveoli in patients with a prolonged course of the disease (up to 20 days).

The main **immune organ lesions** are massive necrosis in the spleen and local necrosis in the lymph nodes.

Systemic vasculitis involves proliferation, swelling, and apoptosis (programmed cell death) of endothelial cells, with infiltration of monocytes, lymphocytes, and plasma cells both around the circumference of small veins and in the vascular walls of the heart, lung, liver, kidney, adrenal gland, and interstitium of striated muscles. Necrosis and fibrinoid thrombosis occurred in parts of small veins.

---

[27] J Thorac Oncol. 2020 May;15(5):700-704. doi: 10.1016/j.jtho.2020.02.010.
Pulmonary Pathology of Early-Phase 2019 Novel Coronavirus (COVID-19) Pneumonia in Two Patients With Lung Cancer.
Tian S1, Hu W2, Niu L1, Liu H1, Xu H3, Xiao SY4. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7128866/pdf/main.pdf

Lancet Respir Med. 2020 Apr;8(4):420-422. doi: 10.1016/S2213-2600(20)30076-X.
Pathological findings of COVID-19 associated with acute respiratory distress syndrome.
Xu Z et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164771/pdf/main.pdf

[28] Lancet. 2020 May 9;395(10235):1517-1520. doi: 10.1016/S0140-6736(20)30920-X.
SARS-CoV-2 and viral sepsis: observations and hypotheses.
Li H1, Liu L2, Zhang D3, Xu J4, Dai H1, Tang N5, Su X6, Cao B7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7168017/

[29] Zhonghua Bing Li Xue Za Zhi. 2020 Apr 8;49(4):291-293. doi: 10.3760/cma.j.cn112151-20200211-00114.
[Analysis of coronavirus disease-19 (COVID-19) based on SARS autopsy].
[Article in Chinese; Abstract available in Chinese from the publisher]
Ding YQ1, Bian XW2.

J Pathol. 2003 Jul;200(3):282-9.
The clinical pathology of severe acute respiratory syndrome (SARS): a report from China.
Ding Y1, Wang H, Shen H, Li Z, Geng J, Han H, Cai J, Li X, Kang W, Weng D, Lu Y, Wu D, He L, Yao K.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7168017/pdf/PATH-200-282.pdf

Systemic toxic reactions included degeneration and necrosis of parenchyma cells in the lung, liver, kidney, heart, and adrenal gland, as well as degeneration of nerve cells in the brain.

Detection of SARS-CoV nucleocapsid protein and RNA in gastrointestinal epithelium, distal renal tubule epithelium, and sweat gland cells has provided direct evidence for the mode of transmission of SARS, and it has been proposed that, **in addition to respiratory transmission, SARS can be transmitted through contact with patient excretions and secretions** (feces, urine, sweat).

In particular, the gastrointestinal tract can be considered a primary target of SARS-CoV, as the virus present in contaminated food and water can enter the human body through the epithelial cells lining the surface of the gastrointestinal tract [30].

In addition, **endocrine glands** (parathyroid and hypophyseal eosinophils, adrenal cortex cells, gastric parietal cells) and **exocrine glands** (skin sweat glands, trachea and tracheal serous glands, pancreatic acinar cells) have also been found to detect capsid protein and RNA polymerase as an index of infection.

It is noteworthy that **in vivo studies with MERS-Cov** showed that inflammation and epithelial degeneration in the small intestine, was associated with the development of pneumonia and brain infection, and in particular that pulmonary infection with MERS-CoV was a secondary manifestation of intestinal infection. [31]

In his commentary to the article "*Covid-19: a puzzle with many missing pieces,*" Dr. G. Ghinga further strengthens the **hypothesis that the primary site of SARS-Cov-2 infection is the gastrointestinal tract.**
Indeed, he observes that if SARS-COV-2 were to spread into the lung following its inhalation, one should expect a predominance of lesions in the upper lobe, whereas in fact **most of the initial lesions are in the periphery**, particularly in the subpleural region in the lower lobe.
The periphery and lower lobe are the most frequently affected even in asymptomatic cases. As demonstrated in the case of SARS virus, the virus could be transported to the right side of the heart through the thoracic duct and superior vena cava and spread mainly to the lower lung through the abundant lymphatic vessels and venules in the lamina propria of the small intestine. [32]

The primary colonization of the intestine by SARS-Cov-2 could also explain its **neuroinvasive potential starting from the enteric nervous** system and through vagal and sympathetic afferents to the

[30] Ding Y, He L, Zhang Q, et al.
Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways.
J Pathol. 2004;203(2):622-630. doi:10.1002/path.1560
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7167761/pdf/PATH-203-622.pdf

[31] Sci Adv. 2017 Nov 15;3(11):eaao4966. doi: 10.1126/sciadv.aao4966.
Human intestinal tract serves as an alternative infection route for Middle East respiratory syndrome coronavirus.
Zhou J et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5687858/pdf/aao4966.pdf

Gastroenterology. 2020 Apr 27. pii: S0016-5085(20)30571-0. doi: 10.1053/j.gastro.2020.04.052.
Is SARS-CoV-2 Also an Enteric Pathogen with Potential Fecal-Oral Transmission: A COVID-19 Virological and Clinical Review.
Ding S1, Liang TJ2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7184994/pdf/main.pdf

Microb Pathog. 2020 Mar 31;144:104177. doi: 10.1016/j.micpath.2020.104177.
Shell disorder analysis predicts greater resilience of the SARS-CoV-2 (COVID-19) outside the body and in body fluids.
Goh GK1, Dunker AK2, Foster JA3, Uversky VN4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7118597/pdf/main.pdf

32 https://www.bmj.com/content/368/bmj.m627/rr-32
Rapid response to: Covid-19: a puzzle with many missing pieces
BMJ 2020; 368 doi: https://doi.org/10.1136/bmj.m627
SARS-COV-2 lung infection could be acquired upon exposure to the virus through the gastrointestinal tract, one important missing piece
Dr. G. Ghirga

central nervous system. Previous experimental work on coronaviruses has demonstrated retrograde neuronal transport as a possible pathway for viral invasion, and this may therefore also be plausible for SARS-CoV-2. [33]

A growing number of studies reinforce the hypothesis that **the gastrointestinal system plays a preponderant role in the pathogenesis and transmission of SARS-CoV-2 infection,** and this is of great relevance both in terms of how to contain and socially distract in the event of an outbreak, and in terms of directing therapy toward enhancing intestinal innate immunity as an effective strategy to overcome the failure of antiviral immunity.

IMMUNOPATHOGENESIS [34]

Aspects of how SARS-Cov-2 infection leads to Covid-19 and the interaction between the virus and the immune system will be analyzed in detail.

Regarding the genetics and structure of the virus, some essential information is given, as this topic will be covered in the chapter on SARS-Cov-2

[33] J. Virol. (2018) 92 (17), e00404-18, DOI: 10.1128/JVI.00404-18
Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43.
Dubé, M., Le Coupanec, A., Wong, A. H. M., Rini, J. M., Desforges, M., Talbot, P. J., and Diamond, M. S.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6096804/pdf/e00404-18.pdf

[34] https://www.covid19cellatlas.org/

J Pathol. 2020 May 17. doi: 10.1002/path.5471.
Angiotensin-converting enzyme-2 (ACE2), SARS-CoV-2 and pathophysiology of coronavirus disease 2019 (COVID-19).
Bourgonje AR et al
https://onlinelibrary.wiley.com/doi/epdf/10.1002/path.5471

Pathogens. 2020 Mar 20;9(3). pii: E231. doi: 10.3390/pathogens9030231.
SARS-CoV-2 and Coronavirus Disease 2019: What We Know So Far.
Rabi FA1, Al Zoubi MS2, Kasasbeh GA3, Salameh DM3, Al-Nasser AD4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7157541/pdf/pathogens-09-00231.pdf.

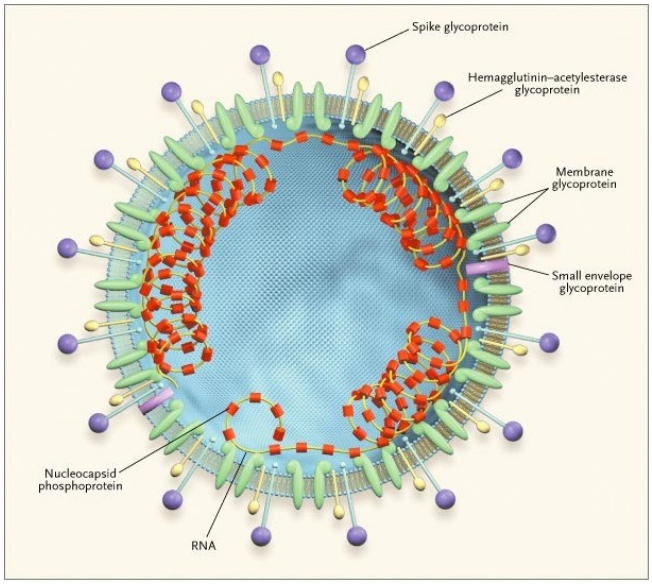
Cell. 2020 Apr 27:S0092-8674(20)30500-6. doi: 10.1016/j.cell.2020.04.035
SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues.
Ziegler CGK et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7252096/pdf/main.pdf

Cell. 2020 Apr 16;181(2):271-280.e8. doi: 10.1016/j.cell.2020.02.052.
SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor.
Hoffmann M1, et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102627/pdf/main.pdf

medRxiv 2020.03.30.20047878; doi: https://doi.org/10.1101/2020.03.30.20047878
ACE2 and TMPRSS2 variants and expression as candidates to sex and country differences in COVID-19 severity in Italy
Rosanna Asselta, Elvezia Maria Paraboschi, Alberto Mantovani, Stefano Duga

bioRxiv 2020.01.31.929042; doi: https://doi.org/10.1101/2020.01.31.929042
The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells
Markus Hoffmann, Hannah Kleine-Weber, Nadine Krüger, Marcel Müller, Christian Drosten, Stefan Pöhlmann
https://www.medrxiv.org/content/10.1101/2020.03.30.20047878v2.full.pdf

Structure of SARS-Cov-2 [35]



Taken from https://www.nejm.org/doi/full/10.1056/NEJMp030078

Coronaviruses are **single-stranded RNA viruses of positive polarity with lipid envelope with pleiomorphic structure** * or spherical in shape, and their distinguishing feature is the club-shaped projections on the surface of the virus, which are called "spikes."

The virus envelope contains four structural protein components, the **S protein (spike)**, **envelope protein (E - envelope)**, **membrane protein (M)** and **nucleocapsid protein (N)**. For SARS-CoV and SARS-CoV2, the S protein is the major determinant of host tropism and pathogenicity.

It is the main target of neutralizing antibodies and therefore of great interest in terms of immunological response and vaccine design. The S protein consists of two subunits, of which S1 constitutes the part involved in receptor recognition and S2 is highly conserved, anchors the protein in the viral membrane and facilitates viral fusion.

***pleiomorphism**: characteristic of cells and microorganisms, including viruses, to change their morphology depending on their environment. The spherical structure of SARS-Cov-2 is the one detectable in culture, while in vivo it can form bacilliform structures.[36]*

To learn more, we suggest reading the article
Journey to the center of the virus: what SARS-CoV-2 looks like

https://www.unisr.it/en/news/2020/3/viaggio-al-centro-del-virus-come-e-fatto-sars-cov-2

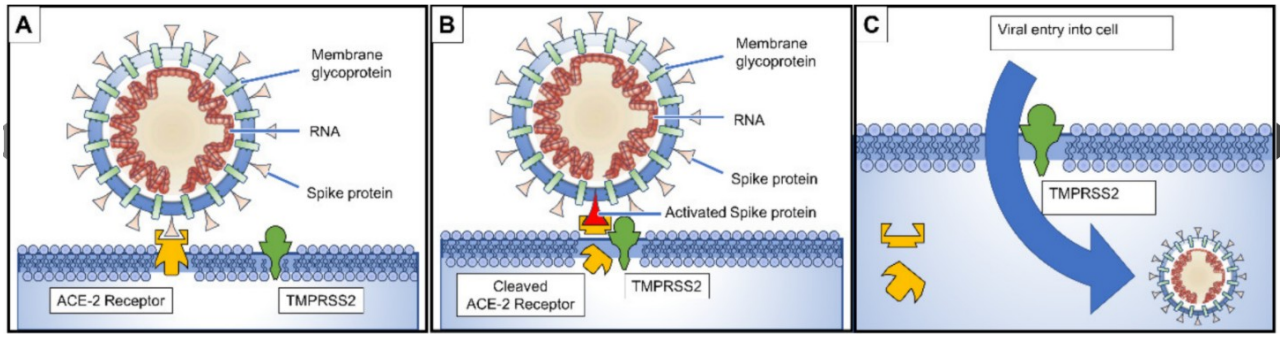
[35] N Engl J Med 2020 Feb 20;382(8):727-733. doi: 10.1056/NEJMoa2001017.
A Novel Coronavirus from Patients with Pneumonia in China, 2019
Na Zhu et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7092803/pdf/NEJMoa2001017.pdf

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

[36] Adv Virus Res. 2016;96:1-27. doi: 10.1016/bs.aivir.2016.08.005.
Supramolecular Architecture of the Coronavirus Particle.
Neuman BW, Buchmeier MJ.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7112365/pdf/main.pdf

SARS-related coronaviruses share the S protein that contains a variable receptor binding domain (RBD).
This RBD binds to the **receptor of angiotensin-converting enzyme-2** (**ACE-2**: it is a zinc-containing carboxypeptidase that converts angiotensin I to angiotensin II in the renin-angiotensin system and regulates cardiac function, mainly by maintaining blood pressure and fluid and electrolyte homeostasis) present in the heart, lungs, kidneys, and gastrointestinal tract thus facilitating viral entry into target cells.

It has been shown that when the spike protein of SARS-Cov-2 binds to the ACE-2 receptor, the complex is proteolytically processed by **the transmembrane type 2 protease TMPRSS2** leading to cleavage of ACE-2 and activation of the spike protein by a mechanism similar to that employed by influenza and human metapneumovirus, thereby facilitating viral entry into the target cell, and thus it has been suggested that cells in which ACE-2 and TMPRSS2 are simultaneously present are more susceptible to SARS-CoV-2 entry.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7157541/pdf/pathogens-09-00231.pdf
(A) Spike protein on the surface of the coronavirus binds to the receptors of angiotensin-converting enzyme 2 (ACE-2) on the surface of the target cell; **(B)** Transmembrane serine type II protease (TMPRSS2) binds to and cleaves the ACE-2 receptor. In the process, spike protein is activated; **(C)** ACE-2 receptor cleavage and activated spike protein facilitate viral entry. Expression of TMPRSS2 increases cellular uptake of coronavirus

The betacoronaviruses, to which SARS-Cov-2 also belongs, also have another structural protein, **hemagglutinin-esterase (HE**), which binds to sialic acid on the cell surface and may be a virulence factor in new hCoVs. [37]

[37] N Engl J Med. 2003 May 15;348(20):1948-51. doi: 10.1056/NEJMp030078.

SARS-associated coronavirus.
Holmes KV1.
https://www.nejm.org/doi/pdf/10.1056/NEJMp030078?articleTools=true

Clin Immunol. 2020 Apr 27;215:108448. doi: 10.1016/j.clim.2020.108448.
COVID-19: Immunology and treatment options.
Felsenstein S1, Herbert JA2, McNamara PS2, Hedrich CM3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf

IN-DEPTH STUDY

THE IMMUNE SYSTEM [38]

THE IMMUNE SYSTEM_LORETTA_BOLGAN

https://www.biopills.net/corso-di-immunologia-cellulare-e-molecolare/

The immune system consists of mechanical structures, chemicals, and cell populations that are highly trained to perform their protective functions. The first characteristic of this sophisticated system is the **ability to identify abnormalities or threats to the organism** (indicated by antigen), and subsequently to **execute a targeted response**.

A classic view in immunology involved the distinction between innate immunity (nonspecific defense mechanisms) and specific or adaptive immunity (targeted against particular threats).
However, current knowledge allows this classification to be revised by blurring the distinctions between the two types of immune responses.

Immunity innate

As can be seen from its presence in all multicellular organisms, it represents the **first level of defense that has evolved** and confers a **generic** type of **protection**, that is, one that does not depend on the type of threat involved.

In detail, the first level of defense is the establishment of a **chemical and physical barrier** against pathogens (e.g., skin and molecules with bactericidal action contained in biological fluids). Other protective mechanisms are the **identification and removal of foreign substances** (antigens) in organs and tissues by leukocytes (or white blood cells).

Other functions exerted include **recognition of the antigen by specific cells** (macrophages, dendritic cells) that are able to carry out its "presentation."

Antigen presentation enables the activation of other key components of the immune system. Another important function is to recruit immune cells to sites of infection through cytokine production and activation of the complement cascade.

Macrophages - mononuclear phagocyte system.

These are cells derived from bone marrow hematopoietic stem cells (and from yolk sac and fetal liver progenitors).
The circulating cells are known as **monocytes**, and they leave the circulation to become **macrophages activated** in inflammation, or **macrophages residing** in districts such as the skin and gastrointestinal tract.
Together they constitute the **mononuclear phagocyte** system.

[38] https://onlinelearning.hms.harvard.edu/hmx/immunity/

J Immunol Res. 2019 Apr 14;2019:1356540. doi: 10.1155/2019/1356540
Intracellular Pathogens: Host Immunity and Microbial Persistence Strategies.
Thakur A, Mikkelsen H, Jungersen G.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6487120/pdf/JIR2019-1356540.pdf

nomenclatura monociti / macrofagi	sede
monociti	midollo osseo / sangue
macrofagi tessutali	tessuti connettivi
cellule di Kupffer	fegato
istiociti	linfonodi
macrofagi alveolari (cellule della polvere)	alveoli polmonari
cellule di Langerhans	cute e mucose
microglia	CNS
cellule di Hofbauer	placenta
mesangio glomerulare	rene
osteoclasti	tessuto osseo
cellule epitelioidi	granulomi (infiammazione cronica)
macrofagi della polpa rossa	milza

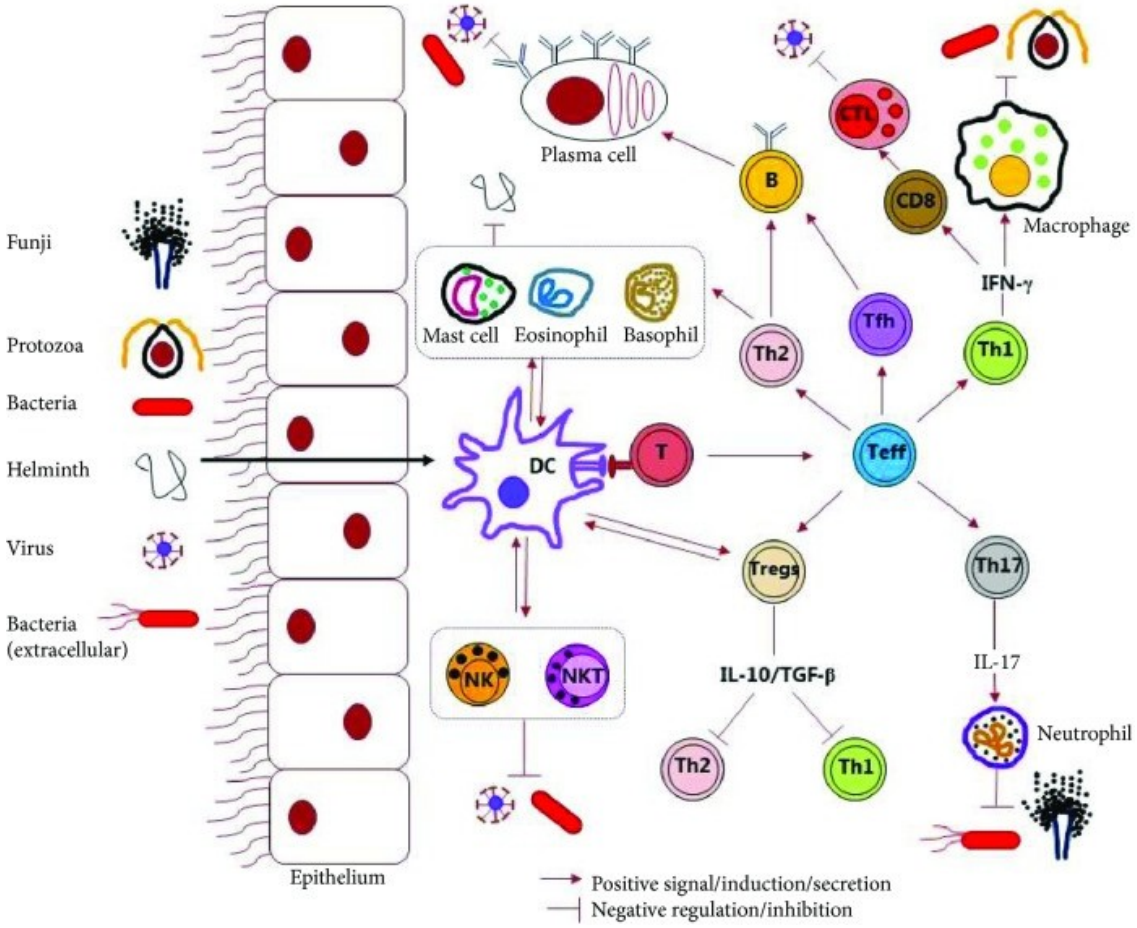
Taken from https://www.uniba.it/docenti/coluccia-mauro/attivita-didattica/materiale-didattico/2-infiammazione.pdf

Immunity adaptive

It is based on the action of certain populations of white blood cells, the **lymphocytes**, which are able to bring about a **specific response to the pathogens involved**. Overall, the pool of lymphocytes in the body ensures that it can recognize virtually all possible antigens, mounting an immune response appropriate to the threat.
Lymphocytes are produced by pluripotent stem cells belonging to the myeloid lineage, located in the bone marrow. Their maturation continues in the body, generating distinct lymphocyte lineages. The 3 main types of resulting lymphocytes are:

- **T** lymphocytes (they mature in the thymus)
- **B** lymphocytes
- Natural killer (**NK**) (maturation in the bone marrow).

They circulate through the blood and lymphatic systems and accumulate in lymph nodes and other tissues (spleen, thymus, bone marrow, tonsils, liver, appendix). Once recognition of the specific antigen has occurred, the lymphocytes begin the processes that lead to a specific immune response.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6487120/

Schematic representation of the host immune response against microbial pathogens. Microbial pathogens or antigens can be internalized by antigen-presenting cells, mostly dendritic cells (DCs), once they cross the epithelial barrier. Antigens are presented to naïve T cells by activated DCs through the interaction between the major histocompatibility complex and the T cell receptor, which leads to the activation and expansion of antigen-specific effector T cells (Teff). Teff differentiates into one of several subtypes, e.g., T helper (Th) cells 1, Th2, follicular T helper (Tfh) cells, Th17 or regulatory T cells (Tregs), depending on the cytokines present in the microenvironment. These cells activate macrophages or $^{CD8+}$ T cells through the production of IFN-γ. Activated macrophages fuse their lysosomes more efficiently with phagosomes, exposing intracellular microbes to a variety of microbicidal lysosomal enzymes and toxic oxygen and nitrogen metabolites. Cytotoxic T cells (CTLs) destroy pathogens through the release of perforins and granzymes or by inducing apoptosis of infected cells. Th2 and Tfh cells activate B cells through cytokine production and induce differentiation of B cells into plasma cells, antibody class switching and maturation of antibody affinity that remove the pathogen by neutralization, opsonization and phagocytosis. Th17 cells participate in neutrophil activation and immune regulation by producing cytokines IL-17A, which are necessary for protection from extracellular pathogens and some intracellular pathogens. Tregs regulate immune responses to pathogens and maintain self-tolerance by negatively regulating Th1 and Th2 cells, for example, by producing cytokines IL-10 and TGF-β. Innate immune cells such as eosinophils, basophils, and mast cells play an important role in protecting against parasitic infections, including helminth infections. Natural killer (NK) and natural killer T (NKT) cells, which form a bridge between innate and adaptive immunity, also contribute to antibacterial and antiviral immunity. NK cells have CTL-like functions while NKT cells produce cytokines to perform their elimination functions.

THE INFLAMMATORY RESPONSE TO INFECTION VIRAL

INFLAMMATION [39]

Inflammation is a protective response involving different types of cells, proteins and lipid mediators; this response is triggered by pathogens and necrotic tissues and is aimed at eliminating the initial cause of cell/tissue damage. However, inflammation itself can also be a source of tissue damage; this is because **immune system** cells such as **neutrophils** produce very high amounts of oxygen and nitrogen **free radicals** that have both microbicidal activity and the ability to damage the **extracellular matrix**.

Inflammation acute

The **acute inflammatory response** rapidly drives leukocytes and plasma proteins to sites of damage. Once here, leukocytes (mostly neutrophils and **monocytes**) will clear invasives and necrotic tissue.
This response is induced by several stimuli including:

- infections by all types of pathogens;
- foreign bodies;
- The blunt traumas or by physical/chemical agents;
- Tissue necrosis of any derivation;
- **hypersensitivity reactions**.

Systemic effects of inflammation

• **Fever**. Elevation of body temperature (1 to 4°C), a common manifestation of acute phase induced by substances called **pyrogens**.

Microbial products (e.g., LPS) stimulate leukocytes to release IL1 and TNF. These act on vascular and perivascular cells at the hypothalamic level by inducing COX2 and increasing the production and release of (PGE_2). PGE stimulates the production of neurotransmitters that act on the thermoregulation center, and this in turn induces a rise in body temperature.

• **Leukocytosis**. Common feature of inflammation, especially bacterial.
The increase in leukocytes initially results from the accelerated release of cells from the marrow, and then stimulation of proliferation.

- Neutrophilia: prevalent increase in PMNs (bacterial infections)
- Lymphocytosis: prevalent increase in lymphocytes (viral infections)
- Eosinophilia: prevalent increase in eosinophils (allergies and parasitic infestations)

In severe bacterial infections (sepsis), bacterial products stimulate the production of large amounts of inflammatory cytokines (TNF and IL1) that cause septic shock: hypotensive shock, insulin resistance hyperglycemia, and disseminated intravascular coagulation.

[39] https://www.uniba.it/docenti/coluccia-mauro/attivita-didattica/materiale-didattico/2-infiammazione.pdf

Polarization of macrophages [40]

Depending on the molecular signals in their environment, monocytes/macrophages can give rise to functionally distinct populations, M1 or M2.

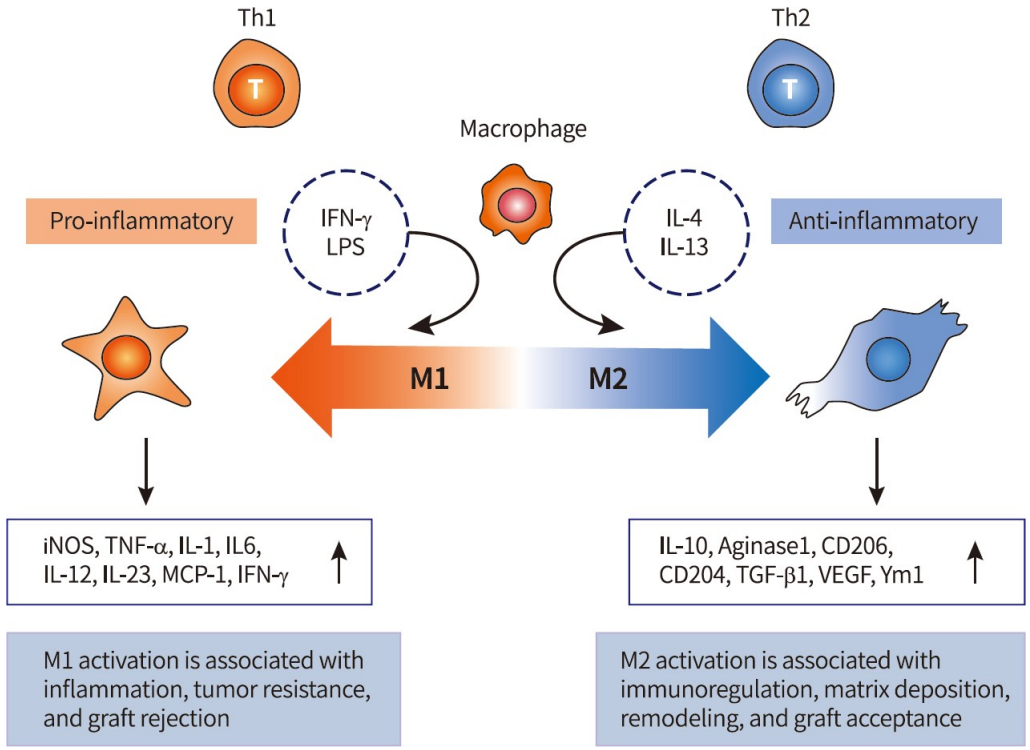
In the presence of microbial products and IFNγ, macrophages become **M1 (classical activation)** and are involved in the Th1 response to infection
- They phagocytose and destroy microbes and cellular debris
- produce and release proinflammatory cytokines.

In the presence of cytokines such as IL-13 and IL-4, **M2** macrophages **(alternative activation)** produce cytokines that:
- They antagonize the inflammatory response
- They promote repair and fibrosis

and are implicated in Th2 response

Programma pro-infiammatorio (attivazione classica, o polarizzazione M1)	Indotto da prodotti microbici (interazione LPS-TLR), o da segnali da parte di cellule T (soprattutto IFN-γ da cellule Th1), e anche da sostanze estranee come cristalli e particolato. Cellule polarizzate M1 producono NO e ROS, e sovraregolano gli enzimi lisosomiali (distruzione del materiale ingerito); secernono citochine pro-infiammatorie (TNF, IL-1, e IL-12 che stimola la differenziazione Th1).
Programma antinfiammatorio (attivazione alternativa, o polarizzazione M2)	Indotto da citochine come IL-4 e IL-13 prodotte da linfociti T (Th2) e da altre cellule. Le cellule M2 non hanno attività microbicida, e producono citochine che possono inibire l'attivazione M1. Cellule attive nella riparazione tessutale (promozione dell'angiogenesi, attivazione di fibroblasti, e stimolazione della sintesi di collagene.

Taken from https://www.uniba.it/docenti/coluccia-mauro/attivita-didattica/materiale-didattico/2-infiammazione.pdf



Taken from https://www.researchgate.net/publication/330966366_M1_and_M2_polarization_of_macrophages_a_mini-review

[40] Lee, Kun
M1 and M2 polarization of macrophages: a mini-review.
Medical Biological Science and Engineering. (2019) 2. 1-5. 10.30579/mbse.2019.2.1.1.

M1 and M2 polarization of macrophages. The pro-inflammatory M1 and anti-inflammatory M2 polarization of macrophages. Interferon-gamma and LPS are the main stimulators of M1 polarization, while interleukin-4 and 13 are inducers of M2 polarization. M1 activation is associated with inflammation, tumor resistance, and transplant rejection. M2 activation is associated with immune regulation, matrix deposition, tissue remodeling and transplantation tolerance. IFN: interferon, LPS: lipopolysaccharide, IL: interleukin, iNOS: inducible nitric oxide synthase, TNF: tumor necrosis factor, MCP: monocyte chemoattractant protein, CD: differentiation cluster,Ym1: chitinase-like 3, TGF: transforming growth factor, VEGF: vascular endothelial growth factor

.

The adaptive Th1 and Th2 response

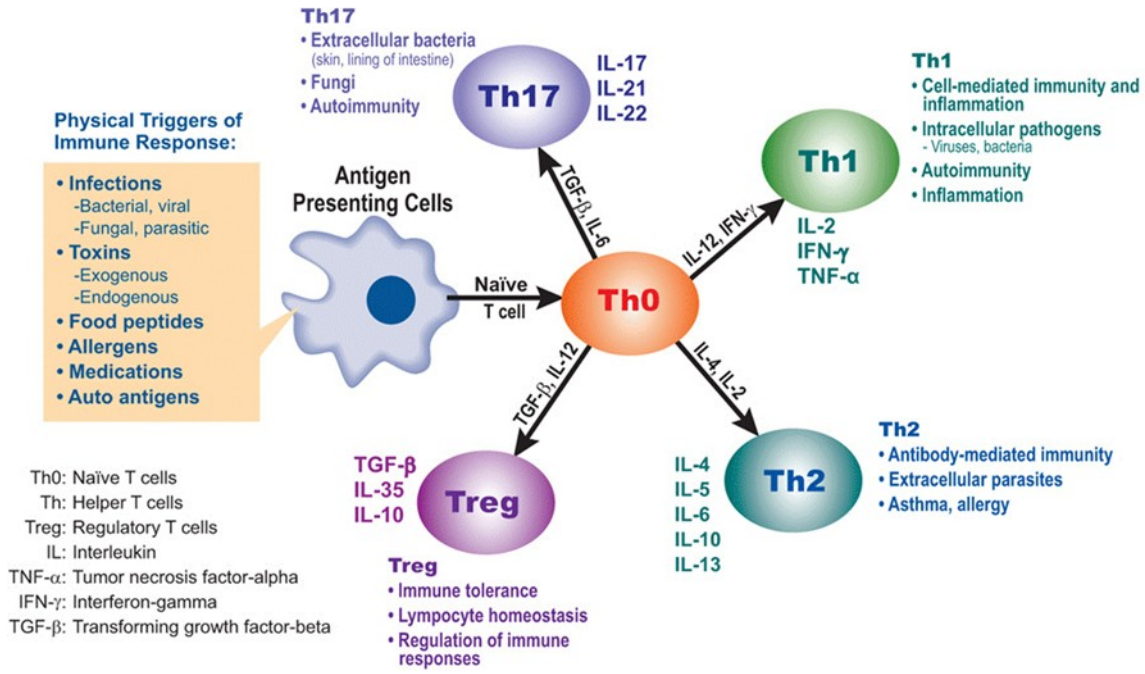
It is important to keep in mind that the immune system responds differently depending on the type of pathogen and particularly depending on whether the agent is intracellular or extracellular.

- In the case of **extracellular infections**, a **Th2 helper T lymphocyte-mediated** response is triggered, leading to **B cell** induction with the production of **antibodies** from plasma cells and memory B cells (**humoral response**).

Bacterial and fungal infections are generally extracellular. Examples of **extracellular protozoa are** Giardia, entamoeba, which cause dysentery, and trypanosoma, which causes sleeping sickness.
Of fundamental importance is the action of the Th2 response toward **worms,** common examples include schistosoma, Onchocercha, and Taenia. Th2 cells and humoral immunity form the basis of the body's immune response to parasitic worms. **Eosinophils** and **IgE are** also very important in killing helminths, since in addition to promoting a potent inflammatory response, they appear to bind to the opsonizing antibodies on the worm's skin to dissolve and subsequently kill them.

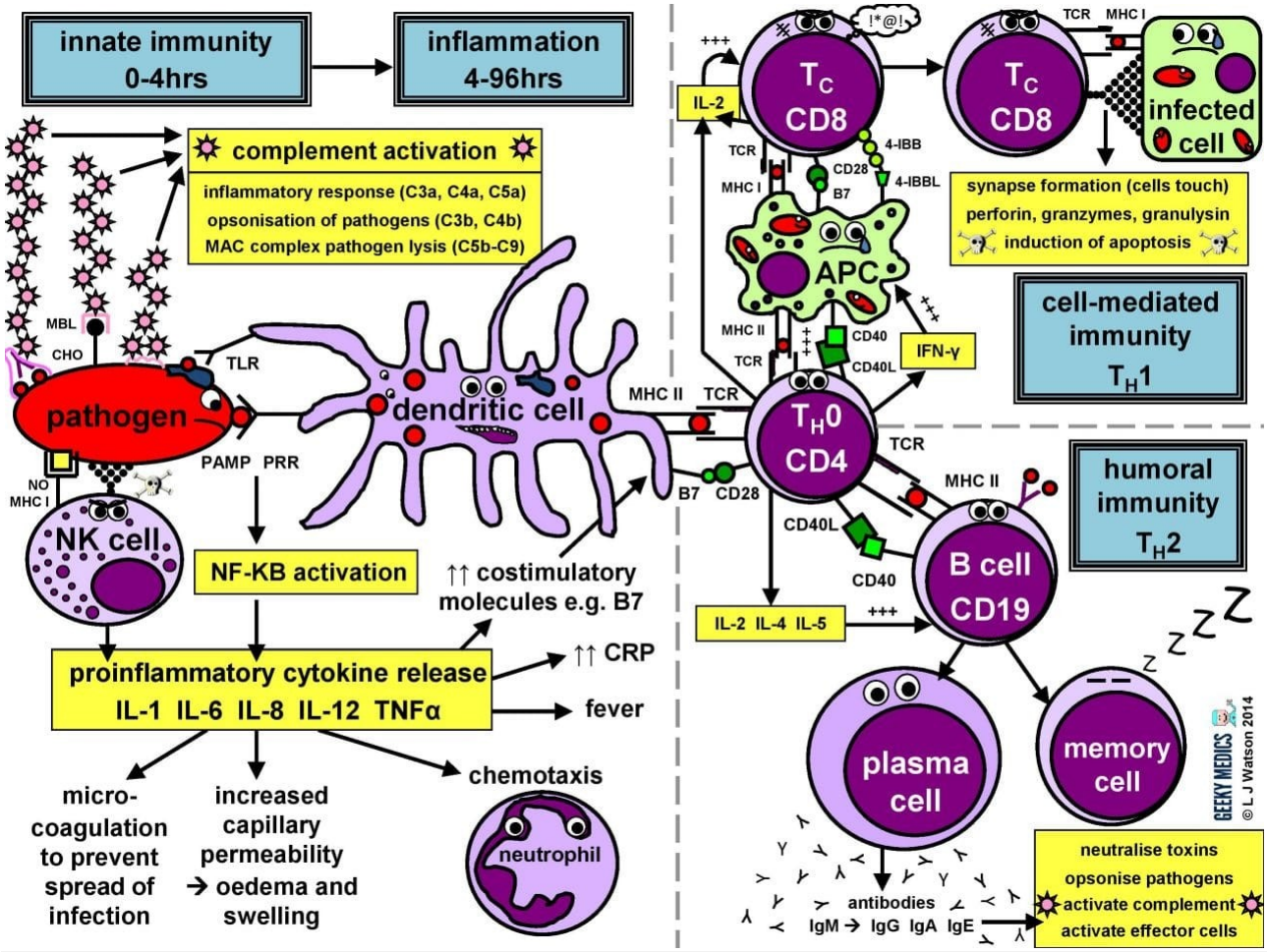
- In the case of **intracellular infections,** on the other hand, a **Th1 helper T lymphocyte-mediated** response is triggered with activation of cell-mediated immunity through **APCs** (antigen-presenting cells) and **cytotoxic T cells.**

Viral infections are intracellular and also **some bacteria** that exist as intracellular organisms, such as Neisseria, Salmonella, Chlamydia, and Mycobacteria.
Intracellular fungi are histoplasma capsulatum, cryptococcus, and pneumocystis, known to cause opportunistic infections in immunocompromised patients lacking cellular immunity. Also intracellular organisms are Plasmodium **protozoa,** which occupies red blood cells and liver cells to cause malaria, leishmania, which survives inside phagocytes, and toxoplasma.



Taken from http://thefunctionalnaturopath.com/autoimmune-disease-th1th2-paradigm/

We summarize the response to viral infection with the following figure. [41]



Taken from https://geekymedics.com/immune-response/

During acute viral infections, peptides of viral origin activate both proliferation and differentiation of $^{CD8+}$ (cytotoxic or killer T cells) and $^{CD4+}$ (helper T cells) T cells. Effective viral clearance (elimination), which occurs within a week of initial infection, requires both $^{CD8+}$ effector T cell-mediated killing of virus-infected cells and $^{CD4+}$ T cell-dependent enhancement of $^{CD8+}$ and B cell responses.

Following viral clearance, most of the virus-specific T cells undergo apoptosis; however, the preservation of a virus-specific memory T cell population is necessary for long-term antiviral immunity. **(Fig. A)**

The importance of the adaptive immune response in viral clearance suggests that chronic viral infections must, by definition, evade or suppress adaptive immunity.

[41] **For a more detailed discussion see**
http://amsacta.unibo.it/3437/18/17_fisiopatologia_delle_infezioni_II_ed_ebook.pdf

https://www.immunopaedia.org.za/immunology/

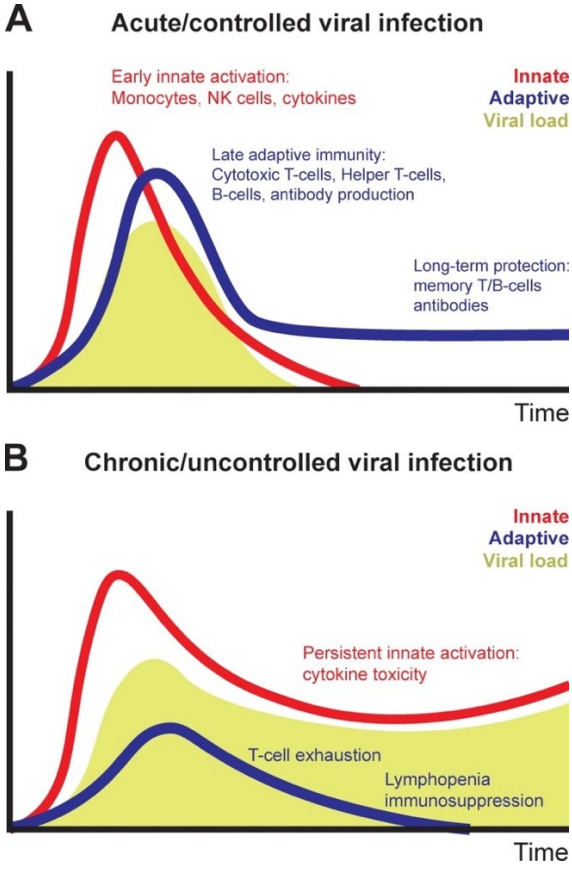
https://www.uniba.it/docenti/coluccia-mauro/attivita-didattica/materiale-didattico/2-infiammazione.pdf

http://www.informasalus.it/it/articoli/immunologia-th1-th2-infiammazione.php

https://www.immunology.org/public-information/bitesized-immunology/pathogens-and-disease/immune-responses-viruses

Rather than failing to activate T-cell responses, these viral infections are characterized by **persistent antigenic T-cell activation**, which eventually leads to a nonresponsive cellular state known as **T-cell "exhaustion"** that allows the virus to replicate and spread more rapidly.
This phenotype has been described in numerous chronic viral infections and is often accompanied by lymphopenia* . **(Fig. B)** [42]

*** lymphopenia Decrease**, absolute or relative, in the number of lymphocytes in the circulating blood (also called lymphocytopenia). Relative *l.* refers to the normal percentage distribution of white blood cells and is observed, among other things, in any case of increased neutrophil leukocytes; *absolute l.* is when the number of lymphocytes per mm^3 of blood (normally in adults 2,000÷3,000) is decreased: it can occur in numerous diseases of immunodepressio ı.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7191310/

The clinical presentation of COVID-19 infection is more consistent with **subacute** rather than acute **viral illness**. Compared with H1N1 influenza infections, in which the median incubation time is 2 days and most intensive care unit admissions occur within 24-48 h of admission [43], patients with COVID-19 infection present to the hospital with a **median incubation time of 5-7 days** and are generally admitted to the hospital for another 3-4 days before requiring admission to the intensive care unit and/or mechanical ventilation [44].

[42] J Exp Med. 2020 Jun 1;217(6). pii: e20200678. doi: 10.1084/jem.20200678.
The many faces of the anti-COVID immune response.
Vardhana SA1,2,3, Wolchok JD3,4,5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7191310/

[43] N Engl J Med. 2010 May 6;362(18):1708-19. doi: 10.1056/NEJMra1000449.
Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection.
Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza, Bautista E. et al
https://www.nejm.org/doi/pdf/10.1056/NEJMra1000449?articleTools=true

[44] Lancet. 2020 Feb 15;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5.
Clinical features of patients infected with 2019 novel coronavirus in Wuhan,
China. Huang C et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159299/pdf/main.pdf

This subacute pattern of progression raises the possibility that **immunosuppression,** due to both depletion (selective elimination of T lymphocytes that have reacted against the virus) and T-cell depletion, contributes to viral persistence and mortality from COVID-19.
Lymphopenia is the most consistent laboratory abnormality in patients with COVID-19 infection. In particular, progressive lymphocyte depletion is observed in patients who deteriorate clinically during COVID-19 infection, while recovery of lymphocyte counts tends to directly precede clinical recovery.[45]

Indeed, the researchers noted that **the number of total T cells, $^{CD4+}$ T cells and CD8 cells was drastically reduced** among patients with COVID-19, particularly in those who required intensive care. Total T cell count, $^{CD8+\ T\ cell\ count}$ or $^{CD4+}$ T cell count lower than 800, 300 or 400/L, respectively, was negatively correlated with patient survival. In addition, T-cell count was negatively associated with serum concentrations of **IL-6, IL-10 and TNF-alpha**. Patients in the period of disease resolution demonstrated reduced IL-6, IL-10 and TNF-alpha concentrations and restored T-cell counts.

T cells among patients with COVID-19 also had significantly higher levels of the depleted cell marker **PD-1**, and increased expression of PD-1 and Tim-3 was noted in the T cells of patients who progressed from the prodromal (nonspecific clinical manifestation that occurs before the typical clinical picture of a given disease arises) to overtly symptomatic stages of disease. This suggests **earlier and more urgent intervention in patients with low T-cell counts**, as due to T-cell depletion and depletion, patients are more vulnerable to secondary infections.[46]

The proposed mechanism is as follows:

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6170629/pdf/fimmu-09-02147.pdf

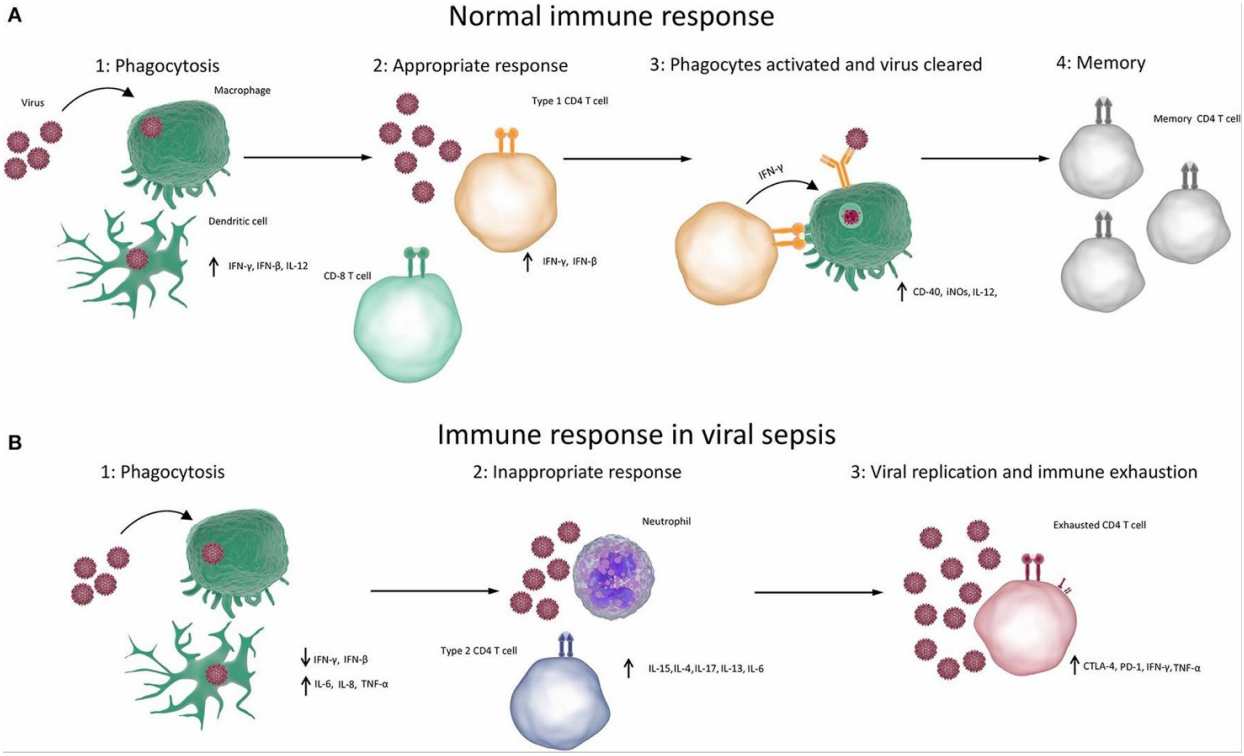
(A) Normal competent response to viral infection resulting in elimination of infection (1) When the immune system is exposed to a virus, the virus infects or is phagocytized by macrophages, dendritic cells, or other phagocytes. Phagocytes fragment, process, and present antigens from the virus and produce type 1 cytokines.(2) Type 1 cytokines cause differentiation of T cells into Th1 cells and CD8 T cells.(3) Th1 cells and CD8 T cells cause apoptosis of infected cells and activate processes such as production of reactive oxygen species in phagocytes, which destroy viruses. Antibody production is elevated, resulting in opsonization, increased phagocytosis, and virus destruction. (4) The virus is eliminated and memory T cells are produced, which can respond rapidly to future infections.

(B) Aberrant immune response resulting in viral sepsis and failure to eliminate the virus. (1) When the immune system is exposed to a virus, the virus infects or is phagocytized by macrophages, dendritic cells, or other phagocytes. Phagocytes fragment, process, and present antigens from the virus. Non-type 1 cytokines are produced. (2) Non-type 1 cytokines provoke inadequate type 2 or type 17 immune responses, which cause inflammation but are unable to eliminate the virus. (3) T cells become exhausted and can no longer competently eliminate pathogens. (CTLA-4, cytotoxic T lymphocyte-associated antigen 4; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; PD-1, programmed death 1; TNF, tumor necrosis factor).

[45] medRxiv 10.1101/2020.03.03.20030437 (Preprint posted March 6, 2020)
Restoration of leukomonocyte counts is associated with viral clearance in COVID-19 hospitalized patients.
Chen X., Ling J., Mo P., Zhang Y., Jiang Q., Ma Z., Cao Q., Hu W., Zou S., Chen L., et al. .
https://www.medrxiv.org/content/10.1101/2020.03.03.20030437v1.full.pdf

[46] Front. Immunol. 11:827. (2020) doi: 10.3389/fimmu.2020.00827.
Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19).
Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, Chen L, Li M, Liu Y, Wang G, Yuan Z, Feng Z, Zhang Y, Wu Y and Chen Y
https://www.frontiersin.org/articles/10.3389/fimmu.2020.00827/full

Front Immunol. 2018 Sep 27;9:2147. doi: 10.3389/fimmu.2018.02147.
Epidemiology and Immune Pathogenesis of Viral Sepsis.
Lin GL1,2, McGinley JP1,2, Drysdale SB1,2,3, Pollard AJ1,2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6170629/

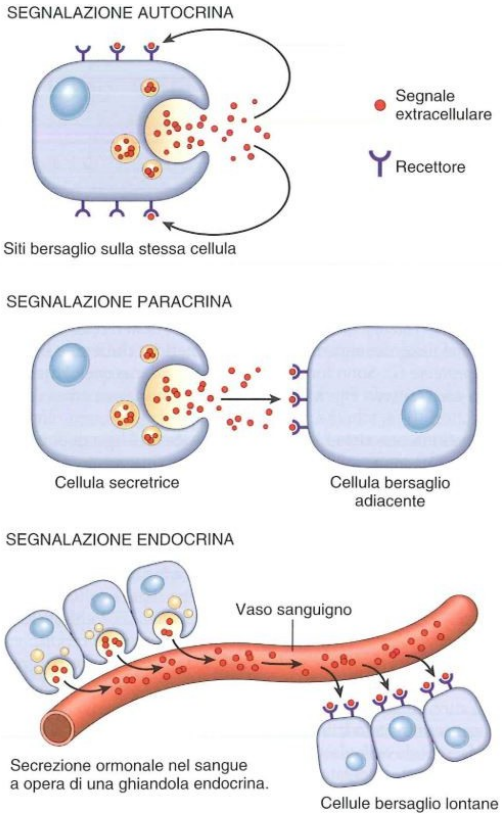


The Cytokines

Innate and adaptive immune responses are key events in the control of infection or chronic disease. The balance between these two systems is mainly orchestrated by cytokines.

Cytokines are low-molecular-weight proteins that contribute to the chemical language that regulates tissue development and repair, hematopoiesis, inflammation, etc., through transduction of signals mediated by binding to cellular receptors.
Cytokines can act on their target cells in an **autocrine, paracrine, and/or endocrine** manner to induce systemic and/or localized immune responses.
In addition, cytokines have **pleiotropic activity**, as well as influence the function of other cytokines in an additive, synergistic, or antagonistic manner.

Cytokines can be secreted by immune cells, but they can also be produced by a wide variety of cells in response to infection or can be produced or released by cells in response to cellular damage when cellular integrity is compromised.
Acting through a number of signaling pathways that control many biological processes, such as cell growth, cell differentiation, apoptosis, development, and survival, they can also reprogram cells in the local tissue environment to enhance certain types of immune responses.



Taken from http://www.bmscience.net/blog/modalita-di-trasduzione-del-segnale/

Property [47]
pleiotropy: a given cytokine can act on different cell types by inducing different effects. redundancy: two or more cytokines can act on the same cell by amplifying their effect. synergy: the combined effect of two different cytokines is greater than the sum of their individual effects. antagonism: the effect of one cytokine can inhibit or counterbalance the effect of another cytokine.
Therefore, cytokines are critical mediators of communication for the immune system and are essential for host defense against pathogens [48]

Definition:
Pleiotropy:
Pleiotropy is the phenomenon whereby a single gene controls more than one character within the same individual. Characters controlled by a pleiotropic gene are found to be closely related to each other. In most cases the pleiotropic gene codes for an enzymatic protein that is upstream of several metabolic pathways or for a structural protein that is used in the construction of different tissues and organs.

[47] http://www.medecovr.it/citochina.html

[48] MOJ Immunology. 4. 10.15406/moji.2016.04.00121
Ray, Arunabha & Joshi, Jagdish. (2016).
Cytokines and their Role in Health and Disease: A Brief
Overview... https://medcraveonline.com/MOJI/MOJI-04-00121.pdf

https://www.sinobiological.com/resource/cytokines/what-are-cytokines
http://www2.nau.edu/~fpm/immunology/lectures/Chapter012.pdf

Cytokines and its receptors in viral infections [49]

In viral infections, cytokines are involved in the **formation of an antiviral state as the first line of nonspecific defense and a subsequent specific response against the virus**. This process begins through the recognition of viral molecules by PRRs, which are present as transmembrane receptors or in different intracellular compartments.

The receptor undergoes a structural change, activating a signaling pathway in the cytoplasm that results in the activation of cytoplasmic transcription factors that are then translocated to the nuclei to promote the expression of various cytokines. The type of cytokine produced can vary depending on the virus and cell type.

The receptors of innate immunity against viruses
(PRR - Pattern Recognition Receptors - molecular profile recognition receptors)

Activation of the inflammatory response can be triggered by two different mechanisms:

- signals from infectious agents: PAMPs (Pathogen Associated Molecular Patterns (PAMPs).

Examples of PAMPs are: double-chain RNAs of viral origin, lipopolysaccharide (LPS) and lipoteichoic acids from Gram-negative and Gram-positive bacteria, respectively,

- signals arising from cellular damage: **DAMPs** (Damage Associated Molecular Patterns profiles).

Examples of DAMPs are Heat Shock Proteins (HSP), High-Mobility Group Box 1 (HMGB-1) and fibrinogen**.**

PAMPs and DAMPs are received by **PRRs** (Pattern Recognition Receptors), low-affinity receptors that result in activation of cellular responses, with release of mediators of the inflammatory response.

[49] Cytokine Profiling Plays a Crucial Role in Activating Immune System to Clear Infectious Pathogens
José Luis Muñoz-Carrillo et al.
November 5th 2018 DOI: 10.5772/intechopen.80843
https://www.intechopen.com/books/immune-response-activation-and-immunomodulation/cytokine-profiling-plays-a-crucial-role-in-activating-immune-system-to-clear-infectious-pathogens

Interazioni PAMP-PRR
(pathogen-associated molecular patterns – pattern recognition receptors)

PAMP	PRR	localizzazione	conseguenze funzionali
	Recettori toll-like		
Acidi lipoteicoici LPS batterico Flagellina batterica Lipopeptidi di micoplasma ssRNA virale dsRNA virale DNA batterico (CpG non metilate)	TLR2 TLR4 TLR5 TLR6 TLR7,8 TLR3 TLR9	monociti, macrofagi, neutrofili, DC immature, cellule NK, alcune cellule T e B, alcune cellule non immuni	Attivazione dei fagociti, produzione di citochine pro-infiammatorie, preparazione delle DC
Componenti parete batterica	Recettori scavenger	monociti, macrofagi, DC, endotelio epatico	Attivazione dei fagociti
Antigeni di cellule infette, stressate o tumorali	Recettore NK	cellule NK	Lisi cellula bersaglio, produzione di citochine pro-infiammatorie
Antigeni di cellule infette, stressate o danneggiate	TCRγδ	cellule Tγδ	Lisi cellula bersaglio, produzione di citochine pro-infiammatorie
Antigeni glicolipidici	Recettore NKT	Cellule NKT	produzione di citochine
	PRM (solubili)		
Polisaccaridi microbici	Collectine (MBL), Proteine di fase acuta	plasma	Attivazione del complemento, Fagocitosi
Peptidoglicani batterici	Proteine NOD	Citoplasma cellulare	Produzione di citochine

Taken from https://www.uniba.it/docenti/coluccia-mauro/attivita-didattica/materiale-didattico/2-infiammazione.pdf
**ss: single strand - single filament; ds: double strand - double filament*

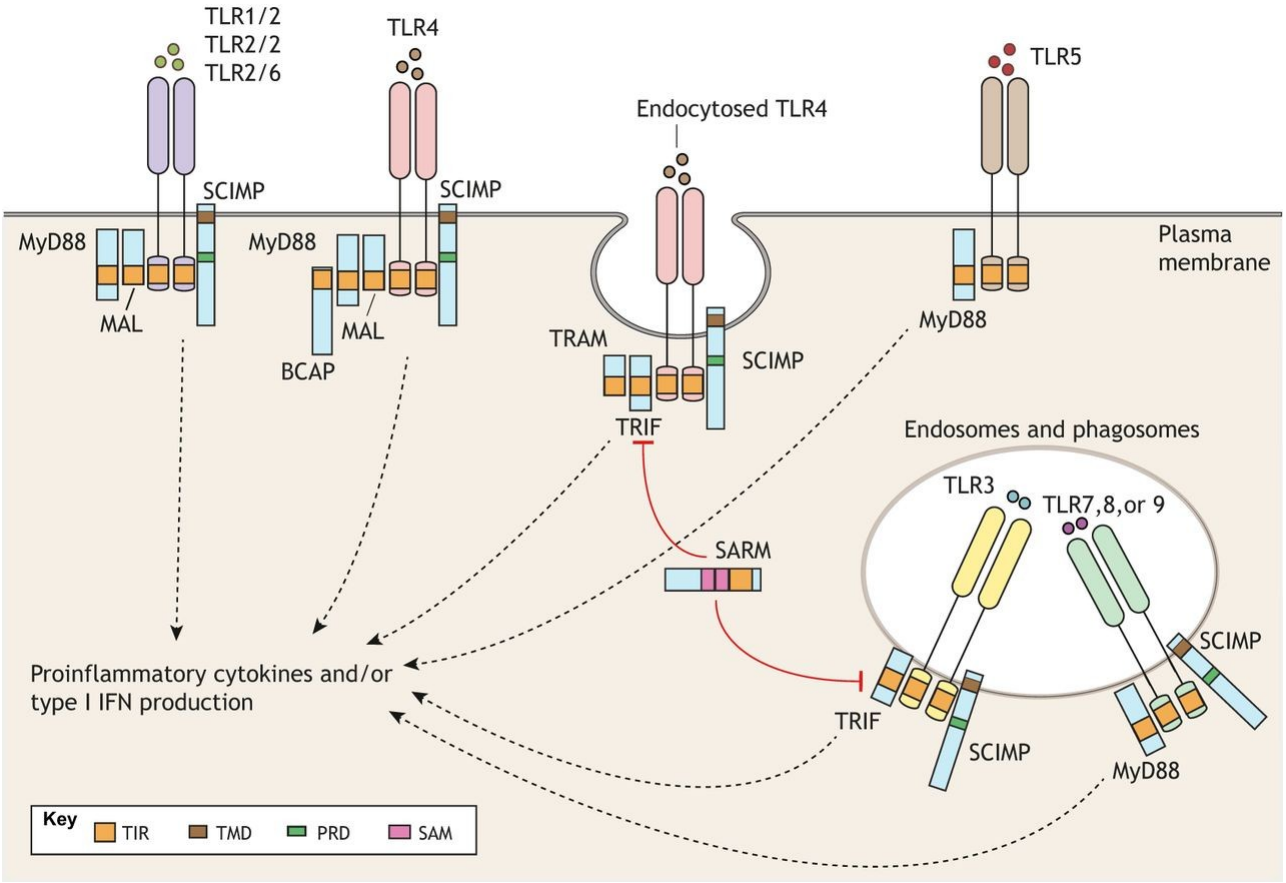
PRR-mediated response to viruses

Viruses can infect virtually any cell in an organism.
Epithelial cells, endothelial cells, fibroblasts, neurons, and innate and adaptive immune cells can be infected. PRRs are present in both cells of nonhematopoietic origin and immune cells.

Some PRRs recognize viral proteins, but others can detect single or double viral RNA or DNA.
In humans, there are **10 TLRs** (Toll-Like Receptor TLRs--in Italian Toll-like receptors, are transmembrane receptors expressed mainly on the membrane of sentinel cells such as macrophages and dendritic cells) distributed in the plasma membrane and endosome membranes. [50]

> *the endosome is a vesicular structure present within the cell that is formed during the process of endocytosis, the cellular mechanism that allows the transit across the membrane of macromolecules and corpuscles, the size of which does not allow entry through the classical mechanisms of membrane transport).*

[50] https://www.chimica-online.it/biologia/endocitosi-mediata-da-recettori.htm



https://jcs.biologists.org/content/joces/133/5/jcs239194.full.pdf
TLRs are positioned on the cell surface or endosomal compartments for signaling. After ligand binding (colored dots), TLR signaling begins with receptor dimerization, which allows recruitment of specific sets of adaptors for different TLRs (MyD88, Mal, TRIF, TRAM, BCAP, and SCIMP). SARM negatively regulates TRIF-dependent TLR signaling (red arrows). Engagement of the signaling adaptor stimulates downstream signaling pathways to drive induction of pro-inflammatory cytokines and, in the case of endosomal TLRs, production of type I interferons (IFNs). The protein domains highlighted are the Toll/interleukin-1 receptor (TIR), transmembrane domain (TMD), proline-rich domain (PRD) and sterile α motif (SAM) (see Key). TLR1 / 2: heterodimer of TLR1 and TLR2; TLR2 / 6: heterodimer or TLR2 and TLR6.

Of these, **TLR-2 and TLR-4** can detect viral surface glycoprotein before viral penetration.
Others such as **TLR-3, TLR-8 and TLR-9** detect different types of viral nucleic acids in endosomes during virus entry. TLR-8 detects genomic ssRNA, TLR-3 detects dsRNA, and TLR-9 detects unmethylated CpG viral DNA.

Another type of receptors that sense viral RNA are **RNA helicase receptors** such as RIG-I and melanoma differentiation-associated gene 5 (MDA5); these receptors have been shown to detect genomic viral dsRNA or an intermediate form during replication, which is formed for virtually all single- or double-stranded RNA viruses during viral replication. There is evidence that some replicative intermediates of dsRNA can translocate to endosomes where TLRs can detect and trigger the signal cascade.

Cytokines produced in viral infections

There are many cytokines with distinct functions. All are molecules of less than 20 KDa and can be pleiotropic or redundant and also can synergize or antagonize each other to ensure virus elimination through regulation of the immune response.

The process includes detection of the pathogen, signaling to neighboring cells, activation and differentiation of innate immune cells, production of adhesion molecules on endothelial cells to extravasate circulating immune cells, and chemotactic molecules to attract cells to foci of infection, increased phagocytosis, and activation of adaptive cells to specifically eliminate infected cells and extracellular virus.

The cytokine network against viruses begins with certain cytokines produced by virus-infected cells. The epithelial cell may produce IFN, IL-8, IL-6, IL-1, GM-CSF, TNFα, IL-18, IL-12, IL-2 and IL-23.
The role of these cytokines is varied, **IFN** induces an antiviral response and **IL-8** is a potent inflammatory that attracts phagocytic cells to the site of infection. **IL-1** can promote apoptosis and is proinflammatory and chemotactic for neutrophils.

GM-CSF is a hematopoietic growth factor that recruits various immune cells for host defense.
In addition, in the course of infection, various cytokines are also produced by innate and adaptive cells that may themselves be infected or activated.

Therefore, the infected cell may upregulate multiple cytokine genes involved in different processes such as activation of Natural Killer, macrophage, and dendritic cells and may increase the production of cytokines that act as a bridge between the innate and adaptive response.

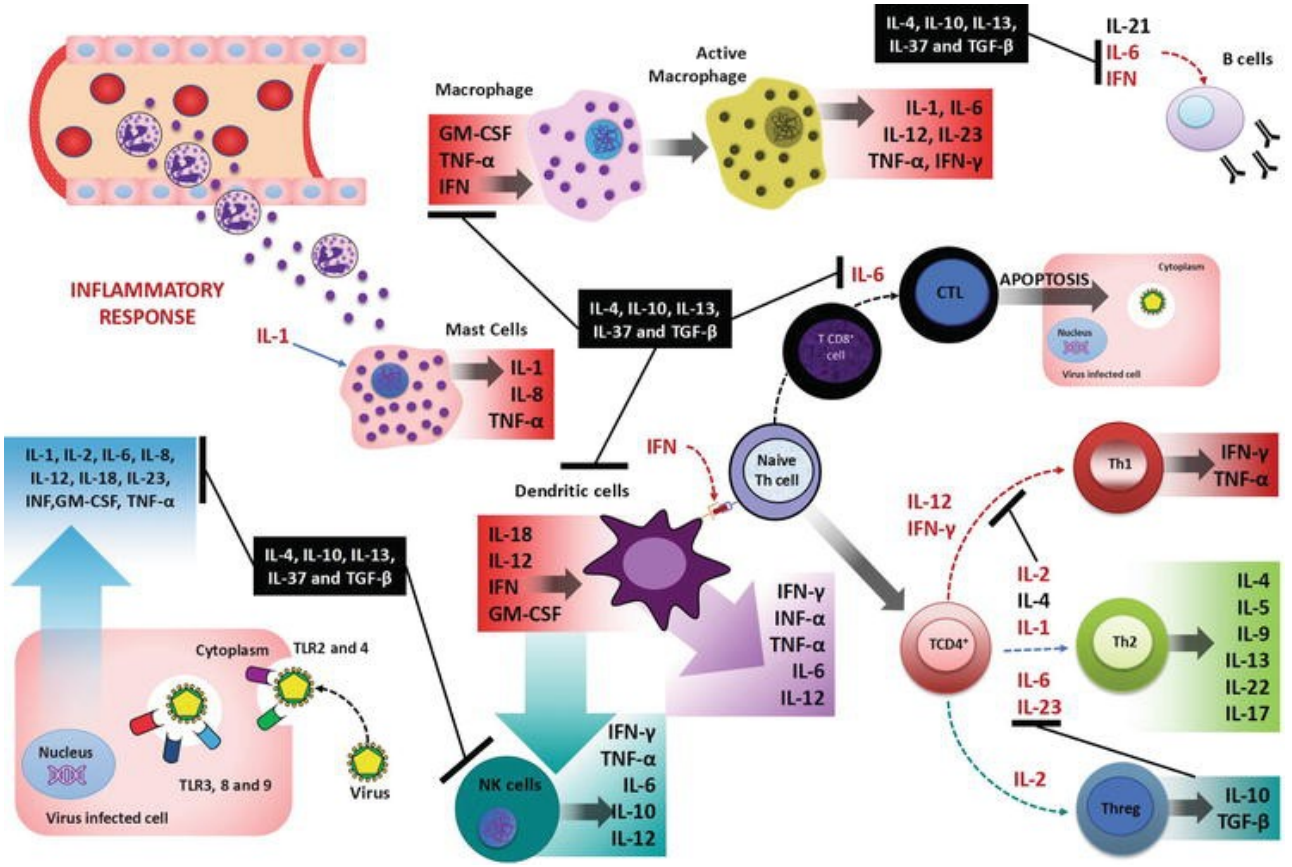
In the process of inflammation, virus-infected cells produce and secrete proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF and IFN, which are **involved in the early defense of the body.** They can activate cells present at the site of infection and recruit leukocyte cells from the circulating system through the process of inflammation.

Table 2: Summary of cytokines and their functions

Cytokine	Family	Main sources	Function
IL-1β	IL-1	Macrophages, monocytes	Pro-inflammation, proliferation, apoptosis, differentiation
IL-4	IL-4	Th-cells	Anti-inflammation, T-cell and B-cell proliferation, B-cell differentiation
IL-6	IL-6	Macrophages, T-cells, adipocyte	Pro-inflammation, differentiation, cytokine production
IL-8	CXC	Macrophages, epithelial cells, endothelial cells	Pro-inflammation, chemotaxis, angiogenesis
IL-10	IL-10	Monocytes, T-cells, B-cells	Anti-inflammation, inhibition of the pro-inflammatory cytokines
IL-12	IL-12	Dendritic cells, macrophages, neutrophils	Pro-inflammation, cell differentiation, activates NK cell
IL-11	IL-6	Fibroblasts, neurons, epithelial cells	Anti-inflammation, differentiation, induces acute phase protein
TNF-α	TNF	Macrophages, NK cells, $CD4^+$ lymphocytes, adipocyte	Pro-inflammation, cytokine production, cell proliferation, apoptosis, anti-infection
IFN-γ	INF	T-cells, NK cells, NKT cells	Pro-inflammation, innate, adaptive immunity anti-viral
GM-CSF	IL-4	T-cells, macrophages, fibroblasts	Pro-inflammation, macrophage activation, increase neutrophil and monocyte function
TGF-β	TGF	Macrophages, T cells	Anti-inflammation, inhibition of pro-inflammatory cytokine production

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5805548/pdf/oncotarget-09-7204.pdf [51]

[51] Oncotarget. 2017 Dec 14;9(6):7204-7218. doi: 10.18632/oncotarget.23208.



Taken from https://www.intechopen.com/books/immune-response-activation-and-immunomodulation/cytokine-profiling-plays-a-crucial-role-in-activating-immune-system-to-clear-infectious-pathogens
Caption: The cytokine network against viruses begins with certain cytokines produced by virus-infected cells, such as IFN, IL-8, IL-6, IL- 1, GM-CSF, TNFα, IL-18, IL-12, IL-2 and IL- 23, which induce a powerful inflammatory response by attracting and activating phagocytic cells (e.g., neutrophils, macrophages, dendritic cells), mast cells and NK cells, at the site of infection. In addition, these cytokines are involved in inducing a Th1 / CTL type of immune response for the purpose of eliminating infected cells and extracellular viruses while cytokines such as IL-4, IL-10, IL-13, IL-37 and TGF- β modulate the immune response to a Th2 and Th17 phenotype to produce immunomodulatory and anti-inflammatory actions.

The cytokine storm [52]

Inflammatory responses and inflammation-associated diseases in organs.
Chen L#1, Deng H#1, Cui H1,2, Fang J1,2, Zuo Z1,2, Deng J1,2, Li Y1,2, Wang X1,2, Zhao L1,2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5805548/pdf/oncotarget-09-7204.pdf

[52] Ye Q, Wang B, Mao J.
The pathogenesis and treatment of the `Cytokine Storm' in COVID-19.
J Infect. 2020;80(6):607-613. doi:10.1016/j.jinf.2020.03.037
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194613/pdf/main.pdf

Immunity 2020 May 19;52(5):731-733. doi: 10.1016/j.immuni.2020.04.003.
COVID-19: A New Virus, but a Familiar Receptor and Cytokine Release Syndrome
Toshio Hirano 1, Masaaki Murakami
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7175868/pdf/main.pdf

EBioMedicine. 2020 May 7;55:102789. doi: 10.1016/j.ebiom.2020.102789.
A glimpse into the eye of the COVID-19 cytokine storm.
Kuppalli K1, Rasmussen AL2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7204696/pdf/main.pdf

Semin Immunopathol. 2017 Jul;39(5):529-539. doi: 10.1007/s00281-017-0629-x
Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology.
Channappanavar R1, Perlman S2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7079893/pdf/281_2017_Article_629.pdf.

Respirology. 2006 Nov;11(6):715-22.
Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome.
Chien JY1, Hsueh PR, Cheng WC, Yu CJ, Yang PC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7192207/pdf/RESP-11-715.pdf

Aging (Albany NY). 2020 May 2;12. doi: 10.18632/aging.103101.
Immune environment modulation in pneumonia patients caused by coronavirus: SARS-CoV, MERS-CoV and SARS-CoV-2.
Yao Z1, Zheng Z1, Wu K1, Junhua Z1.
https://paperchase-aging.s3-us-west-1.amazonaws.com/pdf/vgbdsA4AtLiNjLZgc.pdf
Autoimmun Rev. 2020 Apr 3:102537. doi: 10.1016/j.autrev.2020.102537.
The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease.
McGonagle D1, Sharif K2, O'Regan A3, Bridgewood C4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195002/

Lancet. 2020 Mar 28;395(10229):1033-1034. doi: 10.1016/S0140-6736(20)30628-0.
COVID-19: consider cytokine storm syndromes and immunosuppression.
Mehta P1, McAuley DF2, Brown M3, Sanchez E4, Tattersall RS5, Manson JJ6; HLH Across Speciality Collaboration, UK.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270045/pdf/main.pdf

J Immunother Cancer. 2018 Jun 15;6(1):56. doi: 10.1186/s40425-018-0343-9.
Cytokine release syndrome.
Shimabukuro-Vornhagen A et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6003181/pdf/40425_2018_Article_343.pdf

Arthritis Rheumatol. 2020 Apr 15. doi: 10.1002/art.41285.
On the alert for cytokine storm: immunopathology in COVID-19.
Henderson LA et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262347/pdf/ART-9999-na.pdf

Microbiol Mol Biol Rev. 2012 Mar;76(1):16-32. doi: 10.1128/MMBR.05015-11.
Into the eye of the cytokine storm.
Tisoncik JR1, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294426/pdf/zmr16.pdf

Science. 2020 Apr 17. pii: eabb8925. doi: 10.1126/science.abb8925.
Cytokine release syndrome in severe COVID-19.
Moore BJB1, June CH1.
https://science.sciencemag.org/content/sci/368/6490/473.full.pdf.

J Thromb Haemost. 2020 Apr 15. doi: 10.1111/jth.14844.
Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19.
Dolhnikoff M1, Duarte-Neto AN et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262093/pdf/JTH-9999-na.pdf

J Med Virol. 2020 Apr 16. doi: 10.1002/jmv.25900.
High IL-6/IFN-γ ratio could be associated with severe disease in COVID-19 patients.
Lagunas-Rangel FA1, Chávez-Valencia V2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7262117/pdf/JMV-9999-na.pdf.

Cell Death Differ. 2020 Mar 23. doi: 10.1038/s41418-020-0530-3.
COVID-19 infection: the perspectives on immune responses.
Shi Y1,2, Wang Y3, Shao C4, Huang J4, Gan J4, Huang X4, Bucci E5,6, Piacentini M7, Ippolito G7, Melino G8,9.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7091918/pdf/41418_2020_Article_530.pdf

Clin Infect Dis. 2020 Mar 12. pii: ciaa248. doi: 10.1093/cid/ciaa248.
Dysregulation of immune response in patients with COVID-19 in Wuhan, China.
Qin C1, Zhou L1, Hu Z1, Zhang S1, Yang S1, Tao Y2, Xie C3, Ma K4, Shang K1, Wang W1, Tian DS1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108125/pdf/ciaa248.pdf

Autoimmun Rev. 2020 Apr 3:102537. doi: 10.1016/j.autrev.2020.102537.
The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease.
McGonagle D1, Sharif K2, O'Regan A3, Bridgewood C4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195002/pdf/main.pdf

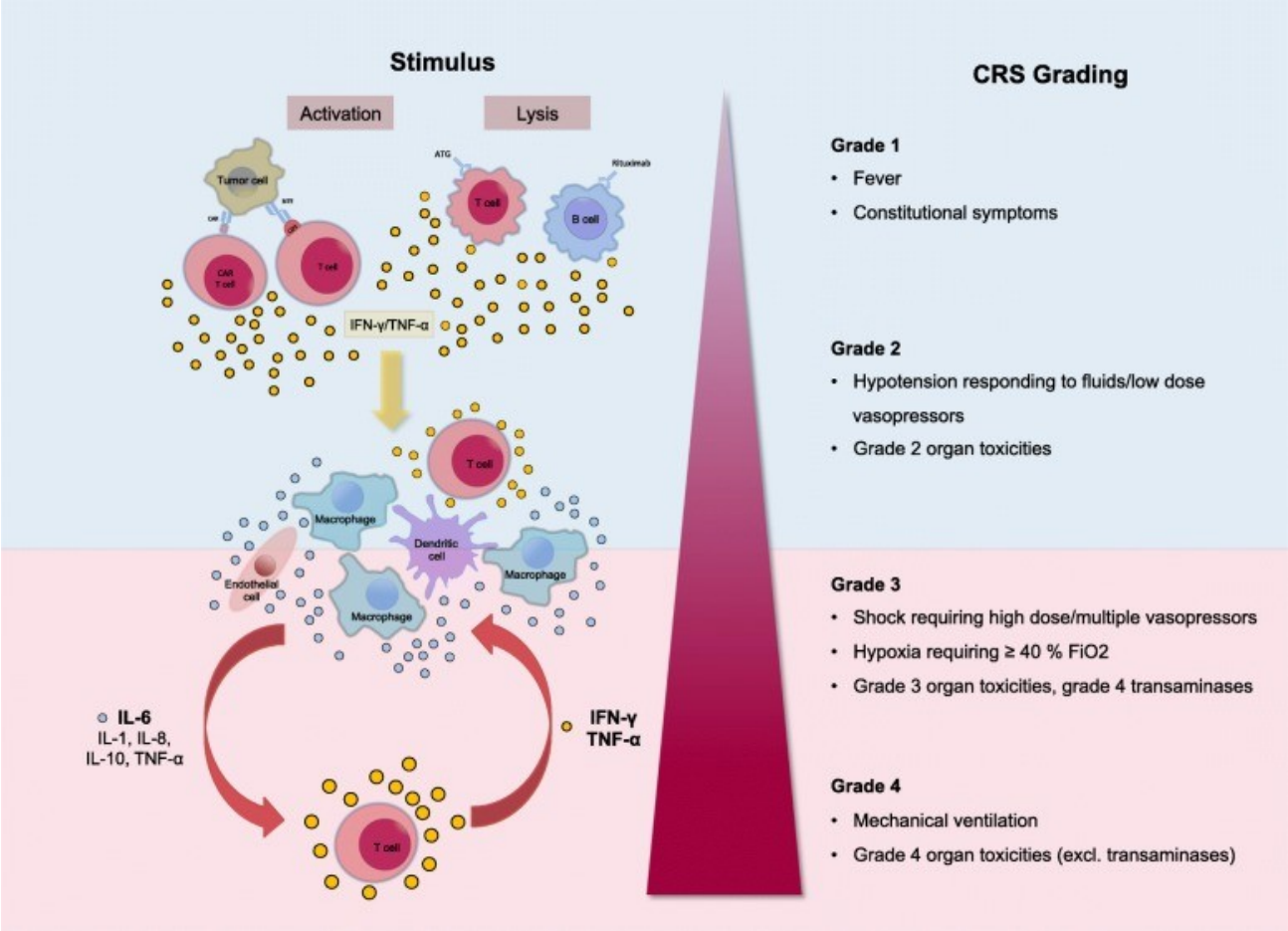
Internet Book of Critical Care (IBCC) COVID-19
https://emcrit.org/ibcc/covid19/

Preventing 'Cytokine Storm' May Ease Severe COVID-19 Symptoms
https://www.hhmi.org/news/preventing-cytokine-storm-may-ease-severe-covid-19-symptoms

The development of a cytokine storm is a **potentially fatal immune condition** characterized by rapid proliferation and hyperactivation of T cells, macrophages, natural killer cells, and overproduction of more than 150 inflammatory cytokines and chemical mediators released by immune or nonimmune cells.

In viral infections, **aberrant release of pro-inflammatory factors in the lungs** leads to apoptosis of pulmonary epithelial and endothelial cells that damages the pulmonary barrier of microvascular and alveolar epithelial cells, leading to vascular leakage, alveolar edema, and hypoxia.

Uncontrolled production of pro-inflammatory factors, containing IL-6, IL-8, IL-1β, and GM-CSF, and chemokines such as CCL2, CCL-5, IP-10, and CCL3, together with reactive oxygen species cause **ARDS** (acute respiratory distress syndrome) leading to lung fibrosis and death.



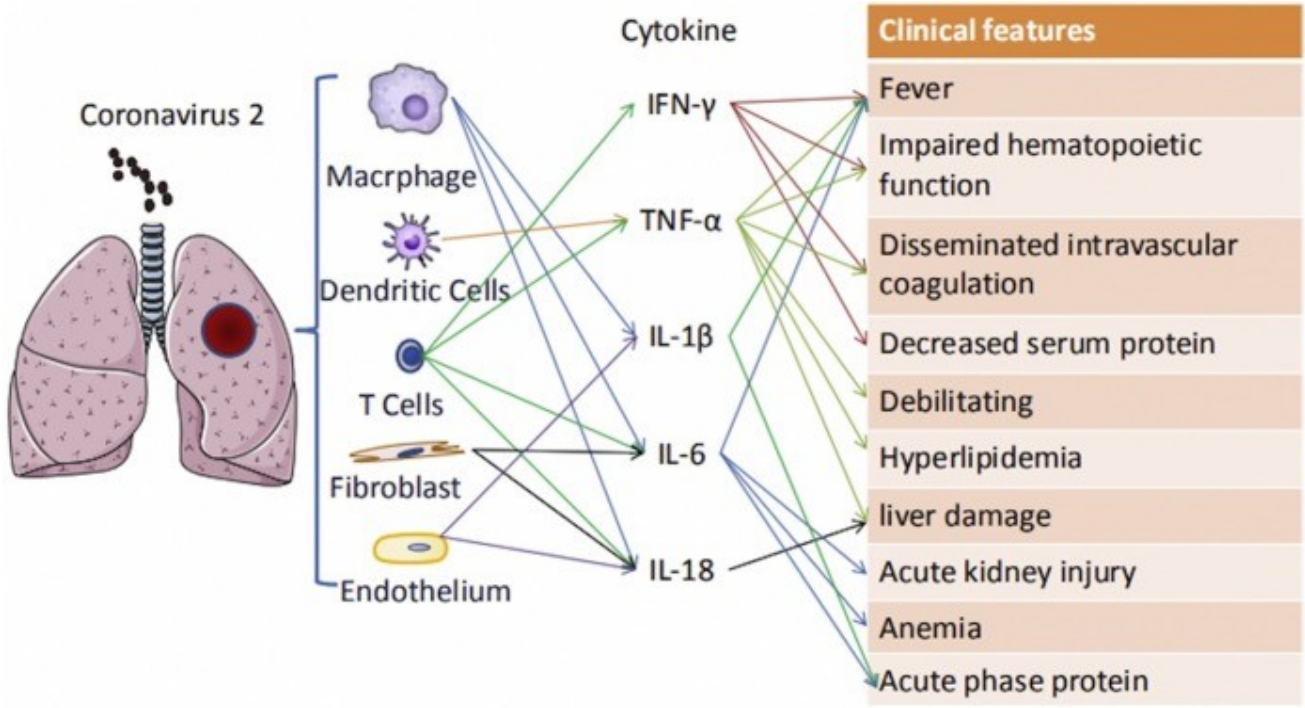
Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6003181/
T-cell activation or immune cell lysis induces a release of interferon gamma (IFN-γ) or tumor necrosis factor alpha (TNF-α). This leads to activation of macrophages, dendritic cells, other immune cells, and endothelial cells, which release pro-inflammatory cytokines, in particular, large amounts of interleukin-6, which, with positive feedback, activates T cells and other immune cells leading to the cytokine storm.
Abbreviations: FiO2: fraction of inspired oxygen; IL-6: interleukin-6; IFN-γ: interferon gamma; TNF-α: tumor necrosis factor alpha

Multiple cytokine secretion is closely related to the development of clinical symptoms; for example:

IFN-γ can cause fever, chills, headache, dizziness, and fatigue;

TNF-α can cause flu-like symptoms like IFN-γ, with fever, general malaise, and fatigue, but it can also cause vascular leakage, cardiomyopathy, lung injury, and acute phase protein synthesis.

IL-6 can lead to vascular leakage, complement activation, and the coagulation cascade, leading to the severe symptoms characteristic of **cytokine release syndrome** (CRS), such as **disseminated intravascular coagulation** (DIC). IL-6 is probably also responsible for the **cardiomyopathy** often observed in patients with CRS.



taken from https://www.ncbi.nlm.nih.gov/pmc/articles/pmc7182527/

THE IMMUNE RESPONSES INDUCED BY SARS-COV-2 INFECTION.

Clinically, the immune responses induced by SARS-CoV-2 infection can be divided into two phases: a

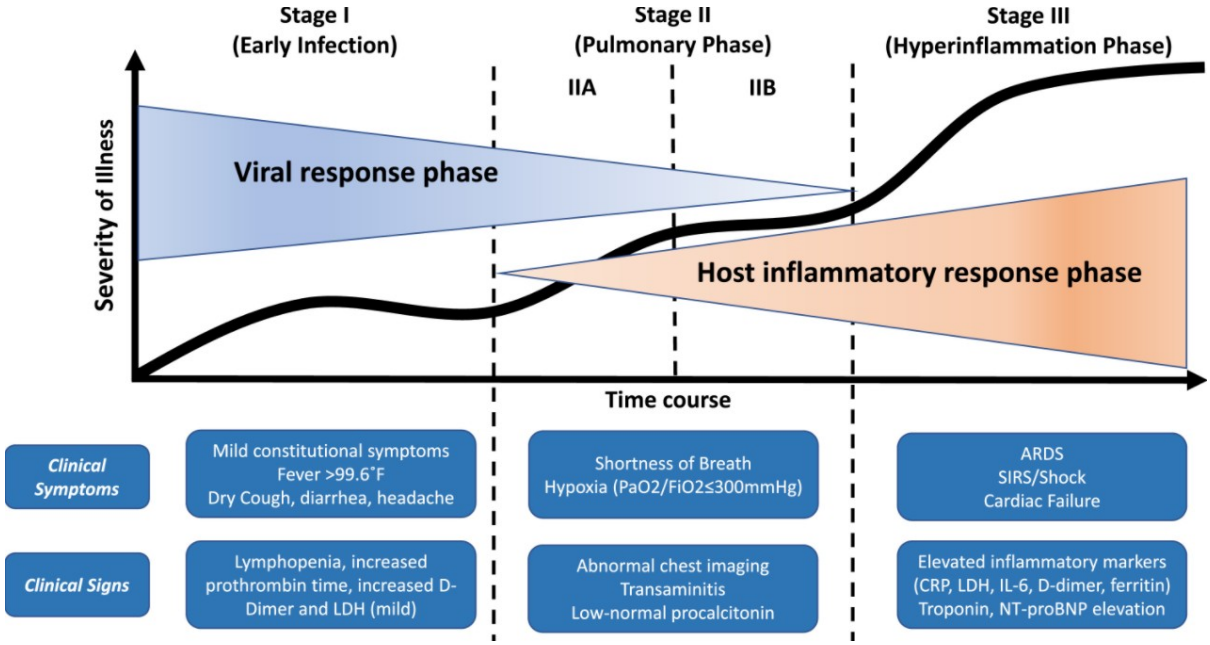
proinflammatory effector phase and

A **regulatory phase of shelter**.

The overall defense response must be able to lead to minimal harm to the organism. [53]
During incubation and the nonsevere phase, a specific innate and adaptive immune response is required to eliminate the virus and preclude progression of the disease to the severe stages.
Therefore, strategies to strengthen the immune defenses at this stage are very important. For the development of an effective endogenous protective immune response during the incubation and nonsevere stages, the host must be in good general health and have an adequate genetic background (e.g., HLA) to elicit antiviral-specific immunity.
In fact, genetic differences are well known to contribute to individual variations in immune response to pathogens.

When a protective immune response is compromised, the virus will spread causing massive destruction of affected tissues, especially in organs that have high ACE2 expression, e.g., the intestines and kidneys. The damaged cells induce secondary inflammation in the lungs mediated largely by macrophages and proinflammatory granulocytes.

Inflammation of the lung is the main cause of life-threatening airway complications in the severe stage, and as could be shown recently, disseminated intravascular coagulation is one of the diseases that leads to death if not treated appropriately in the early stage.[54]



[53] Annu Rev Immunol. 2019 Apr 26;37:405-437. doi: 10.1146/annurev-immunol-042718-041739.
Disease Tolerance as an Inherent Component of Immunity.
Martins R1, Carlos AR1, Braza F1, Thompson JA1, Bastos-Amador P1, Ramos S1, Soares MP1.
https://www.annualreviews.org/doi/pdf/10.1146/annurev-immunol-042718-041739

[54] J Clin Virol. 2020 Apr 9;127:104362. doi: 10.1016/j.jcv.2020.104362.
Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past.
Giannis D1, Ziogas IA2, Gianni P3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7195278/pdf/main.pdf

Adapted from https://www.jhltonline.org/article/S1053-2498(20)31473-X/pdf
Classification of disease states COVID-19
Legend: Figure shows 3 increasing stages of disease progression with COVID-19, with stage-specific signs and symptoms. ARDS = acute respiratory distress syndrome; CRP = C-reactive protein; IL = Interleukin; JAK = Janus Kinase; LDH = lactate dehydrogenase; SIRS = systemic inflammatory response syndrome;

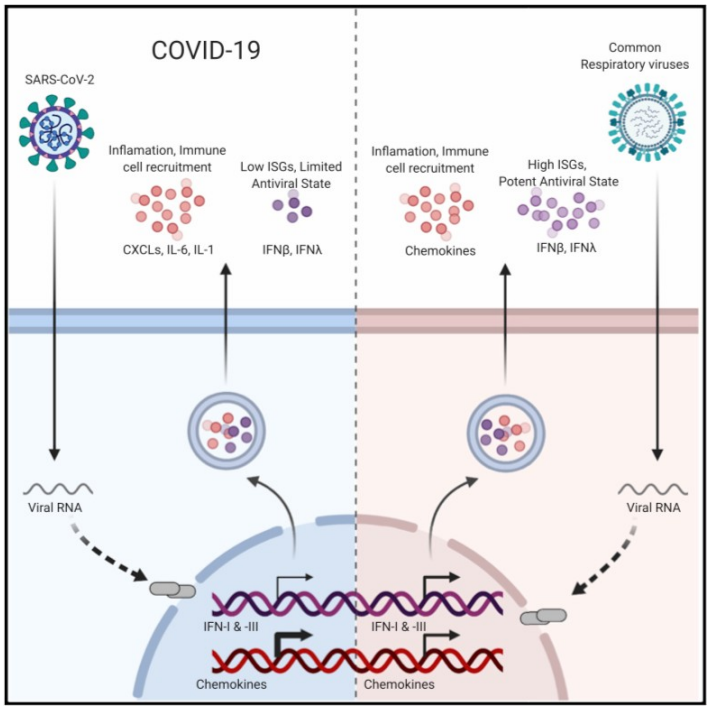
In viral infection, immune cells participate in the three stages:

- **innate immune cells** detect the presence of the virus and initiate the first antiviral responses and trigger the adaptive response;
- **effector or adaptive immune cells** eliminate the virus by killing infected cells and producing antiviral antibodies, followed by the conversion of a subset into memory lymphocytes;
- **innate immune cells** act in concert with epithelial regeneration pathways to repair damaged tissue and produce mediators that return the immune system to homeostasis.

Viruses typically elicit **strong type 1 immune responses** involving the production of **type I and III interferons by** myeloid cells with antiviral activity and **proinflammatory mediators** such as IL-12, TNFα, and CCL2 and the production of IFNγ by lymphocytes necessary for the subsequent immune response.

Type 2 regulatory immune responses are important in the later stages of viral infection. It is now recognized that while type 1 responses are important for viral clearance, type 2 responses promote repair of damaged tissue and resolution of the immune response.

It is important to point out that compared with other respiratory viruses, SARS-CoV-2 infection results in a lower antiviral transcriptional response characterized by low levels of IFN-I and IFN-III and elevated expression of chemokines and IL-6, which could explain the **coexistence of a** COVID-19-associated **state of immunosuppression and hyperinflammation**, as will be discussed in more detail below (see: CYTOKINE TEMPESTER SUBSTYLES) [55]



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7227586/

[55] Cell 2020 May 13;S0092-8674(20)30489-X. doi: 10.1016/j.cell.2020.04.026.
Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19
Daniel Blanco-Melo et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7227586/

IN BRIEF

Three phases can be distinguished during the response to SARS-Cov-2 viral infection:

***Symptomatic phase of viral infection**: symptoms are flu-like and last about 7 to 10 days. The innate immune system reacts to block virus replication. A peculiarity of this infection is loss of taste and smell, gastrointestinal and neurological symptoms. If the person has an efficient immune system, the infection resolves without complications.*

***Complication of infection:** onset of pulmonary symptoms. The adaptive immune system overreacts to uncontained infection during the first phase with the production of high amounts of inflammatory mediators. If pharmacologic intervention is not taken, the complication can rapidly progress to the most severe phase.*

***Very severe/fatal phase:** onset of respiratory distress syndrome, multi-organ failure, disseminated thromboembolism. These manifestations are related to the attack of the body's structures by the immune system.*

*during the complication the person may go **through bacterial co-infection***
(especially hospital antibiotic-resistant) that further aggravate the clinical picture.

***Post-infectious phase**: it will be important to monitor patients who have overcome complications, because as will be seen below, long-term autoimmune reactions are possible*

FEVER IN THE RESPONSE TO SARS-COV-2 VIRAL INFECTION AND ITS COMPLICATIONS

The progression of fever over the course of viral infection to fatal complication is of great relevance to understanding the stage of disease.

Definition of Fever, pyrexia and hyperthermia [56]

Normal human temperature is considered 37°C, but can vary by up to 1°C in healthy subjects. **Elevated core temperature is a common symptom in intensive care, affecting up to 70% of patients.**
Despite general usage, the terms "pyrexia," "fever," and "hyperthermia" are not yet universally defined.

The American College of Critical Care Medicine, the International Statistical Classification of Diseases and the Infectious Diseases Society of America define **fever** as an internal temperature of 38.3°C or higher, that is, just above the upper limit of a normal human temperature, regardless of the cause.

Fever has its etymological basis in Latin, meaning simply "heat," and pyrexia comes from the Greek "pir," meaning fire or fever. Some sources use the terms interchangeably, while others retain "fever" to mean an increase in temperature caused by the action of thermoregulatory pyrogens on the hypothalamus, for example in sepsis and inflammatory conditions.

Hyperthermia also has no agreed-upon definition, but is usually defined as an internal temperature above 38.2°C, regardless of the cause.

- **FEVER** (Pyrexia) It is an increase in body temperature above the normal circadian range of daily variation as a result of a change in the thermoregulatory center located in the anterior hypothalamus and pre-optic area (i.e., an increase in the hypothalamic limit of 37° C) due to infection, metabolic disorders, or increased cellular destruction.

- **HYPERTHERMIA** is a state of elevated internal temperature rising rapidly above 40°C, secondary to failure of thermoregulation, which occurs when a body produces or absorbs more heat than it dissipates.

---

[56] For further study: http://amsacta.unibo.it/3067/124/41_fp_termoregolazione_I_ed_ebook.pdf
Pathophysiology of thermoregulation.

Crit Care. 2016 Jul 14;20(1):200. doi: 10.1186/s13054-016-1375-5.
The pathophysiological basis and consequences of fever.
Walter EJ1, Hanna-Jumma S2, Carraretto M2, Forni L2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4944485/pdf/13054_2016_Article_1375.pdf

Crit Care Med. 2008 Apr;36(4):1330-49. doi: 10.1097/CCM.0b013e318169eda9.
Guidelines for evaluation of new fever in critically ill adult patients: 2008 update from the American College of Critical Care Medicine and the Infectious Diseases Society of America.
O'Grady NP1, Barie PS, Bartlett JG, Bleck T, Carroll K, Kalil AC, Linden P, Maki DG, Nierman D, Pasculle W, Masur H; American College of Critical Care Medicine; Infectious Diseases Society of America.
https://journals.lww.com/ccmjournal/Fulltext/2008/04000/Guidelines_for_evaluation_of_new_fever_in.40.aspx

J Infect Public Health. 2011 Aug;4(3):108-24. doi: 10.1016/j.jiph.2011.05.002.
Fever, fever patterns and diseases called 'fever'--a review.
Ogoine D1.
https://www.sciencedirect.com/science/article/pii/S1876034111000256?via%3Dihub

https://semmelweis.hu/belgyogyaszat3/files/2018/04/Patient-with-fever.pdf

Arch Intern Med. 1998 Sep 28;158(17):1870-81.
Concepts of fever.
Mackowiak PA1.
https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/209609

Patient Assessment in Clinical Pharmacy pp 121-132 29 March 2019 DOIhttps://doi.org/10.1007/978-3-030-11775-7_10
Diachinsky M. (2019) Fever. In: Mahmoud S. (eds) Patient Assessment in Clinical Pharmacy. Springer, Cham

In contrast to fever, the regulation of the thermoregulatory center during hyperthermia remains unchanged at normothermic levels, while in the body the temperature rises uncontrollably and overrides the ability to lose heat. External heat exposure and endogenous heat production are two mechanisms by which hyperthermia can cause a dangerously high internal temperature. It can be rapidly fatal and its treatment differs from that of fever. The underlying cause must be removed, and antipyretics do not reduce the elevated temperature.

- **HYPERPYRESSIA** This is the term for an extraordinarily high fever (> 41.5°C), which can be observed in patients with severe infections, but most cases occur in patients with hemorrhages in the CNS.

Table 1. Normal and febrile body temperature ranges (rectal temperatures).

Body temperature	°C	°F
Normal	37–38	98.6–100.4
Mild/low grade fever	38.1–39	100.5–102.2
Moderate grade fever	39.1–40	102.2–104.0
High grade fever	40.1–41.1	104.1–106.0
Hyperpyrexia[a]	>41.1	>106.0

Data from Refs. [31], [32]. NB–hypothermia = rectal temperature <35 °C (<95 °F).

a Hyperpyrexia in severe malaria is defined as rectal temperature above 40 °C [33]. C, centigrade; F, Fahrenheit.

Tratta da https://reader.elsevier.com/reader/sd/pii/S1876034111000256?token=AC31C2C9E32AE728E17E26C69BF8E70B65CB89C5A661621D922C227EA6E38D5CC7A67AA679ACAA010C2A9D59CD011EF4

Depending on the temperature measured at **the axillary level**, fever can be classified into:

Sub-fever (37-37.5°C) Fever (37.5-37.9°C) Moderate fever (38-38.9°C)

High fever (39-39.9°C) Hyperpyrexia (>40°C)

Compared with older children and adults, **infants and toddlers have higher and more prolonged fevers**, more rapid temperature rises, and greater temperature fluctuations.

In the **geriatric group (> 65 years), which is** likely to have lower body temperatures, IDSA (Infectious Diseases Society of America) defines **fever** as:

- single oral temperature > 100°F (> 37.8°C);
- Repeated oral temperatures > 37.2°C (99°F) or rectal temperatures > 37.5°C (99.5°F);
- A temperature increase of > 2°F (> 1.1°C) from the baseline temperature.

Pyrogens [57]

Pyrogens are fever-inducing substances. They act on the hypothalamic thermoregulatory center. They inhibit heat-sensitive neurons while stimulating cold-sensitive neurons, thus causing the body temperature in the hypothalamus to rise above the normal range. Pyrogens can be exogenous or endogenous.

• **Exogenous pyrogens**: mainly microbes or their products (bacteria, viruses, prions, toxins ect)

- Endotoxins are microbial products called Toll-like receptor (TLR) ligands, e.g., lipopolysaccharide endotoxin (produced by all gram-negative bacteria); toxic shock syndrome toxin (TSST-1 associated with Staphylococcus aureus strains)
- Group A streptococcus exotoxins act as both direct toxins and superantigens *

> **Superantigens, or type I toxins, are protein antigens of bacteria that provoke a very intense immune response, with the release from T lymphocytes of large amounts of cytokines.*

• **Endogenous** pyrogens: Pyrogenic cytokines-they are specific cytokines produced after activation of TLR that causes fever

- IL-1, TNF, IF-alpha, and IL-6 are released by monocytes, neutrophils, lymphocytes, glial endothelial cells, and mesenchymal cells. Pyrogenic cytokines act on the fenestrated endothelium of circumventricular organs by releasing prostaglandin E2, which in turn acts on the thermoregulatory center of the hypothalamus. In response, the hypothalamus raises the body temperature above the normal range resulting in fever.

The stages of fever [58]

Fever manifestations can be divided into three phases, corresponding to the rise, peak period and fall of body temperature.

1. **Prodromal or rising phase**: coincides with the beginning of the production of prostaglandins, proinflammatory substances produced by the body in response to infection. The prostaglandins in the blood reach the brain and signal a specific group of neurons in the hypothalamus to raise the body temperature to, for example, 38° or 39°C above the basal value, normally set at 37°C. Hypothalamic neurons function as a biological thermostat: their reprogramming by prostaglandins triggers a series of reactions that lead to the onset of fever (chills, vasoconstriction, stimulation of thyroid activity resulting in acceleration of basal metabolism). At this stage, **one** generally **feels cold**.

2. **Fastigium phase or febrile acme**: lasts throughout the period of prostaglandin production. Hypothalamic neurons maintain the temperature at the new value, and fever persists steadily or with fluctuations throughout the day. During this phase, **one feels** warm, the skin appears hot and flushed, and headache, muscle aches, restlessness appear; heart rate and respiratory rate may increase.

3. **Defervescence phase**: one continues to be hot, but fever decreases progressively (defervescence by lysis) or very rapidly (defervescence by crisis) concomitant with the reduction in prostaglandin levels. Hypothalamic neurons are recalibrated to the normal mean temperature value. This phase is often accompanied by sweating.

[57] https://www.biologyonline.com/dictionary/endogenous-pyrogen

[58] https://www.nonfartiinfluenzare.it/tutto-sulla-febbre/che-cos%C3%A8-la-febbre/

Classification of fever

Depending on the course and duration of the different phases, different types of fever are distinguished.

- **Continuous fever**: body temperature reaches 40°C and remains almost constant during the period of febrile fastigium. This is a typical form of fever in **pneumonia**. There is usually rapid defervescence with profuse sweating.

- **Remittent or discontinuous fever**: body temperature during the period of febrile fastigium goes through daily fluctuations of 2-3 degrees, but never reaches defervescence. It is a typical form of fever in **septicemias** (severe systemic infections) and **viral diseases**. It is common in tuberculosis.

- **Intermittent fever**: In this form, there is an alternation between periods of hyperthermia and periods without fever within the same day. This is what occurs in septicemia, neoplasia, and iatrogenic drug-induced fever. Oscillations that develop over several days, on the other hand, are observed in malaria (if the **peak of hyperthermia is** observed every four days it is called a *quartan fever*, if it is observed every three days a *tertian fever*), Hodgkin's lymphoma and other lymphomas. A high fever (around 40°C or between 37 and 38°C when sweating is present), intermittent and associated with **chills** is a symptom of septic fever, originating from **a bacterial infection**.

- **Rippling fever**: the fever period ranges from 10 to 15 days.

- **Recurrent and familial fever**: familial Mediterranean fever (FMF), in which the fever period ranges from 3 to 5 days

Mechanisms of fever damage [59]

There are numerous pathophysiological mechanisms for the deleterious effects of fever, classified as follows:

- **Direct cellular damage**:

Hyperthermia is directly cytotoxic and affects cell membrane stability and transmembrane transport function of proteins. As a result, ion transport is disrupted leading to an increase in intracellular sodium and calcium with a reduction in intracellular potassium concentration. Protein and DNA synthesis is disrupted at various stages of the pathway; while RNA and protein synthesis can recover rapidly after the cessation of hyperthermia, DNA synthesis remains disrupted longer. Direct cell death in humans occurs at temperatures of about 41°C, with the rate of cell death increasing dramatically with even modest further increases in temperature. The thermal energy required for cell death is similar to that required for protein denaturation, suggesting that hyperthermic cell death may occur primarily through its effects on protein structure.

- **Local effects**, e.g., stimulation of cytokines and inflammatory response:

The levels of pro-inflammatory and anti-inflammatory cytokines are elevated at the time of heatstroke hyperthermia. Of these, some (e.g. INFγ, IL-1β) are increased in a proportion of patients, while IL-6 is elevated in all patients. In addition, there is some correlation with outcome: increased IL-6 and its duration correlate with mortality, regardless of the maximum internal temperature reached. In addition to cytokines, heat shock proteins (HSPs), a family of cellular proteins that offer protection against a range of insults, including heat, are also produced. Regarding vascular changes, on the other hand, most organs show similar changes consisting of capillary dilatation, vascular stasis and extravasation into the interstitium, which can be observed as early as 30 minutes after 40.5°C.

[59] Crit Care. 2016 Jul 14;20(1):200. doi: 10.1186/s13054-016-1375-5.
The pathophysiological basis and consequences of fever.
Walter EJ1, Hanna-Jumma S2, Carraretto M2, Forni L2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4944485/pdf/13054_2016_Article_1375.pdf

Braz J Med Biol Res. 2001 Mar;34(3):301-14.
Fever induction pathways: evidence from responses to systemic or local cytokine formation.
Roth J1, De Souza GE.
https://www.scielo.br/pdf/bjmbr/v34n3/4079m.pdf

- **Systemic effects**, e.g., intestinal bacterial translocation [60]:

Bacterial translocation is the spread of viable and non-viable germs, and their metabolites, through the mucosa of the intestine to the mesenteric lymph nodes, spleen, liver, and peritoneum. Bacterial translocation may be a normal phenomenon that occurs even in healthy individuals without consequence. However, when the immune system is stressed extensively it results in septic complications (septicemia or sepsis) that localize to sites far from the original site of infection. Therefore, the relationship between bacterial translocation and multi-organ failure" (MOF) lies in the fact that, having passed the hepatic filter and then the pulmonary filter, dissemination into the circulatory stream of germs occurs resulting in bacteremia, fungemia, and endotoxemia causing (and maintaining) systemic sepsis and MOF.

Pyrogenic fever is a common response to sepsis in critically ill patients, and its generation occurs through several mechanisms. The interaction of exogenous pyrogens (e.g., microorganisms) or endogenous pyrogens (e.g., interleukin (IL) -1, IL-6, tumor necrosis factor (TNF)-α) with the vascular organ of the lamina terminalis (OVLT) leads to the production of fever. Exogenous pyrogens can stimulate cytokine production or act directly on OVLT. The OVLT is one of seven predominantly cellular structures in the anterior hypothalamus, a highly vascularized organ lacking a blood-brain barrier (BBB), allowing it to be stimulated directly by pyrogenic substances. Its stimulation leads to an increase in the synthesis of prostanoids including prostaglandin (PG) E2, which acts in the pre-optic nucleus of the hypothalamus and slows the discharge rate of heat-sensitive neurons and causes an increase in body temperature. Neural pathways may explain the rapid onset of fever, with production of pyrogenic cytokines responsible for the maintenance, rather than the onset, of fever.

Benefits of fever [61]

From studies in intensive care units (ICUs), it was found that **a high temperature in patients with infection in the first 24 hours after admission was associated with a better outcome** than normothermia or hyperthermia above 40°C, and a temperature between 37.5°C and 39.4°C tended to give a better outcome than normothermia.

In elderly patients with community-acquired pneumonia, the observed mortality rate was significantly higher in patients without fever (29%) than in patients who developed a febrile response (4%). A temperature higher than 38.2°C also played a protective role against invasive fungal infections.

High temperature can provide protection through several mechanisms.
First, human infectious pathogens often demonstrate **optimal replication at temperatures below 37°C**; therefore, in a high-temperature organism, replication is inhibited.
An increase in temperature is also associated with the **induction of innate immunity** associated in turn with microbial destruction.

---

[60] https://www.pagepress.org/journals/index.php/wpph/article/view/6735/6172
Amendola, G. (2014).
Translocation of intestinal bacterial flora: its role in sepsis and HIV infection. Working Paper of Public Health, 3(1). https://doi.org/10.4081/wpph.2014.6735

[61] Intensive Care Med. 2012 Jan 31.
Early peak temperature and mortality in critically ill patients with or without infection.
Young PJ1, Saxena M, Beasley R, Bellomo R, Bailey M, Pilcher D, Finfer S, Harrison D, Myburgh J, Rowan K.

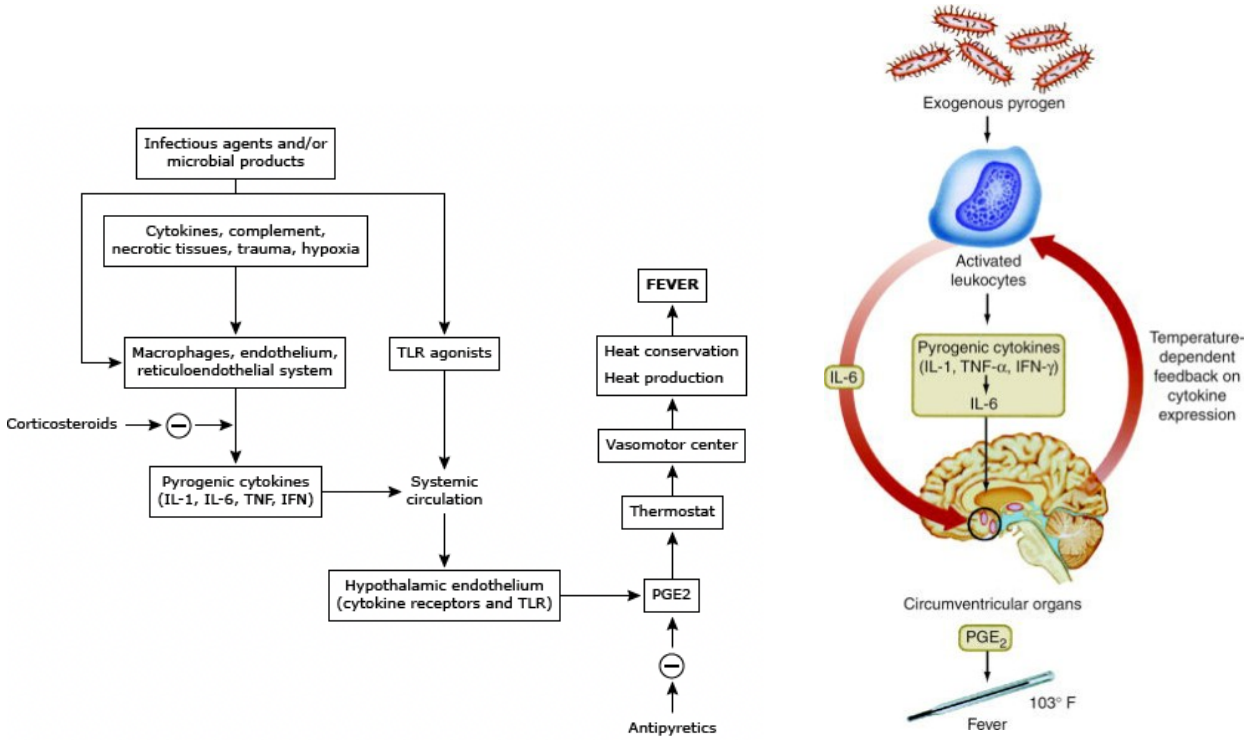
Crit Care. 2012 Feb 28;16(1):R33. doi: 10.1186/cc11211.
Association of body temperature and antipyretic treatments with mortality of critically ill patients with and without sepsis: multi-centered prospective observational study.
Lee BH et al Fever and Antipyretic in Critically ill patients Evaluation (FACE) Study Group.

South Med J. 1997 Mar;90(3):296-8.
Community-acquired pneumonia in the elderly: association of mortality with lack of fever and leukocytosis.
Ahkee S1, Srinath L, Ramirez J.

Intractable Rare Dis Res. 2016 May;5(2):97-102. doi: 10.5582/irdr.2016.01009.
Fever as an important resource for infectious diseases research.
González Plaza JJ1, Hulak N2, Zhumadilov Z3, Akilzhanova A3.

Interestingly, at temperatures above about 40°C there is again an increase in mortality, suggesting that at this stage the deleterious effects of hyperthermia on organ and cell function outweigh any benefit conferred by hyperpyrexia in acute sepsis.

In contrast to fever in response to sepsis, non-pyrogenic fever is not beneficial in any way.
A temperature of 37.5°C or higher at any time during ICU admission tends to give a worse outcome that becomes significant at temperatures higher than 38.5°C.



Taken from https://semmelweis.hu/belgyogyaszat3/files/2018/04/Patient-with-fever.pdf
https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/209609

It follows, therefore, that the assessment of the course of fever in the course of SARS-Cov-2 infection and COVID-19 in its severe complications allows us to understand the stage and level of severity and to intervene with a more targeted therapeutic approach.

As can be seen from the table below, moderate symptomatic patients or at the beginning of hospitalization have a temperature of about ***37.5°C****, while with aggravation the average temperature during the hospital stay* ***exceeds 38°C***

- *The best data to date suggest that* ***only about 30-50% of patients are febrile on admission*** [62]
- ***The absence of fever does not exclude acute COVID-19 infection.***

Characteristic	All Patients (N = 1099)	Disease Severity		Presence of Primary Composite End Point†	
		Nonsevere (N = 926)	Severe (N = 173)	Yes (N = 67)	No (N = 1032)
Fever on admission					
Patients — no./total no. (%)	473/1081 (43.8)	391/910 (43.0)	82/171 (48.0)	24/66 (36.4)	449/1015 (44.2)
Median temperature (IQR) — °C	37.3 (36.7–38.0)	37.3 (36.7–38.0)	37.4 (36.7–38.1)	36.8 (36.3–37.8)	37.3 (36.7–38.0)
Distribution of temperature — no./total no. (%)					
<37.5°C	608/1081 (56.2)	519/910 (57.0)	89/171 (52.0)	42/66 (63.6)	566/1015 (55.8)
37.5–38.0°C	238/1081 (22.0)	201/910 (22.1)	37/171 (21.6)	10/66 (15.2)	228/1015 (22.5)
38.1–39.0°C	197/1081 (18.2)	160/910 (17.6)	37/171 (21.6)	11/66 (16.7)	186/1015 (18.3)
>39.0°C	38/1081 (3.5)	30/910 (3.3)	8/171 (4.7)	3/66 (4.5)	35/1015 (3.4)
Fever during hospitalization					
Patients — no./total no. (%)	975/1099 (88.7)	816/926 (88.1)	159/173 (91.9)	59/67 (88.1)	916/1032 (88.8)
Median highest temperature (IQR) — °C	38.3 (37.8–38.9)	38.3 (37.8–38.9)	38.5 (38.0–39.0)	38.5 (38.0–39.0)	38.3 (37.8–38.9)
<37.5°C	92/926 (9.9)	79/774 (10.2)	13/152 (8.6)	3/54 (5.6)	89/872 (10.2)
37.5–38.0°C	286/926 (30.9)	251/774 (32.4)	35/152 (23.0)	20/54 (37.0)	266/872 (30.5)
38.1–39.0°C	434/926 (46.9)	356/774 (46.0)	78/152 (51.3)	21/54 (38.9)	413/872 (47.4)
>39.0°C	114/926 (12.3)	88/774 (11.4)	26/152 (17.1)	10/54 (18.5)	104/872 (11.9)

Taken from https://www.nejm.org/doi/full/10.1056/NEJMoa2002032

CYTOKINE STORM SYMPTOMATOLOGY

Before going into detail about the symptomatology of COVID-19, it is useful to summarize the differences and similarities between COVID-19 and seasonal human coronaviruses and the other two animal coronaviruses that have made the species leap (SARS-Cov- 1 and MERS). [63]

Coronaviruses are highly prevalent animal pathogens with a wide host spectrum. Altogether, thousands of coronavirus species are known [1]. Currently, seven CoVs are recognized as human pathogens. The family Coronaviridae is divided into two subfamilies**, Coronavirinae** and **Torovirinae**. The Coronavirinae include the genera **Alpha- and Betacoronavirus**, which infect only mammals, and **Gamma- and Deltacoronavirus**, which infect both mammals and

[62] Lancet 2020 Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3.
Clinical Course and Risk Factors for Mortality of Adult Inpatients With COVID-19 in Wuhan, China: A Retrospective Cohort Study
Fei Zhou et al
https://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736(20)30566-3.pdf

JAMA 2020 Mar 19;323(16):1612-1614. doi: 10.1001/jama.2020.4326.
Characteristics and Outcomes of 21 Critically Ill Patients With COVID-19 in Washington State.
Matt Arentz 1, Eric Yim 2, Lindy Klaff 2, Sharukh Lokhandwala 2, Francis X Riedo 2, Maria Chong 3, Melissa Lee
https://jamanetwork.com/journals/jama/fullarticle/2763485

N Engl J Med 2020 May 21;382(21):2012-2022. doi: 10.1056/NEJMoa2004500.
Covid-19 in Critically Ill Patients in the Seattle Region - Case Series
Pavan K Bhatraju et al
https://www.nejm.org/doi/full/10.1056/NEJMoa2004500

N Engl J Med 2020 Apr 17;NEJMc2010419. doi: 10.1056/NEJMc2010419.
Clinical Characteristics of Covid-19 in New York City
Parag Goyal et al
https://www.nejm.org/doi/full/10.1056/NEJMc2010419

JAMA 2020 Apr 22;e206775. doi: 10.1001/jama.2020.6775.
Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area
Safiya Richardson et al
https://jamanetwork.com/journals/jama/fullarticle/2765184

[63] see literature attached to the article superscripted in the text in square brackets Clin Immunol. 2020 Apr 27;215:108448. doi: 10.1016/j.clim.2020.108448.
COVID-19: Immunology and treatment options.
Felsenstein S1, Herbert JA2, McNamara PS2, Hedrich CM3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf

birds. **Human CoVs E229 and NL63 are alpha-coronavirus,** while **OC43 and HKU1 and all new CoVs (including SARS-CoV2) are beta-coronavirus**. [64]

The first hCoVs E229-CoV and OC43-CoV were described in 1966 [5,6], and they are part of a group of four seasonal hCoVs (shCoVs) that also includes HKU1-CoV and NL63-CoV, which were only discovered in 2005 [7,8]. **All shCoVs are globally endemic and often cause common colds, accounting for 2-18% of all respiratory tract infections** [9- 13]. **By age 4, 75% of children show antibodies directed against at least one of the shCoV** [65].

Anti-shCoV antibodies provide cross immunity and some antibody-mediated protection against infection by other species within group [66]. While their overall pathogenic potential is relatively low, in immunocompromised infants, the elderly, and those with pre-existing pulmonary disorders, shCoV can cause severe respiratory or sepsis-like symptoms [17-21]. OC43 shows some neurotropism and can cause demyelination and CNS infections in vulnerable patient groups [22,23]. While estimates of their contribution to annual respiratory disease vary, **shCoVs remain asymptomatic in about 50 percent of cases** [24-26]

This is in sharp contrast to the clinical presentation seen in infections with the "new coronaviruses" SARS-CoV, MERS-CoV and SARS-CoV2, which are associated with morbidity and fatality ratios that far exceed those of shCoV. In previous outbreaks of the new coronaviruses (SARS in 2003 and MERS in 2012), the severity of the clinical manifestation puzzled physicians.

Common features included massive inflammatory cell infiltration of the lungs resulting in acute lung injury (ALI) and ARDS, highly elevated inflammatory markers in serum, evidence of monocyte/macrophage activation, activated coagulation, and proinflammatory cytokine and chemokine profiles [33-38]. This soon led to the study of the host response as a determining factor in the onset of this fulminant disease process [67]. Animal models of SARS suggest that lung inflammation intensifies after viral clearance, peaking up to 14 days after infection [39] and similar observations have been made in human SARS patients.
This suggests that **the clinical deterioration in the more advanced course of the disease was probably not due to uncontrolled viral replication, but rather to uncontrolled immune responses and associated damage** [40,41].

The pulmonary pathology in COVID-19 is characterized by diffuse alveolar damage and focal reactive hyperplasia of pneumocytes with irregular inflammatory cellular (monocytes, macrophages, and lymphocytes) infiltration and evidence of intravascular thrombosis [47,48] preventing alveolar gas exchange. In addition, one-fifth of hospitalized patients develop significant cardiovascular morbidity, characterized by increased troponin, tachyarrhythmias, and thromboembolic events, which is strongly associated with mortality risk [49-51].

Thus, the common features of COVID-19 patients requiring hospitalization and ICU support are severe pneumonia with hypoxic respiratory failure of subacute onset evolving into ARDS, with a clinical picture characterized by fever, lymphopenia, very elevated C-reactive protein, proinflammatory cytokines, serum ferritin and D-dimer. [52,53]

[64] Viruses. 2019 Jan 14;11(1):59. doi: 10.3390/v11010059.
From SARS to MERS, Thrusting Coronaviruses into the Spotlight.
Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6357155/pdf/viruses-11-00059.pdf

[65] J Clin Microbiol. 2008 Jul;46(7):2368-73. doi: 10.1128/JCM.00533-08
Human coronavirus NL63 and 229E seroconversion in children.
Dijkman R, Jebbink MF, El Idrissi NB, Pyrc K, Müller MA, Kuijpers TW, Zaaijer HL, van der Hoek L.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2446899/pdf/0533-08.pdf

[66] Clin Virol. 2012 Feb;53(2):135-9. doi: 10.1016/j.jcv.2011.11.011
The dominance of human coronavirus OC43 and NL63 infections in infants.
Dijkman R, Jebbink MF, Gaunt E, Rossen JW, Templeton KE, Kuijpers TW, van der Hoek L.J
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7108278/pdf/main.pdf

[67] Semin Immunopathol 2017 Jul;39(5):529-539. doi: 10.1007/s00281-017-0629-x.
Pathogenic Human Coronavirus Infections: Causes and Consequences of Cytokine Storm and Immunopathology
Rudragouda Channappanavar 1, Stanley Perlman
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7079893/pdf/281_2017_Article_629.pdf

As previously discussed, the host response and clearance of viral infections are highly dependent on the expression of type I interferon (T1IFN) [84]. T1IFN expression and downstream signals modulate cellular responses and reprogram cells into an "anti-viral state," subsequently promoting infection control and pathogen clearance [85].
However, in a proportion of infected individuals, SARS-CoV, MERS-CoV and probably SARS-CoV2 evade recognition by the immune system through suppression of these mechanisms, causing more severe disease and worse prognosis. Taken together, **the suppression of innate immune mechanisms in infected epithelial cells and monocytes/macrophages allow the new coronaviruses to proliferate without triggering the innate antiviral response mechanism in these cells**.

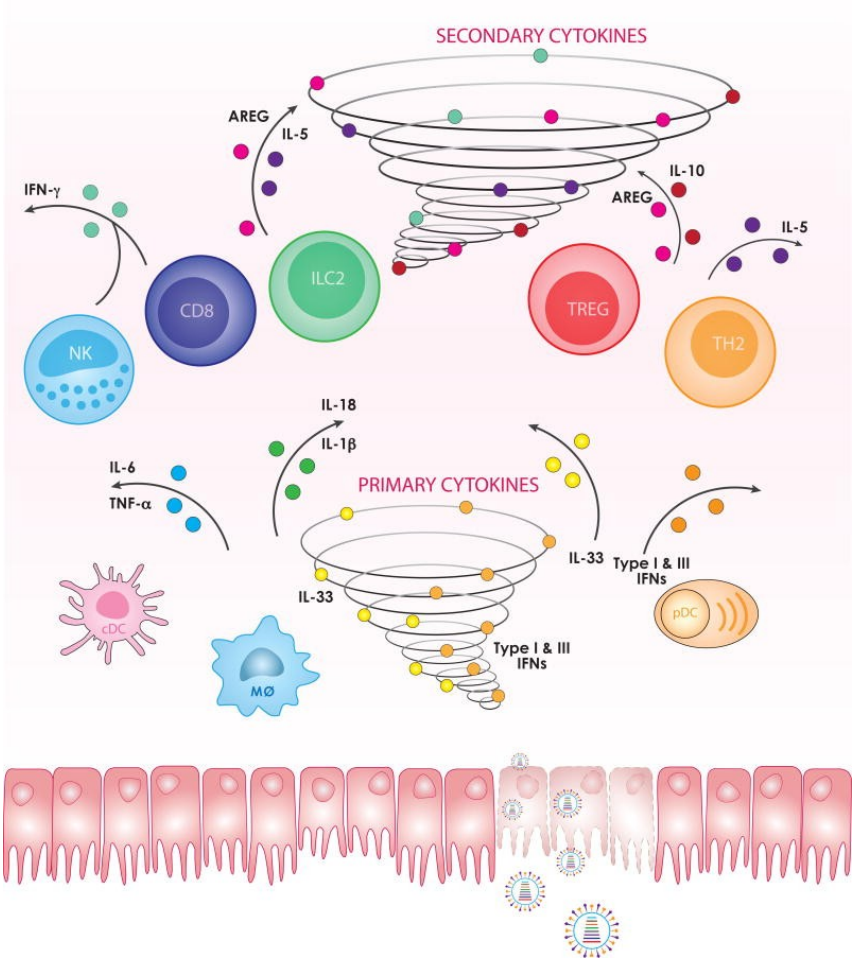
However, **at a later stage, infected cells undergo cell death and release virus particles along with intracellular components** that trigger innate inflammatory mechanisms through their recognition by PRRs present in/on innate immune cells. **As a result of this innate immune activation and the resulting expression of pro-inflammatory cytokines (including IL-1β, IL-6, TNF-α, etc.), adaptive immune cells become involved in host defense against viral infections**.

T lymphocytes play a central role in this antiviral response through the production of $^{CD4+}$ T-cell-derived cytokines, $^{CD8+}$ T-cell-mediated cytotoxicity, and B-cell activation resulting in antibody production. [68]
Again, coronaviruses are able to overcome the antiviral action of T lymphocytes by inducing their death by apoptosis or exhaustion.
When adaptive immune cells are activated, they trigger a **"second wave" of inflammation**, **which can be seen in COVID-19 patients who worsen after 7-10 days of infection**. Similar mechanisms have been reported in influenza and other viral infections [69].

Overall, severely ill COVID-19 patients manifesting cytokine storm have lymphopenia and sometimes atrophy of lymphatic tissues, i.e., lymph nodes and spleen. This is in line with reports of primary and secondary forms of **hemophagocytic lymphohistiocytosis** (HLH) and associated **cytokine storm**, resulting in inflammatory-based cell death and hypo-cellularity of lymphatic organs.

[68] Int J Biol Sci 2020;16(10):1753-1766. doi:10.7150/ijbs.45134.
COVID-19: what has been learned and to be learned about the novel coronavirus disease.
Yi Y, Lagniton PNP, Ye S, Li E, Xu RH.
http://www.ijbs.com/v16p1753.htm

[69] Semin Immunopathol. 2017 Jul;39(5):541-550. doi: 10.1007/s00281-017-0636-y.
New fronts emerge in the influenza cytokine storm.
Guo XJ1,2, Thomas PG3,4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5580809/pdf/nihms880362.pdf



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5580809/pdf/nihms880362.pdf

Spatial and temporal segregation of cytokine production after influenza infection

After internalization in epithelial cells, influenza virus can be detected by innate immune sensors and trigger downstream immune responses, including huge cytokine production, sometimes called "cytokine storm." Cytokines induced directly in a virus-infected cell versus those downstream of other cytokine signaling can be segregated as primary cytokines and secondary cytokines, respectively. In the primary cytokine wave, virus-infected epithelial, endothelial, and other lung immune cells produce type I and III IFNs, IL-1β, IL-18, TNF-α, IL-6, IL-33, and other cytokines in order to limit viral replication and spread and initiate downstream immune responses (lower panel). Following their recruitment and activation by the primary cytokines, CD8 T cells, NK cells, ILC2, Treg and Th2 cells can secrete the secondary cytokines IFN-γ, IL-10, anfiregulin and IL-5 to eliminate the virus and infected cells, dampen inflammation and restore lung function (upper panel).

Subtypes of cytokine storm syndrome

As seen above, to date, three phenotypes have been observed in COVID-19 patients, indicating three stages of infection progression and extension:

- **"Mild"** (benign infection: 80%) in patients with minor, nonspecific symptoms that do not progress to more severe disease;
- **"Moderate"** (full-blown pneumonia with or without hypoxia and localized inflammation: 15%) in patients requiring hospitalization
- **"severe"** (systemic hyperinflammation and ARDS (acute respiratory distress syndrome: 5%) in patients requiring critical care management and at risk of fatal outcome (1-2%)

As the pandemic progresses, the pathophysiology of COVID-19 is becoming clearer and the potential for differentiated therapeutic interventions has increased. Indeed, it has been reported that COVID-19 associates states of both immunodeficiency and hyperinflammation, the latter manifested by a cytokine storm.

In the recent article "*Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions,* "[70] the authors go into detail about the mechanism of COVID-19 immunopathology in the lungs by dividing it into four stages, an excerpt of which is given below:

1. **Virus entry**: early onset of rapid viral replication can cause massive epithelial and endothelial apoptosis, vascular leakage, and release of pro-inflammatory mediators

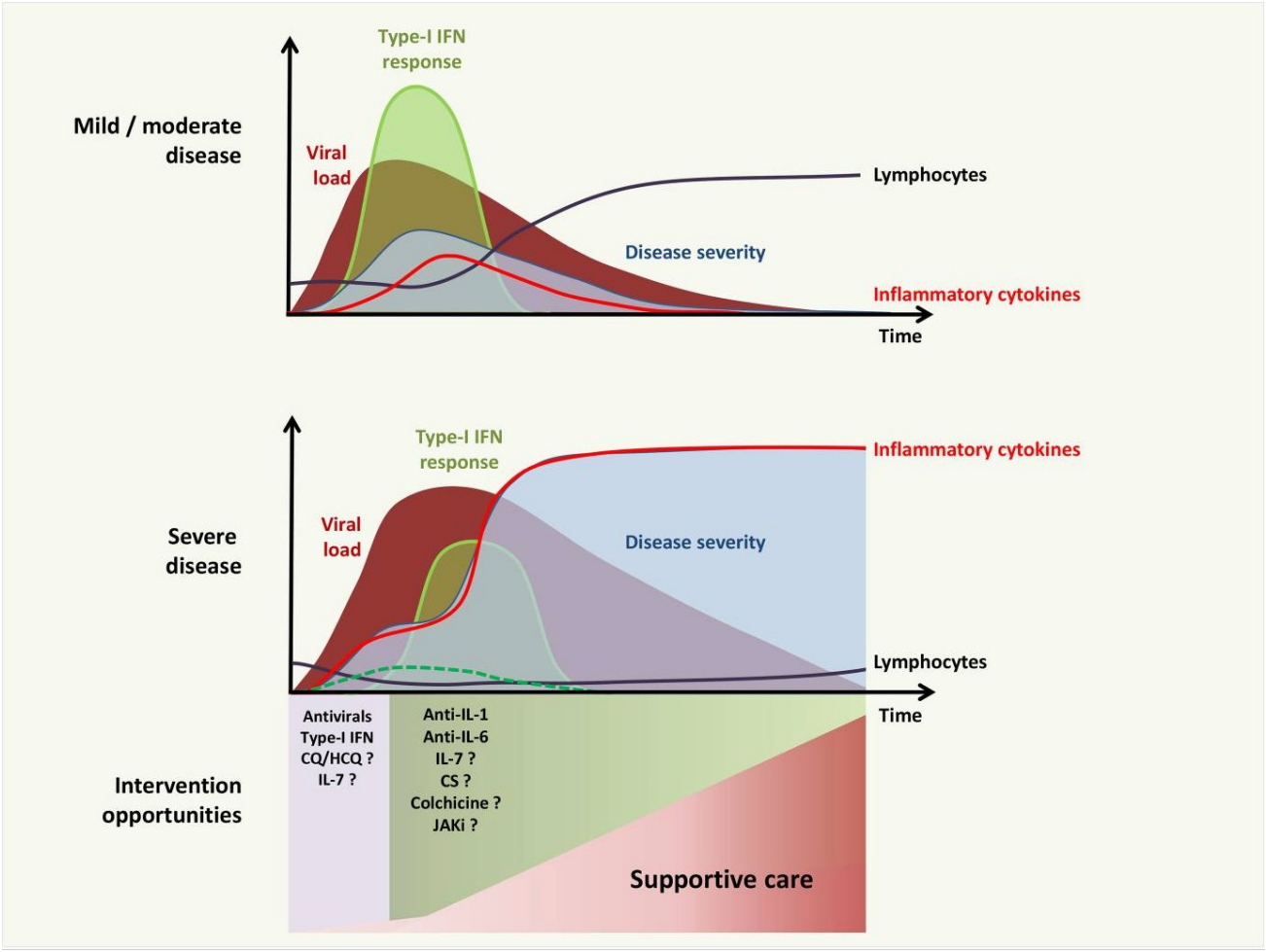
2. **Innate immune response, first wave of cytokines**: epidemiological studies have demonstrated increased acute phase markers in patients with COVID-19, including ESR, C-reactive protein (CRP), serum amyloid A, and ferritin, suggesting rapid activation of the innate immune response. As a result, COVID-19 patients have high levels of circulating TNF-α, IL-1β, IL-1Ra, sIL-2Rα, IL-6, IL-10, IL-17, IL-18, IFN-γ, MCP-3 , M-CSF, MIP- 1a, G-CSF, IP-10 and MCP-1.

> *These results suggest **hypercytokinemia** as a hallmark of COVID-19. However, only serum concentrations of some of these cytokines allow discrimination between mild, moderate and severe cases (mainly IL-1 ß, IL-1Ra, IL-6, IL-7, IL-10, IP-10 and TNF-α).*
> *Furthermore, the levels of these cytokines in mild/moderate cases are generally lower than the levels observed in normal macrophage activation syndrome/reactive hemophagocytic lymphohistiocytosis (MAS/reHLH) or severe cytokine release syndrome (CRS).*
> *Therefore, **hypercytokinemia should be considered a general marker of SARS-CoV-2**, while the term "**cytokine storm**" should be retained for those **situations of excessively exuberant inflammation leading to critical conditions,** such as ARDS, disseminated intravascular coagulation, or multiorgan failure.*

3. **Immunosuppression:** pDCs (plasmacytoid dendritic cells) are circulating immune cells that act as sentinels and are activated after physical contact with virus-infected cells and transfer of PAMPs to TLR7 sensors present in the pDCs in a process called **interferogenic synapsis.** This synapsis enables robust production of type I IFNs at the infected site, thereby limiting viral replication and systemic deleterious response. Data derived from SARS-CoV and MERS-CoV outbreaks revealed that **coronaviruses suppress the type I IFN response** by interfering with PRRs or type I IFN receptor signaling pathways, a finding also confirmed for SARS- Cov-2 [71]

[70] Jamilloux Y, et al.
Should we stimulate or suppress immune responses in COVID-19? Cytokine and anti-cytokine interventions
Autoimmun Rev. 2020;19(7):102567. doi:10.1016/j.autrev.2020.102567
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196557/pdf/main.pdf
[71] Cell 2020 May 13;S0092-8674(20)30489-X. doi: 10.1016/j.cell.2020.04.026.
Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19
Daniel Blanco-Melo et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7227586/

*In mouse models of SARS-CoV and MERS-CoV infections, **a delayed type I IFN response may explain more severe disease**, with impaired virus control and hyperinflammation induced by the same type I IFN. This leads to an influx of neutrophils and monocyte-macrophages (the main sources of pro-inflammatory cytokines) and further apoptosis of T cells, epithelial cells, and endothelial cells. These acute inflammatory mechanisms damage the pulmonary microvascular and alveolar barrier and cause vascular leakage and alveolar edema, converging in ARDS. Therefore, not only the intensity of the response but also its timing appear to play a critical role in coronavirus infection.*



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196557/
The kinetics and intensity of the antiviral response are decisive in the outcome of COVID-19. In mild to moderate COVID-19, early antiviral response, mainly of type I interferon (IFN), allows rapid reduction of viral load and prevents T-cell depletion and hypercytokinemia. In severe cases of COVID-19, delayed (solid green line) or low (dashed green line) antiviral response results in elevated lung cytokine/chemokine levels, altered virus-specific T-cell responses, and acute clinical deterioration. Optimal times for therapeutic interventions are proposed.

4. **Cytokine storm**, a **lethal second wave**: Sudden and rapid clinical deterioration has been widely mentioned in the advanced stages of COVID-19 (approximately 7-10 days). This often manifests as an unexpected worsening of symptoms (fever, dyspnea) and is correlated with increased levels of acute phase markers (ESR, CRP, ferritin), coagulopathy (elevated D-dimer titers, disseminated intravascular coagulation) and cell lysis (CK, LDH). In the most severe patients, clinical and laboratory parameters were correlated with increased levels of proinflammatory cytokines (IL-1β, IL-1Ra, IL-6, TNF-α, and sIL2-Rα), suggestive of a cytokine storm.

Interestingly, ARDS occurs in patients with SARS-CoV despite a decrease in viral load, suggesting that the host's excessive immune response may be responsible for this outcome rather than viral virulence.

*This cytokine profile strongly resembles both cytokine release syndrome (CRS, seen in CAR T-cell therapy) and **hemophagocytic lymphohistiocytosis** (HLH).*
Numerous authors have paralleled COVID-19 cytokine storm with primary or reactive HLH (reHLH) because of its close similarity, including high fever, cytopenia, hyperferritinemia, abnormal liver tests, coagulopathy, and pulmonary involvement (including ARDS), which occur in about 50% of patients with reHLH. In adults, reHLH is often triggered by viral infections and is observed in 3-4% of sepsis cases.

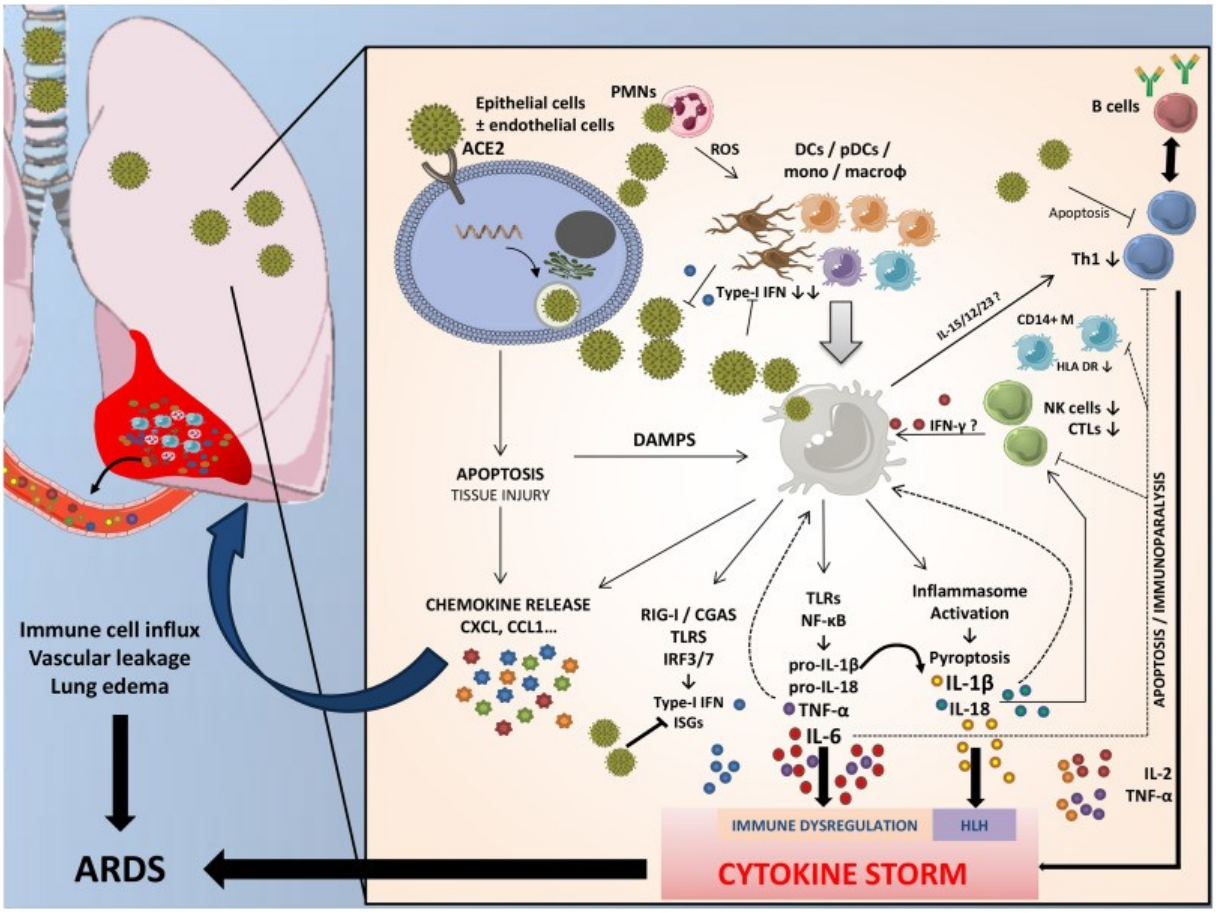
Although an alternative pathway of hyperactivation of primary innate immunity is possible, it is more likely that **this cytokine storm occurs due to the combination of a defective (or delayed) first line of defense, followed by persistent hypercytokinemia (IL-6, IL-1β, and TNF-α) and a dysfunctional T-cell response (usually of cytotoxicity).**
This results in impaired clearance of apoptic cells or infected/activated macrophages, increased viral replication and spread, followed by an IL-18/IFN-γ self-feeding cycle that activates macrophages, culminating in multiple cytokine release, hemophagocytosis, coagulopathy, and ARDS.
Some of these mediators may further fuel this vicious cycle, including impairment of NK cell function by IL-6 or activation of macrophages by ferritin H-chain. Most importantly, hemophagocytosis has been reported in lung tissues from patients with inauspicious outcome to SARS-CoV infection.

Analyzing the immunopathology of SARS-CoV2-related ARDS in more detail, Giamarellos-Bourboulis et al. concluded that there are two patterns of immune dysfunction in the aggravation of COVID-19 [72]:

- A highly suggestive pattern of **macrophage activation syndrome** (hyperferritinemia and elevated H score: 25% of patients), which is mediated by **IL-1β**

- a model with **IL-6-mediated immune dysregulation**, characterized by a **combination of hypercytokinemia, immunoparalysis** (as indicated by the reduction of HLA-DR molecules on CD14 monocytes) and **global lymphopenia** (including $^{CD4+}$ and NK cells). Interestingly, blockade of IL-6 with tocilizumab partially restored HLA-DR expression on CD14 monocytes and increased circulating lymphocyte counts.

[72] Giamarellos-Bourboulis EJ, et al.
Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure
Cell Host Microbe. 2020;S1931-3128(20)30236-5. doi:10.1016/j.chom.2020.04.009
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7172841/pdf/main.pdf



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196557/

The immunopathology of COVID-19.

Entry of SARS-CoV-2 into epithelial/endothelial cells, through binding to ACE2 (and CD147), induces apoptotic and necroptotic pathways resulting in lung injury and release of numerous chemokines that drive recruitment of large numbers of immune cells within the lungs. Dendritic cells (DCs) and plasmacytoid DCs (pDCs, the main source of type I interferon (IFN)), along with macrophages and alveolar neutrophils, promote the innate immune response by secreting alarmins and antiviral or proinflammatory cytokines, as well as presenting the antigen to adaptive immune cells. SARS-CoV-2 may have developed strategies to upregulate the type I IFN response and induce T-cell apoptosis. Recognition of molecular profiles (viral RNA, particles or danger signals) by various Toll-like receptors (TLRs), NOD-like receptors (NLRs) or RIG-I-like receptors (RLRs) activates transcription and release of proinflammatory mediators, such as interleukin (IL) -1β, -6, -18 and tumor necrosis factor (TNF)
-α. These mediators further orient naïve T cells to Th1 or cytotoxic lymphocytes (CTL or CD8+), which in turn secrete quantities of cytokines. A self-feeding proinflammatory cytokine cycle on innate immune cells results in cytokine storm, coagulopathy, and acute respiratory distress syndrome (ARDS). The COVID-19 cytokine storm may be intertwined in two mechanisms: one highly suggestive of macrophage activation syndrome (hemophagocytic lymphohistiocytosis, HLH) driven by IL-1β, and another pattern characterized by IL-6-driven immune dysregulation, which triggers immunoparalysis (decreased HLA-DR on CD14 monocytes) and global lymphopenia.

The second article that is cited is "*COVID-19 gone bad: A new character in the spectrum of the hyperferritinemic syndrome?* "[73], in which the authors analyze the possible epidemiological and molecular mechanisms responsible for hyperinflammation in patients with severe COVID-19, and point out the similarities between this condition and "**hyperferritinemic syndromes**," which would allow COVID-19 severe to be considered a new member of this spectrum of inflammatory conditions.

Hyperferritinemia is the hallmark of "hyperferritinemic syndromes," and over the past decade, a growing body of evidence supports the idea that **elevated circulating ferritin may not only reflect a phase inflammatory response**

[73] Colafrancesco S, Alessandri C, Conti F, Priori R.
COVID-19 gone bad: A new character in the spectrum of the hyperferritinemic syndrome?
Autoimmun Rev. 2020;19(7):102573. doi:10.1016/j.autrev.2020.102573
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7199723/pdf/main.pdf

acute but also play a critical role in inflammation. Ferritin is a major intracellular iron storage protein, and the ratio of its two subunits, H and L, may differ depending on tissue type and physiological state of the cell. **H-ferritin** appears to exhibit pro-inflammatory activity culminating in the induction of the expression of several inflammatory mediators, including IL-1β.

> *Once released, ferritin loses some of its internal iron content giving rise to extremely high serum levels of "**free iron.**" It appears that excess circulating "free iron" detectable in severe inflammatory conditions can deteriorate the inflammatory reaction with the particular ability to induce a **marked pro-coagulant state**. This ability is related to changes in the morphology of red blood cells and fibrin induced by "free iron" that can promote the production of hydroxyl radicals. Oxidative stress on red blood cells and fibrin can induce the production of dense clots responsible for the development of stroke.*

These data were further strengthened by two other publications by Prof. Shoenfeld's research group. [74]

IMMUNE EVASION STRATEGIES OF SARS-COV2

As discussed above, **SARS-Cov-2's suppression of innate immune mechanisms** (inhibition of IFN I and III release and of the proinflammatory cytokines IL-6, IL-1 and TNF-alpha) in infected epithelial cells and, to some extent, infected monocytes/macrophages allow new coronaviruses to proliferate without triggering the innate antiviral response mechanism of these cells.

Subsequently, however, infected cells undergo cell death and release virus particles along with intracellular components that **trigger innate inflammatory mechanisms** through their recognition by PRRs in/on uninfected innate immune cells.

As a result of this immune activation and the resultant **expression of pro-inflammatory cytokines** (including IL-1β, IL-6, TNF-α, etc.), **adaptive immune cells become involved in host defense**, and T lymphocytes play a central role in this second phase of the antiviral response, which includes the release of $^{CD4+}$ T-cell-derived cytokines, $^{CD8+}$ T-cell-mediated cytotoxicity, and B-cell activation resulting in antibody production.

Novel coronaviruses can also escape (partially) these mechanisms through the induction of T-cell apoptosis. However, as already seen, lymphocytes can also become depleted as a result of the expression of proinflammatory cytokines by uninfected innate immune cells that trigger hyperinflammation, observed during the development of a "cytokine storm." [75]

---

[74] Shoenfeld Y.
Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning.
Autoimmun Rev. 2020;19(6):102538. doi:10.1016/j.autrev.2020.102538
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7131471/pdf/main.pdf

Ruscitti Piero, Berardicurti Onorina, Cipriani Paolo, Iagnocco Annamaria, Shoenfeld Yehuda.
Severe hyper-inflammatory COVID-19, another piece in the puzzle of the "hyperferritinemic syndrome."
Rheumatol Point View. 2020 (Submitted for publication)

[75] Cardone M, Yano M, Rosenberg AS and Puig M
Lessons Learned to Date on COVID-19 Hyperinflammatory Syndrome: Considerations for Interventions to Mitigate SARS-CoV-2 Viral Infection and Detrimental Hyperinflammation.
Front. Immunol. (2020) 11:1131. doi: 10.3389/fimmu.2020.01131

Below we go further into the details of the mechanism of evasion of the antiviral response and cellular damage caused by SARS-COV-2 as detailed in the article "*COVID-19: Immunology and treatment options*" [76] as this is useful in understanding the possible therapeutic approaches to be addressed in the various stages of infection.

A) SARS-CoV2 infects airway **epithelial cells** through interaction with the trans-membrane enzyme ACE2 **(a)**.

While RNA viruses usually activate TLR3 and/or 7 in endosomes **(b)** and cytosolic RNA sensors RIG-I and MDA-5 **(c)**, **SARS-COV2 effectively suppresses** the activation of TNF receptor-associated factors (TRAF) 3 and 6, limiting the activation of the transcription factors NFκB and IRF3 and 7 and suppressing **the early pro-inflammatory responses mediated by type I interferons (IFNs) and the pro-inflammatory effector cytokines IL-1, IL-6 and TNF-α** (red symbols).
In addition, coronaviruses inhibit the activation of STAT**(d)** transcription factors, which further limits antiviral response mechanisms.

Overall, this hinders the containment of the virus by blocking the activation of antiviral programs and the recruitment of immune cells**.**

B) **Monocytes/tissue macrophages** express ACE2 significantly lower, making infection through this pathway less likely **(a)**.

However, **immune complexes consisting of ineffective antibodies against e.g., seasonal coronaviruses and viral particles can be engulfed by macrophages through Fcγ receptors** causing them to become infected **(b)**.

In a process known as **antibody-dependent enhancement (ADE),** which will be discussed in detail below, **virions on the one hand inhibit type I IFN signaling in infected macrophages and on the other hand allow pro-inflammatory expression of IL-1, IL-6, and TNF-α, which may contribute to hyperinflammation and cytokine storm syndrome (c, d)**.

Inhibition of type 1 IFN signaling suppresses antiviral programs, while increased expression of IL-1, IL-6, and TNF-α is self-amplified through positive feedback loops **(f)**.

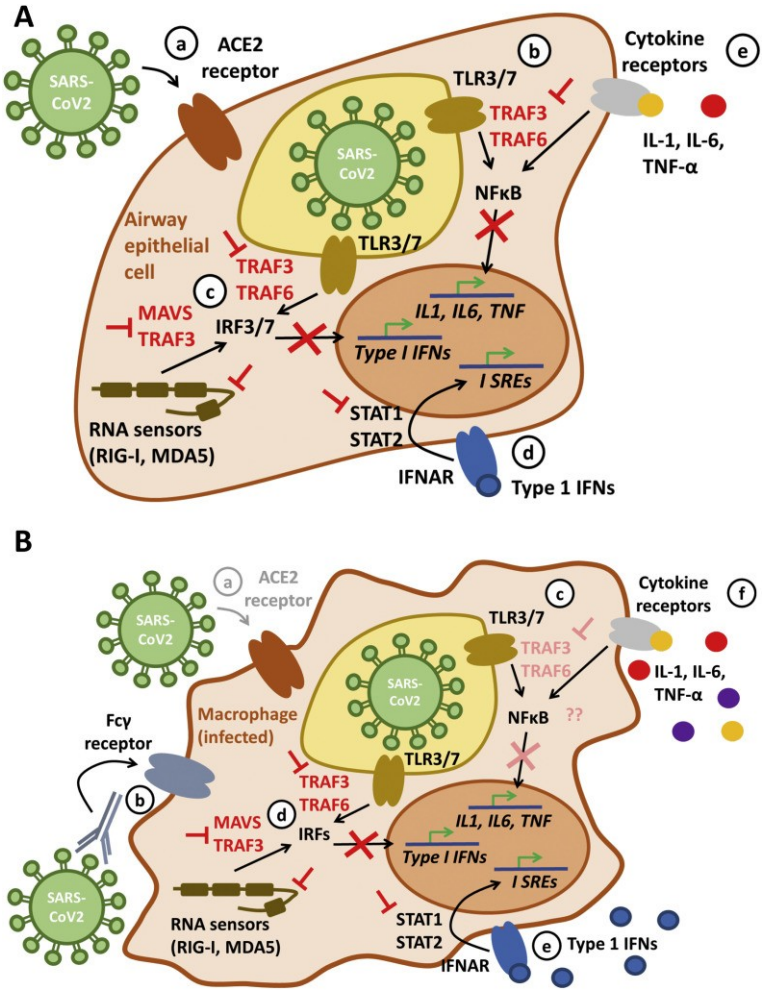
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7272717/pdf/fimmu-11-01131.pdf

Tay, M.Z., Poh, C.M., Rénia, L. et al.
The trinity of COVID-19: immunity, inflammation and intervention.
Nat Rev Immunol 20, 363-374 (2020). https://doi.org/10.1038/s41577-020-0311-8
https://www.nature.com/articles/s41577-020-0311-8.pdf

Girija ASS, Shankar EM and Larsson M (2020) Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? Front. Immunol. 11:1206. doi: 10.3389/fimmu.2020.01206
https://www.frontiersin.org/articles/10.3389/fimmu.2020.01206/full

[76] Felsenstein S, Herbert JA, McNamara PS, Hedrich CM.
COVID-19: Immunology and treatment options.
Clin Immunol. 2020;215:108448. doi:10.1016/j.clim.2020.108448
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf

Bindoli S, Felicetti M, Sfriso P, Doria A.
The amount of cytokine-release defines different shades of Sars-Cov2 infection
Exp Biol Med (Maywood). 2020;1535370220928964. doi:10.1177/1535370220928964
https://journals.sagepub.com/doi/pdf/10.1177/1535370220928964



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf
(see article for more details)

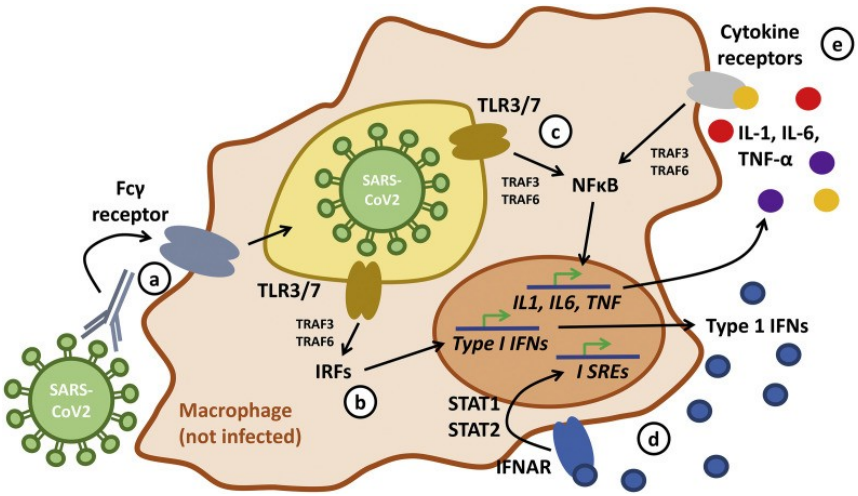
Endothelial and vascular cell damage

Although in apparent contradiction to the mechanisms of immune evasion discussed above, increased innate immune activation, including increased expression of T1IFN, IL-1β, IL-6, and TNF-α contributes consistently to morbidity and mortality in COVID-19, MERS, and SARS.
One possible explanation is the **induction of endothelial and vascular cell damage and cell death following viral replication**.

Virus-induced inflammatory cell death results in the expression of pro-inflammatory cytokines, recruitment and activation of uninfected immune cells.

> *From this we hypothesize that uninfected monocytes/macrophages and neutrophils recruited at the site of infection manifest strong and poorly controlled inflammatory responses, resulting in tissue damage and systemic inflammation, both of which contribute to morbidity and mortality* [77].

[77] Clin Immunol 2020 May;214:108393. doi: 10.1016/j.clim.2020.108393.
The Use of Anti-Inflammatory Drugs in the Treatment of People with Severe Coronavirus Disease 2019 (COVID-19): The Perspectives of Clinical Immunologists from China
Wen Zhang et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102614/pdf/main.pdf



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf

Uninfected monocytes/macrophages escape from the bloodstream and invade the lungs where they detect viral particles and/or cytoplasmic and nuclear components.

These particles are delivered in the form of immune complexes inside the cell **(a)** where they are presented to TLRs, activating NFκB- and/or IRF-dependent pro-inflammatory pathways **(b, c)**.
As **a** result, **uninfected monocytes/macrophages produce significant amounts of pro-inflammatory cytokines (d, e) that recruit additional innate and adaptive immune cells and cause further tissue damage**.

Antibody-dependent enhancement (ADE) and immune complex vasculitis

Another factor believed to contribute to organ damage and adverse outcomes is the **early production of antibodies to coronaviruses**.

Antibody-dependent enhancement (ADE) is a phenomenon that has been shown to contribute to accumulation damage during viral infections.
It has been shown to **promote cellular uptake of viral particles bound in immune complexes through their binding to Fcγ receptors (FcγR)**.

This may contribute to the aforementioned persistent viral replication in immune cells (including newly infected antigen-presenting cells), but also to immune complex-mediated inflammatory responses, which contribute to tissue and organ damage, including acute respiratory distress syndrome (ARDS) [78].

COVID-19-related inflammatory responses could also be induced by **dysregulation of the complement system**, a critical component of host innate immunity. Although intended to prevent viral replication, excessive activation of complement components such as C3, C3a, C5, C5a and serine protease

[78] Fu, Y., Cheng, Y. & Wu, Y.
Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools.
Virol. Sin.(2020). https://doi.org/10.1007/s12250-020-00207-4
https://link.springer.com/content/pdf/10.1007/s12250-020-00207-4.pdf

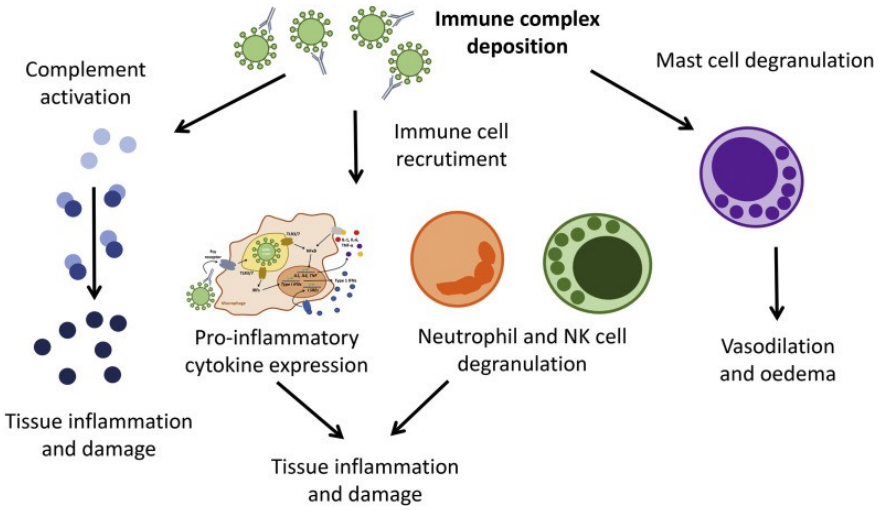
Takada A, Kawaoka Y.
Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications.
Rev Med Virol. 2003;13(6):387-398. doi:10.1002/rmv.405

Jin, Y.; Yang, H.; Ji, W.; Wu, W.; Chen, S.; Zhang, W.; Duan, G.
Virology, Epidemiology, Pathogenesis, and Control of COVID-19.
Viruses 2020, 12, 372.
https://www.mdpi.com/1999-4915/12/4/372/htm

associated lectin (MASP2), has been associated with increased inflammation in both SARS- CoV-1 and SARS-CoV-2 infection.

Overexpression of C3a and C5a can activate alveolar macrophages through their respective receptors leading to the release of pro-inflammatory cytokines such as IL-6. This lack of immune control can exacerbate respiratory and vascular diseases. [79]

A subgroup of patients with COVID-19 is known to develop **vasculitic lesions, vessel occlusion, and infarcts**. Histopathologic reports from tissue sections suggest features associated with immune complex-mediated vasculitis, including infiltration of monocytes and lymphocytes in and around blood vessels, wall thickening, and focal hemorrhage [80].



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pd

[79] Gralinski LE, et al.
Complement activation contributes to severe acute respiratory syndrome coronavirus pathogenesis.
mBio. (2018) 9:e01753-18. doi: 10.1128/mBio.01753-18
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6178621/pdf/mBio.01753-18.pdf

Risitano AM, Mastellos DC, Huber-Lang M, Yancopoulou D, Garlanda C, Ciceri F, et al.
Complement as a target in COVID-19?
Nat Rev Immunol. (2020). doi: 10.1038/s41577-020-0320-7.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7187144/pdf/41577_2020_Article_320.pdf

Magro C, Mulvey JJ, Berlin D, New G, Salvatore S, Harp J, et al.
Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases.
Transl Res. [Preprint]. (2020). doi: 10.1016/j.trsl.2020.04.007. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7158248/pdf/main.pdf

Mastellos DC, Ricklin D, Lambris JD.
Clinical promise of next-generation complement therapeutics.
Nat Rev Drug Disc. (2019) 18:707-29. doi: 10.1038/s41573-019-0031-6
https://www.nature.com/articles/s41573-019-0031-6.pdf

[80] Zhang W, Zhao Y, Zhang F, et al.
The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China.
Clin Immunol. 2020;214:108393. doi:10.1016/j.clim.2020.108393
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102614/pdf/main.pdf

Xu Z, Shi L, Wang Y, et al.
Pathological findings of COVID-19 associated with acute respiratory distress syndrome [published correction appears in Lancet Respir Med. 2020 Feb 25]. Lancet Respir Med. 2020;8(4):420-422. doi:10.1016/S2213-2600(20)30076-X
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164771/pdf/main.pdf

Yao XH, Li TY, He ZC, et al.
[A Pathological Report of Three COVID-19 Cases by Minimal Invasive Autopsies].
Zhonghua Bing Li Xue Za Zhi. 2020;49(5):411-417. doi:10.3760/cma.j.cn112151-20200312-00193

s

IN BRIEF

COVID-19 is the complication of infection with the SARS-Cov-2 coronavirus and manifests with a similar timing and symptomatology as other viruses in the same family (SARS-Cov-1, MERS)

From studying the immune mechanism leading to the severe/fatal complication, it has been observed that multi-organ failure and death are linked to an exaggerated response of the immune system to the virus. This occurs through the induction of multiple mechanisms:

SARS-Cov-2 a f t e r entering cells induces an ***initial wave of proinflammatory cytokines*** *(hypercytokinemia) produced by infected and innate response cells.*

This is followed subsequently by a ***state of immunosuppression*** *due to virus-induced blockade of interferon I and III production in epithelial cells and depletion/depletion of T lymphocytes that promote persistence of viral infection.*

If the infection is not resolved by innate immunity there is a ***second wave of cytokines,*** *caused by an exaggerated response by both the innate and adaptive immune systems in an attempt to thwart viral replication, which can lead* ***to cytokine storm syndrome and its subtypes*** *(IL-1ß-mediated macrophage activation syndrome, IL-6-mediated cytokine release syndrome, and hyperferritinemia syndrome).*

When the virus infects epithelial cells bearing the surface ACE2 receptor, it is able to evade the antiviral immune response by blocking the production of type I interferons and inflammatory cytokines (IL-1, IL-6 and TNF-α).
Excessive viral replication and epithelial cell death summon cells of the innate immune system from the bloodstream that internalize the virus and activate the inflammatory response, with the aim of blocking virus replication, which, however, leads to more tissue damage.

When the virus infects immune system cells (monocytes and tissue macrophages) it enters as a complex with antibodies (immunocomplex) through binding to the Fcy receptor present on the surface of these cells, blocks the production of interferons and other antiviral mediators but stimulates high production of proinflammatory cytokines leading to cytokine storm and severe tissue damage (antibody-mediated disease enhancement - ADE)

The mechanism of ADE in the induction of COVID-19 [81]

Sensitization of the humoral immune response to pathogenic viruses and production of antiviral antibodies are part of the host's antiviral repertoire.
Paradoxically, **for a number of viral pathogens, under certain conditions, antibodies provide a means for improved entry and increased virus replication in numerous cell types**.

> *Known as **antibody-dependent enhancement of infection (ADE)**, the phenomenon occurs when virus-antibody immune complexes interact with cells that are either complement-bound (IgM-virus binding to the complement receptor (CR) on macrophages) or that have Fc receptors on their membrane, promoting internalization of the virus and increasing its infection.*

Frequently associated with exacerbation of viral disease, the ADE of infection presents a serious obstacle to prevention of viral disease by vaccination and is believed to be partially responsible for the adverse effects of newer antiviral therapies such as intravenous immunoglobulins (monoclonal antibodies and hyperimmune sera).

There is a growing body of work examining the intracellular signaling pathways and epitopes responsible for mediating ADE to aid in the rational design of antiviral strategies. Despite studies including in vitro studies confirming ADE as a hallmark of infection for a growing number of viruses, difficulties remain in understanding the molecular mechanisms of ADE and its effects on viral pathogenesis.

Two models have been proposed to describe the kinetics of virus neutralization by antibodies.
The first is a single-hit [**single-hit] model**, in which the binding of a single antibody molecule to a critical site on the virion is sufficient to neutralize the virus, while the second**, more widely accepted, multi-hit [multi-hit] model** proposes that neutralization is achieved only once the single virion is bound by enough antibodies to exceed the stoichiometric threshold of neutralization [82].

Since the number of epitopes available for a specific antibody on the virion is known, the stoichiometry of neutralization can be calculated. However, the stoichiometric threshold of neutralization is determined by antibody affinity and epitope accessibility. For example, antibodies specific for poorly accessible epitopes require a higher concentration to exceed the occupancy threshold for neutralization. [83]

Paradoxically, however, it is now recognized that sub-neutralizing concentrations of antibodies, under certain conditions, can act to enhance viral infection by promoting viral entry into target cells.

[81] Kulkarni R. (2020) Antibody-Dependent Enhancement of Viral Infections. In: Bramhachari P. (eds) doi: 10.1007/978-981-15-1045-8_2

Dynamics of Immune Activation in Viral Diseases. Springer, Singapore
https://link.springer.com/content/pdf/10.1007%2F978-981-15-1045-8_2.pdf

Immunol Rev 2015 Nov;268(1):340-64. doi: 10.1111/imr.12367.
Fc Receptors in Antibody-Dependent Enhancement of Viral Infections
Adam Taylor 1, Suan-Sin Foo 1, Roberto Bruzzone 2 3, Luan Vu Dinh 4, Nicholas J C King 4, Suresh Mahalingam
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7165974/pdf/IMR-268-340.pdf

[82] J Gen Virol 2002 Sep;83(Pt 9):2091-2108. doi: 10.1099/0022-1317-83-9-2091.
Occupancy and Mechanism in Antibody-Mediated Neutralization of Animal Viruses
P J Klasse 1, Q J Sattentau 1
https://www.microbiologyresearch.org/content/journal/jgv/10.1099/0022-1317-83-9-2091#tab2

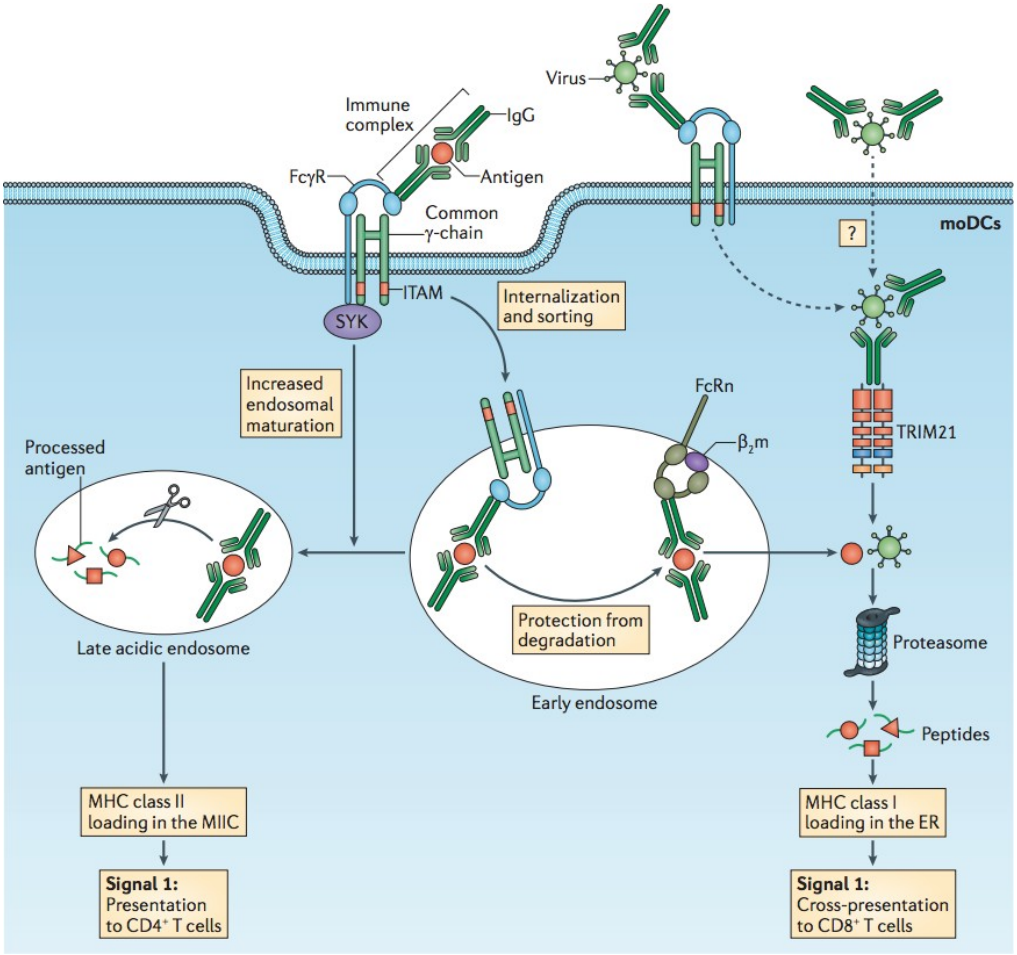
[83] PLoS Pathog 2011 Jun;7(6):e1002111. doi: 10.1371/journal.ppat.1002111.
A Dynamic Landscape for Antibody Binding Modulates Antibody-Mediated Neutralization of West Nile Virus
Kimberly A Dowd 1, Christiane A Jost, Anna P Durbin, Stephen S Whitehead, Theodore C Pierson
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3128118/pdf/ppat.1002111.pdf

This mechanism of enhanced virus uptake (ADE), is thought to occur when the virus is bound by ***nonneutralizing IgG*** *antibodies* ***or sub-neutralizing concentrations of IgG antibodies*** *that facilitate cell entry through a mechanism dependent on the Fcγ* ***receptor*** *(FcγR).*

The function of Fcγ receptors in dendritic cells and macrophages [84]

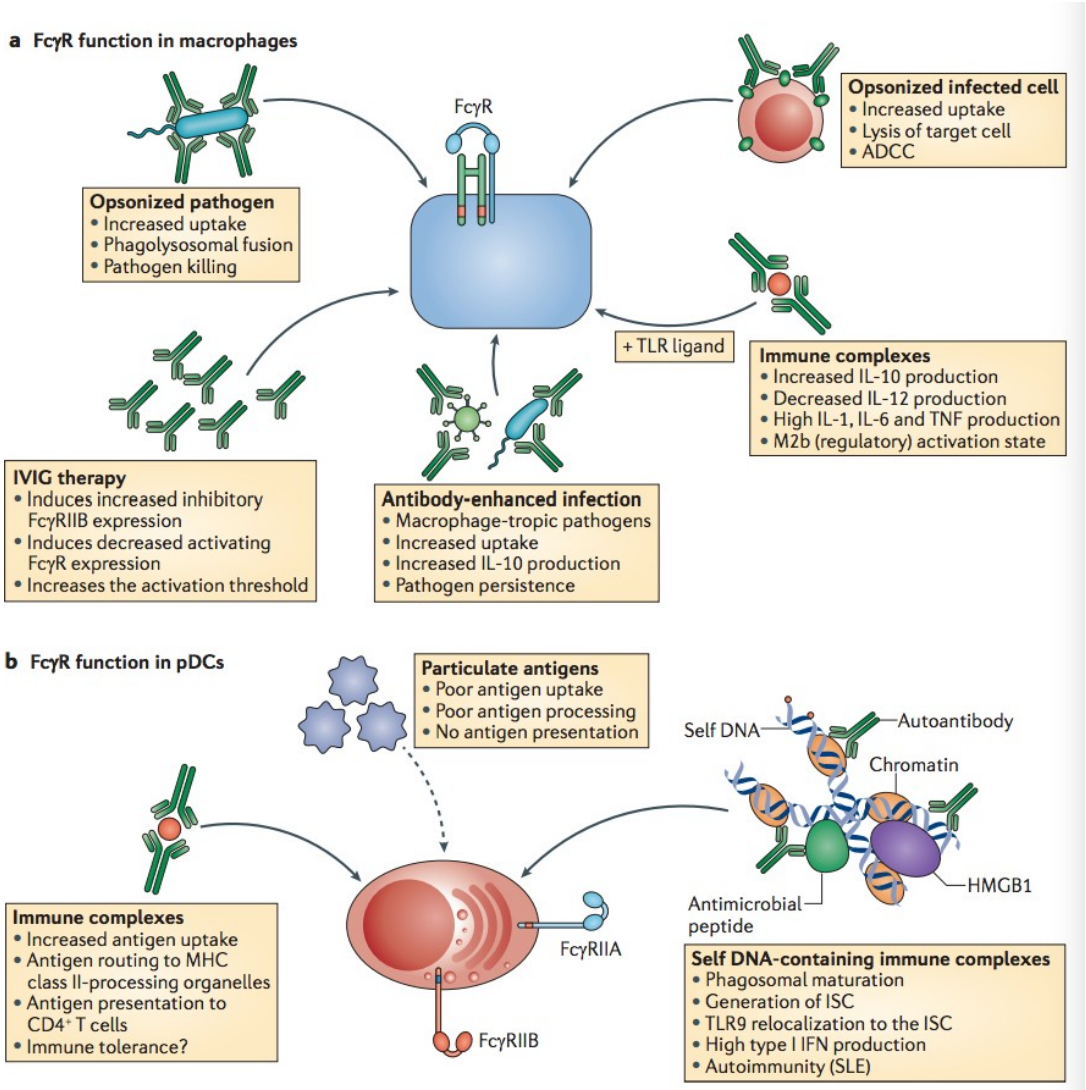
Fc gamma (γ) receptors (FcγR) belong to a large family of proteins that include classical membrane-bound surface receptors, atypical intracellular receptors, and cytoplasmic glycoproteins**. Classical receptors can be divided into inhibitory (FcγRIIB) and activating (FcγRI, FcγRIIA, FcγRIIC, FcγRIIIA and FcγRIIIB)**.

Immune complexes that are internalized through FcγRs present on antigen-presenting cells (particularly dendritic cells) are an important part of antigen presentation for the development of effective immune responses.
This process also increases the efficiency of T-cell activation, particularly in response to low antigen concentrations.



Efficient processing of antibody-bound antigens by moDC (monocyte-derived dendritic cells). β2m, β2 microglobulin; ER, endoplasmic reticulum.

[84] Guilliams, M., Bruhns, P., Saeys, Y. et al.
The function of Fcγ receptors in dendritic cells and macrophages.
Nat Rev Immunol 14, 94-108 (2014). https://doi.org/10.1038/nri3582
https://www.researchgate.net/publication/259826273_The_function_of_Fc_gamma_receptors_in_dendritic_cells_and_macrophages/link/0a85e53c9011b7e1790000/download



FcγR-mediated macrophages and pDC activation. **(A)** Antibody-coated pathogens (opsonized pathogens) are often killed more efficiently by macrophages because of the Fc receptor for IgG-mediated activation (FcγR) of macrophages, which induces increased immunoreceptor-dependent uptake of the tyrosine activation motif (ITAM) and increases phagolysosomal fusion, thus producing more efficient killing of pathogens. Similarly, opsonized infected cells can be killed through a mechanism called antibody-mediated cellular cytotoxicity (ADCC). However, immune complexes induce a particular macrophage activation state called the M2b macrophage activation state (regulatory), which is characterized by increased production of interleukin-10 (IL-10), IL-1, IL-6, tumor necrosis factor (TNF) and decreased production of IL-12. This state of M2b activation may facilitate the survival of macrophage-tropic pathogens, such as Leishmania spp. which have developed strategies to subvert macrophage function and utilize macrophages as a preferred cellular niche. Increased uptake of these macrophage-tropic pathogens results in antibody enhancement of infection. Finally, manipulation of macrophage activation by immune complexes has suggested that they are one of the main mechanisms behind intravenous immunoglobulin therapy (IVIG therapy). A high dose of immune complexes is thought to induce higher expression of inhibitory FcγRIIB and lower expression of activating FcγR, which produces an increased activation threshold for macrophages.
B) plasmacytoid dendritic cells (pDCs) have poor abilities to capture and present particulate antigens on CD4+ T cells compared with conventional DCs (cDCs) (dashed arrow). Antigens with antibody coating are more efficiently taken up by pDCs and subsequently more efficiently targeted to MHC class II processing organelles than cDCs, which results in better antigen presentation to CD4+ T cells. Because pDCs have been shown to be tolerogenic at steady state, we hypothesize that, in the absence of danger signals, FcγR-mediated uptake and presentation of antigen immunocomplexes by pDCs induces the development of tolerance. PDCs have also been implicated in the pathogenesis of systemic lupus erythematosus (SLE). In patients with SLE, immune complexes containing self-DNA associated with antimicrobial peptides, high mobility group 1 box protein (HMGB1) and autoantibodies are recognized by FcγR on pDCs. This triggers phagosomal maturation and generation of the interferon signaling compartment (IFN) (ISC). The triggering of FcγR by self-DNA-containing immune complexes has been shown to be crucial in the relocalization of Toll-like receptor 9 (TLR9) to ISC, which then results in high levels of type I IFN production by pDCs and exacerbates the autoimmune response in SLE patients.

Please refer to the text of the article for further discussion

Figures taken from
https://www.researchgate.net/publication/259826273_The_function_of_Fc_gamma_receptors_in_dendritic_cells_and_macrophages/link/0a85e53c9011b7e1790000/download

The neonatal Fc receptor (FcRn) [85]

Among the atypical FcγRs, the neonatal Fc receptor (FcRn) has gained increasing interest given its intimate influence on IgG biology and its ability to bind to albumin as well.

FcRn is distinguished by being a **beta (β)-2-microglobulin (β2m) protein** structurally related to the major histocompatibility complex class I (MHC-I) family, which is, however, unable to present antigenic peptides to T cells.

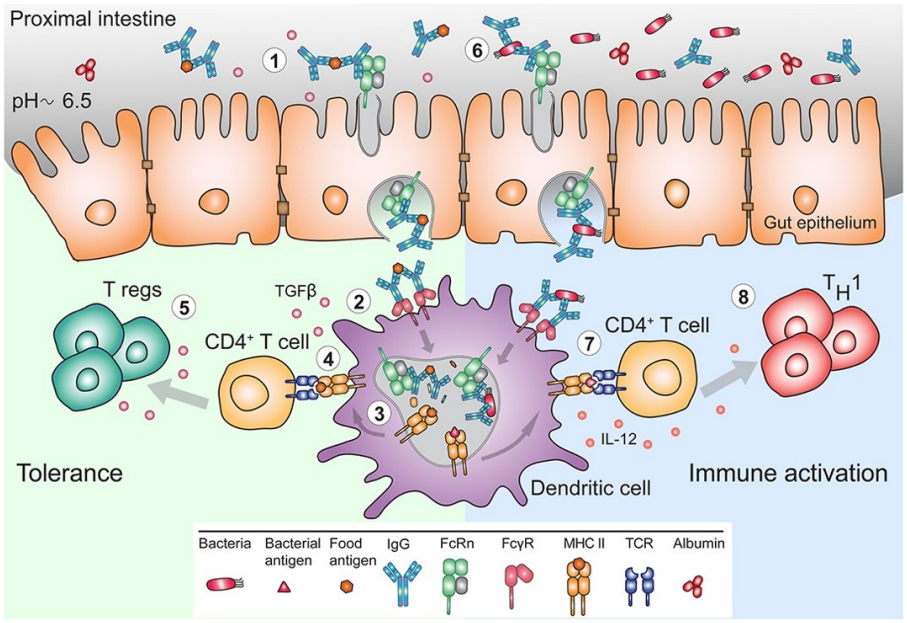
Furthermore, FcRn has **a nearly ubiquitous expression profile**, possesses a predominantly intracellular localization, is monomorphic, and **binds to IgG another structurally and functionally unrelated protein, albumin**.

While IgG subtypes are critical in immune responses, albumin functions as a carrier protein in addition to being an important regulator of oncotic blood pressure (i.e., reabsorption into the bloodstream of electrolytes, water, and substances produced by tissue catabolism) .
Despite these differences, IgG and albumin are the two most abundant serum proteins that possess a long half-life in serum because of their interaction with FcRn, which rescues them from intracellular degradation through a **cellular recycling mechanism**.

Another of the functions of FcRn is to **transport IgG from mother to offspring** thus providing the naïve and immature immune system of the newborn with the experience and protection developed in the adult progenitor.
This functional expression of FcRn and its ability to transcyte IgG (transcytosis: "shuttle" transport that occurs within the cell) is not limited to the newborn but **persists throughout life and allows for the targeted release of IgG at sites where the presence of this type of antibody strengthens immunity, a process widely exploited by IgG-based therapy** .

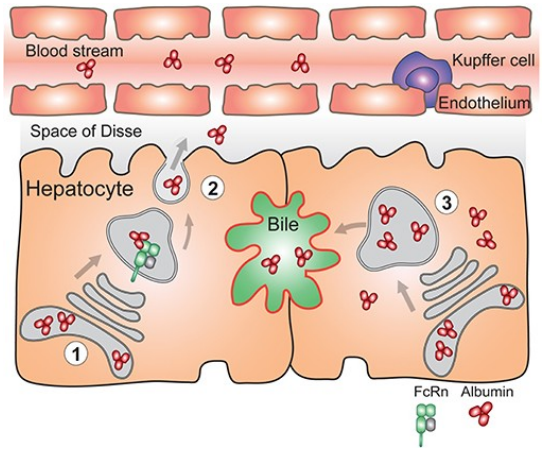
Finally, the functions of FcRn are differentially determined depending on whether the IgG is a single molecule, and therefore monomeric, or is present as an immune complex (IC).
In the latter case, FcRn has been shown to critically regulate innate immune responses as well as the processing and presentation of antigens contained in IgG IC.

[85] For further discussion see bibliography attached to this article Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I and Blumberg RS The Neonatal Fc Receptor (FcRn): A Misnomer?
Front. Immunol. (2019) 10:1540. doi: 10.3389/fimmu.2019.01540
https://www.frontiersin.org/articles/10.3389/fimmu.2019.01540/full



Taken from https://www.frontiersin.org/articles/10.3389/fimmu.2019.01540/full

FcRn mediates the bidirectional transport and immune response of IgG and to IgG immune complexes in the intestine. **(1)** The pH of the mucosal surface of the proximal intestine may be slightly acidic, so that FcRn can bind maternal IgG and IC IgG already on the cell surface and transcyte them on the basolateral side. **(2)** APC like DC, can actively bind and internalize IC IgG via FcγR. **(3)** FcRn in APC helps in antigen processing and delivery of IC IgG to antigen loading compartments where peptides derived from these complexes can be loaded onto MHC II for presentation on $^{CD4+}$ T cells. **(4)** In early infancy, presentation of the antigen-derived peptide on MHC II in the presence of other factors derived from breast milk creates **(5)** a tolerogenic environment to $^{CD+}$ T cells. In these cases, the expression of FcRn by APCs is crucial for the induction of $^{CD4+\ Foxp3+}$ regulatory T cells (Treg). **(6)** In adulthood, during infection, pathogen-derived antigens bound by luminal IgG will be transported across the mucosa in an FcRn-dependent manner and **(7)** delivered to APCs, which process and present antigens, **(8)** for subsequent activation of immune responses. Therefore, FcRn in the intestine can release IgG into the lumen and also transcytate monomeric IgG or IC IgG in the reverse direction into the lamina propria. This process ensures the specific release of luminal antigens in the form of IgG IC to mucosal dendritic cells that can then regulate immune responses.



Taken from https://www.frontiersin.org/articles/10.3389/fimmu.2019.01540/full

FcRn in the liver is essential for vector transport of albumin into the bloodstream. (1) Hepatocytes are polarized epithelial cells of which the apical side (red) faces the bile duct and the basolateral side (black) faces the fenestrated sinusoidal endothelium. The sinusoidal endothelium is populated by liver-specific macrophages called Kupffer cells. Albumin is produced exclusively by hepatocytes. **(2)** FcRn in hepatocytes is required for the release of newly synthesized albumin on the basolateral side of the cells and subsequent secretion of albumin into the bloodstream (left) **(3)** Absence of FcRn expression in hepatocytes results in increased levels of albumin in the bile, its intracellular accumulation, and lower circulating albumin levels (right). For simplicity, FcRn-mediated albumin recycling in hepatocytes is not depicted. Expression of FcRn in the liver has two main purposes: to maintain monomeric IgG and albumin in the circulation and to direct albumin to the circulation rather than to the bile.

Other important anatomical localizations of FcRn are:

- **the kidneys, where it is** expressed in podocytes (filtration barrier cells) for the purpose of removing IgG and IgG complexes from the filter membrane in order to provide protective IgG in the urinary tract (its absence causes nephritis), and is also expressed by the epithelial cells of the proximal tubules where it assists in albumin reabsorption.

- **Endothelium**: Endothelial cells line the entire vascular system and control the passage of numerous cells and molecules in and out of the circulation and are a major cellular location where FcRn controls the levels and persistence of IgG and albumin. Expression of FcRn by these cells is well documented in intracellular vesicular compartments.

- **The blood-brain barrier:** Microscopy studies have shown that FcRn is expressed in the cerebral microvascular endothelium and choroid plexus epithelium, where it has been suggested to mediate the active transport of IgG from the brain into the bloodstream

- **Cells of hematopoietic origin:** FcRn is expressed by monocytes, macrophages (both tissue-resident and splenic), neutrophils, DC and B lymphocytes but not by T cells or natural killer (NK) cells. The presence of FcRn mainly in antigen-presenting cells (APCs) indicates that it might provide functional benefits to these cells and implicates FcRn directly in IgG-mediated immune responses

---

The interaction between the exposed Fc part of the antibody constituting the antibody-virus immune complex with the FcγR present on the membrane of myeloid cells such as monocytes, macrophages, dendritic cells (DCs), and some granulocytes typically leads to phagocytosis, resulting in an increase in the number of infected cells-the so-called **extrinsic ADE.**

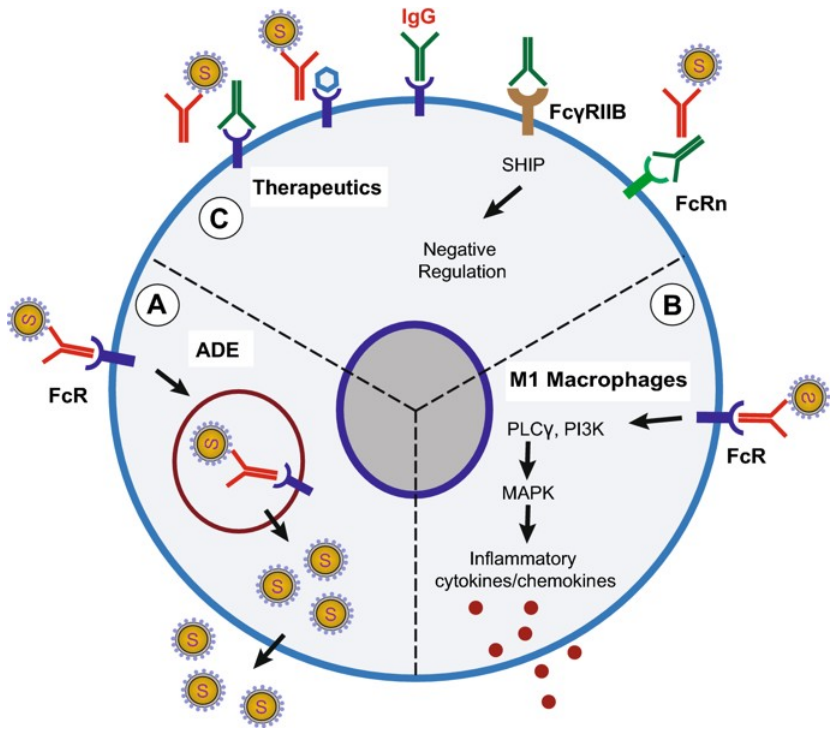
This form of ADE thus requires a **prior sensitization of the humoral immune response**, whereby circulating antibodies produced during primary infection recognize and bind to a heterologous serotype of the virus and increase viral infectivity through internalization of virus-antibody immune complexes by FcγR-bearing cells, rather than promoting viral neutralization.

Once internalized, these immune complexes can modulate the cells' innate antiviral responses to increase virus production in each cell, a process called **intrinsic ADE**.

Extrinsic and intrinsic ADE together are thought to induce the massive release of inflammatory and vasoactive mediators that ultimately contribute to disease severity. [86]

[86] Lancet Infect Dis. 2010;10(10):712-722. doi:10.1016/S1473-3099(10)70166-3
Intrinsic antibody-dependent enhancement of microbial infection in macrophages: disease regulation by immune complexes.
Halstead SB, Mahalingam S, Marovich MA, Ubol S, Mosser DM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3057165/

Trends Immunol. 2003;24(4):165-168. doi:10.1016/s1471-4906(03)00065-6
Suppression of antiviral responses by antibody-dependent enhancement of macrophage infection.
Suhrbier A, La Linn M.



Tratta da https://link.springer.com/article/10.1007/s12250-020-00207-4
Antibody-dependent enhancement (ADE) mediated by Fc receptor of viral infection and inflammatory responses.
A) ADE occurs when antiviral neutralizing antibodies cannot completely neutralize the virus. Instead, the virus-NAb complex attaches to the Fc receptor (FcR), leading to viral endocytosis and infection of target cells. The result is increased overall virus replication and increased disease severity.
B) Binding of the virus-NAb complex to FcR can also activate proinflammatory signaling, shifting macrophage responses to the accumulation of proinflammatory macrophages (M1 or classically activated) in the lungs. M1 macrophages secrete inflammatory cytokines such as MCP-1 and IL-8, causing lung injury.
C) Potential therapies based on targeting Fc receptors to block SARS-CoV-2-induced inflammatory responses. From left to right, FcR can be blocked using specific anti-Fc antibodies, small molecules, or intravenous immunoglobulin (IVIG). The inhibitory FcR, FcγRIIB, can also be targeted to inhibit FcR activation. FcRn can also be blocked by specific antibodies or competitively inhibited through binding of IVIGs.

Studies of the antigenic determinants mediating ADE allowed the role of epitopes of structural surface proteins to be demonstrated; indeed, antibodies generated against virus envelope proteins were seen to potentiate infection in vitro.

Examples of such epitopes include the spike protein of SARS-CoV [87].

[87] Yip MS, et al.
Antibody-dependent enhancement of SARS coronavirus infection and its role in the pathogenesis of SARS.
Hong Kong Med J. 2016;22(3 Suppl 4):25-31.
https://www.hkmj.org/system/files/hkm1603sp4p25.pdf

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

J Virol 2011 Oct;85(20):10582-97 doi: 10.1128/JVI.00671-11
Anti-severe Acute Respiratory Syndrome Coronavirus Spike Antibodies Trigger Infection of Human Immune Cells via a pH- And Cysteine Protease-Independent FcγR Pathway
Martial Jaume et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187504/pdf/zjv10582.pdf

Biochem Biophys Res Commun 2014 Aug 22;451(2):208-14 doi:10.1016/j.bbrc.2014.07.090.
Antibody-dependent SARS Coronavirus Infection Is Mediated by Antibodies Against Spike Proteins
Sheng-Fan Wang et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7092860/pdf/main.pdf

Virus (family)	Mechanism of ADE	Clinical significance of ADE	Implications for vaccines
Influenza virus (family *Orthomyxoviridae*)	1. Antibodies against viral hemagglutinin (HA) and neuraminidase (NA) mediate virus uptake via FcRs into macrophages, possibly leading to increased antigen presentation and T-cell activation 2. Enhancement of viral fusion by anti-HA2 antibodies suggested	Increased risk of medically attended illness among individuals with prior influenza-like illness during 1918 pandemic, and among seasonal influenza vaccine recipients during 2009 H1N1 pandemic, is suggestive of ADE	ADE suggested to be responsible for vaccine-associated enhanced respiratory disease in immunized pigs and ferrets, but mechanism not yet clearly understood
Respiratory syncytial virus (family *Paramyxoviridae*)	Antibodies against viral glycoproteins G and F mediate virus uptake via FcRs into monocytes, macrophages, dendritic cells leading to immune response modulation	Clinical relevance remains unclear in light of contradictory reports regarding association of maternal antibody-induced ADE with severe disease in infants	Enhanced disease in formalin-inactivated RSV vaccine recipients initially attributed to ADE, but other mechanisms also recently suggested
Ebola virus (family *Filoviridae*)	Antibodies against viral glycoprotein (GP) promote virus internalization by FcR-mediated or complement component C1q/C1q receptor-mediated process into monocytes/macrophages, endothelial, epithelial cells, and hepatocytes	Clinical relevance not yet understood	Vaccine-related ADE not yet demonstrated, but remains a concern. Avoiding induction of known infectivity-enhancing antibodies, while retaining T-cell epitopes in vaccines proposed for ADE mitigation
SARS—Coronavirus (family *Coronaviridae*)	Antibodies against viral spike (S) glycoprotein mediate virus uptake via FcRs into immune cells such as B cells, monocytes, macrophages	Clinical relevance still debated	Impact on vaccine safety not yet understood
Chikungunya virus (family *Togaviridae*)	FcR-mediated internalization into B cells, monocytes suggested	Clinical relevance not yet understood	Impact on vaccine safety not yet understood

EDI envelope domain I, *EDII* envelope domain II, *prM* precursor membrane, *FcR* Fc receptor, *LILR-B1* leukocyte immunoglobulin-like receptor B1, *TLR* Toll-like receptor, *RIG-I* retinoic acid-inducible gene I, *MDA5* melanoma differentiation-associated antigen 5, *DHF* dengue hemorrhagic fever, *DSS* dengue shock syndrome, *EDIII* envelope domain III

Tratta da https://link.springer.com/content/pdf/10.1007%2F978-981-15-1045-8_2.pdf

In deceased SARS patients and in animal models, extensive lung damage has been associated with elevated initial viral loads, increased accumulation of inflammatory monocytes/macrophages in the lungs, and elevated levels of serum proinflammatory cytokines (i.e., IL-1, IL-6, IL -8, CXCL-10, and MCP1), a situation that as already seen has also been found in severe cases of COVID-19.

The development of acute respiratory disease coincided with antiviral IgG seroconversion in 80% of patients. In addition, it was found that patients who developed neutralizing anti-S antibody more rapidly were more likely to die from the disease; it took an average of only 14.7 days for deceased patients to reach their peak levels of neutralizing antibody activity, compared with 20 days for cured patients.
In addition, **significantly higher levels of anti-Spike antibodies were detected in the sera of patients who died** (n = 6) during infection than in the sera of cured patients (n = 8).

Considering these results, ***it is plausible that anti-Spike IgG promoted the production of proinflammatory cytokines, leading to cytokine storm syndrome via FcγRI and/or FcγRIIA receptors.*** [88]

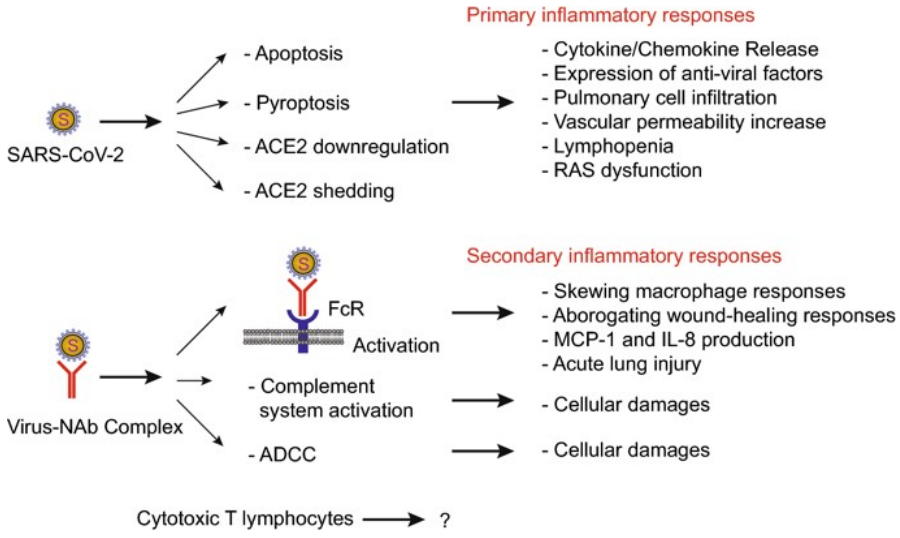
Virol J 2014 May 6;11:82 doi: 10.1186/1743-422X-11-82.
Antibody-dependent Infection of Human Macrophages by Severe Acute Respiratory Syndrome Coronavirus
Ming Shum Yip et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4018502/pdf/1743-422X-11-82.pdf

[88] Host Microbe. 2016. 19(2):181-193. doi: 10.1016/j.chom.2016.01.007.
Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice Cell
Channappanavar R, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4752723/pdf/main.pdf

Lancet. 2003. 361(9371):1767-1772. doi: 10.1016/S0140-6736(03)13412-5.
Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study.
Peiris JS, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7112410/pdf/main.pdf

J Med Virol 78:1-8 (2006)
Antibody responses against SARS coronavirus are correlated with disease outcome of infected individuals.
Zhang L, Zhang F, Yu W, He T, Yu J, Yi CE, Ba L, Li W, Farzan M, Chen Z, Yuen KY, Ho D
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7166884/pdf/JMV-78-1.pdf

JCI Insight. 2019. 4(4). pii:doi:10.1172/jci.insight.123158.
Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection.



Tratta da https://link.springer.com/article/10.1007/s12250-020-00207-4

Possible mechanisms of inflammatory response mediated by SARS-CoV-2. Based on previous studies on SARS-CoV, we separate inflammatory responses in SARS-CoV-2 infection into primary and secondary responses. Primary inflammatory responses occur early after viral infection, before the appearance of neutralizing antibodies (NAb). These responses are mainly driven by active viral replication, virus-mediated ACE2 upregulation and dispersion, and host anti-viral responses. Secondary inflammatory responses begin with the generation of adaptive immunity and NAb. The virus-NAb complex can also trigger FcR-mediated inflammatory responses and acute lung injury (ADE). ADCC: antibody-dependent cell-mediated cytotoxicity

Liu L, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478436/pdf/jciinsight-4-123158.pdf

THE IMMUNOPATHOLOGY OF SARS VACCINES AND ADE FROM SARS-COV-2 [89]

Vaccine immunopathology

During testing of early experimental SARS-CoV vaccines, following vaccination and challenge testing (reinfection), some experimental animals developed lung or liver histopathology characterized by significant tissue infiltration of lymphocytes, monocytes, and eosinophils [90].

A key feature of eosinophilic immunopathology is the appearance of **inflammatory infiltrates consisting of mononuclear cells, particularly eosinophils,** in histopathologic sections of the lungs or livers of vaccinated experimental animals following challenge testing with infectious viruses.

The importance of pulmonary eosinophils has led some researchers to conclude that immune enhancement (ADE) occurs through Th2-type immunity suggesting hypersensitivity to vaccine components [91].
Indeed, a paper titled "*Consensus Considerations on Assessing the Risk of Disease Enhancement with COVID-19 Vaccines: Outcome of an Epidemic Preparedness Coalition from the CEPI Alliance* "[92] questioned the use of aluminum and other adjuvants that could promote Th2 responses.

However, some of the published literature questions the primary role of Th2 cells in directly promoting immune enhancement.

In fact, it has been seen that **ADE occurs mainly as a result of the use of vaccines with viral vectors** expressing coronavirus antigens.

---

[89] Nat Rev Immunol. 2020 Apr 21. doi: 10.1038/s41577-020-0321-6.
The potential danger of suboptimal antibody responses in COVID-19.
Iwasaki A1,2, Yang Y3.
https://www.nature.com/articles/s41577-020-0321-6

Swiss Med Wkly. 2020 Apr 16;150:w20249. doi: 10.4414/smw.2020.20249.
Is antibody-dependent enhancement playing a role in COVID-19 pathogenesis?
Negro F1.
https://smw.ch/article/doi/smw.2020.20249

Medical Countermeasures Analysis of 2019-nCoV and Vaccine Risks for Antibody-Dependent Enhancement (ADE) (2/27/2020).
Ricke, Darrell and Malone, Robert W.,
http://dx.doi.org/10.2139/ssrn.3546070

Front Microbiol. 2018 Dec 5;9:2991. doi: 10.3389/fmicb.2018.02991.
Viral-Induced Enhanced Disease Illness.
Smatti MK, Al Thani AA, Yassine HM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6290032/pdf/fmicb-09-02991.pdf.

[90] Nat Rev Immunol 2005 Dec;5(12):917-27. doi: 10.1038/nri1732.
Immunopathogenesis of Coronavirus Infections: Implications for SARS
Stanley Perlman 1, Ajai A Dandekar
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7097326/pdf/41577_2005_Article_BFnri1732.pdf

[91] J Virol 2011 Dec;85(23):12201-15. doi: 10.1128/JVI.06048-11.
A Double-Inactivated Severe Acute Respiratory Syndrome Coronavirus Vaccine Provides Incomplete Protection in Mice and Induces Increased Eosinophilic Proinflammatory Pulmonary Response Upon Challenge
Meagan Bolles et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3209347/pdf/zjv12201.pdf

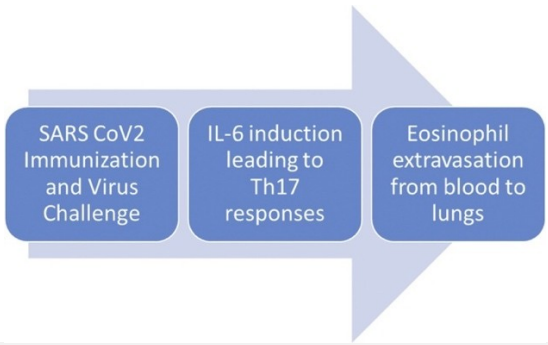
PLoS One 2012;7(4):e35421. doi: 10.1371/journal.pone.0035421.
Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge With the SARS Virus
Chien-Te Tseng et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3335060/pdf/pone.0035421.pdf

[92] https://media.tghn.org/articles/FINAL2 summary_considerations_ED_with_COVID-19_vac-March_20_20.pdf

In at least one study, it was noted that mice showing immune enhancement following challenge testing with SARS virus overregulated Th1 cytokines and underregulated anti-inflammatory cytokines such as IL- 10, despite exhibiting eosinophilic infiltrates. [93]

New information has revealed a critical role of Th17 for host inflammatory responses in the pathogenesis of COVID-19 pneumonia and edema.

In particular, **IL-17 can induce pulmonary eosinophilic responses and allergic diseases** by promoting eosinophil production from the bone marrow and recruitment and extravasation into the lungs. [94]
Th17 cells differentiate in part through the actions of IL-6, which has been shown to play an important role in lung pathology associated with SARS infection [95]



Excerpted from https://www.sciencedirect.com/science/article/pii/S1286457920300721?via%3Dihub#bbib16
Mechanisms of eosinophilic immunopathology related to vector-borne viral coronavirus vaccines.

[93] Nat Rev Immunol 2020 Apr 28;1-2. doi: 10.1038/s41577-020-0323-4.
COVID-19 Vaccine Design: The Janus Face of Immune Enhancement
Peter J Hotez 1 2 3 4, David B Corry 5 6, Maria Elena Bottazzi
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7187801/pdf/41577_2020_Article_323.pdf

JCI Insight 2019 Feb 21;4(4):e123158. doi: 10.1172/jci.insight.123158.
Anti-spike IgG Causes Severe Acute Lung Injury by Skewing Macrophage Responses During Acute SARS-CoV Infection
Li Liu et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478436/

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

[94] J Microbiol Immunol Infect 2020 Mar 11;S1684-1182(20)30065-7. doi: 10.1016/j.jmii.2020.03.005.
TH17 Responses in Cytokine Storm of COVID-19: An Emerging Target of JAK2 Inhibitor Fedratinib
Dandan Wu 1, Xuexian O Yang 2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7156211/

J Immunol 2008 Apr 15;180(8):5625-35. doi: 10.4049/jimmunol.180.8.5625.
Molecular Mechanisms of Cytokine and Chemokine Release from Eosinophils Activated by IL-17A, IL-17F, and IL-23: Implication for Th17 Lymphocytes-Mediated Allergic Inflammation
Phyllis F Y Cheung 1, Chun K Wong, Christopher W K Lam
https://www.jimmunol.org/content/180/8/5625.long
[95] Eur J Immunol. 2010 Jul;40(7):1830-5. doi: 10.1002/eji.201040391.
IL-6: regulator of Treg/Th17 balance.
Kimura A, Kishimoto T.
https://onlinelibrary.wiley.com/doi/full/10.1002/eji.201040391

Virology 2007 Sep 1;365(2):324-35. doi: 10.1016/j.virol.2007.04.009.
Nucleocapsid Protein of SARS-CoV Activates interleukin-6 Expression Through Cellular Transcription Factor NF-kappaB
Xue Zhang 1, Kailang Wu, Di Wang, Xin Yue, Degui Song, Ying Zhu, Jianguo Wu
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7103332/

Despite the fact that COVID-19 results in severe pulmonary dysfunction, the contribution of cytokine storm, enhancement due to Th17 or pulmonary eosinophilia on end-stage mortality is still not fully elucidated.

In fact, **new evidence suggests that the severe morbidity and mortality associated with COVID-19 may be more due to cardiac dysfunction rather than pulmonary failure**, as severe cardiac failure may be the main cause of respiratory and other organ failure leading to severe life-threatening disease.

In this regard, myocarditis heart failure and cardiomyopathy has also been linked to IL-17-producing T cells and IL-17-promoting cytokines [96]

Furthermore, **it is known that platelets, in addition to their common function in regulating thrombosis and hemostasis, also contribute to tissue inflammation that affects adaptive immunity**.
Platelets have pro-inflammatory and regulatory mediators stored in their α granules and dense granules, which are rapidly released at sites of inflammation or tissue injury, and platelet-derived factors are highly effective in directly or indirectly modulating the function of various T-cell subgroups, particularly Th17 cell differentiation in the early phase of inflammation and platelet-T-cell aggregate formation in the resolution phase of inflammation. [97]

ADE from SARS-Cov-2

ADE modulates the immune response and can cause prolonged inflammation, lymphopenia, and/or cytokine storm, all or one of which have been documented in severe cases and deaths from SARS-Cov-2.
ADE also requires previous exposure to similar antigenic epitopes presumably circulating in local viruses, making it a **possible explanation for the observed geographic limitation of severe cases and deaths**.[98]

> *It has been shown that pre-existing IgG anti-coronavirus antibodies that cross-react with SARS-CoV-2, **including those against common and less pathogenic coronavirus strains**, can increase the risk of ADE and disease complication [99].*

[96] JCI Insight 2016 Jun 16;1(9):e85851. doi: 10.1172/jci.insight.85851. Cardiac myosin-Th17 Responses Promote Heart Failure in Human Myocarditis
Jennifer M Myers et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4924810/

[97] Front Immunol 2018 Mar 2;9:406. doi: 10.3389/fimmu.2018.00406.
Fresh Evidence for Platelets as Neuronal and Innate Immune Cells: Their Role in the Activation, Differentiation, and Deactivation of Th1, Th17, and Tregs During Tissue Inflammation
Eugene D Ponomarev 1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5863511/

[98] Microbes Infect 2020 Mar;22(2):72-73. doi: 10.1016/j.micinf.2020.02.006.
Is COVID-19 Receiving ADE from Other Coronaviruses?
Jason A Tetro 1
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102551/

[99] J Virol. 2020 Feb 14;94(5):e02015-19. doi: 10.1128/JVI.02015-19
Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry.
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.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7022351/

Journal of Clinical and Translational Science. April 13, 2020 doi: 10.1017/cts.2020.39
The Potential for Antibody-Dependent Enhancement of SARS-CoV-2 Infection: Translational Implications for Vaccine Development
Jiong Wang, Martin S. Zand
https://doi.org/10.1017/cts.2020.39
https://www.cambridge.org/core/journals/journal-of-clinical-and-translational-science/article/potential-for-antibodydependent-enhancement-of-sarscov2-infection-translational-implications-for-vaccine-development/504FE38E2475590EFE93872BC6D67D3D/core-reader

Regarding the trend with age, it has been shown that **anti-CoV IgG increases with age in childhood**, with high titers **~75% in children >4 years old**, and **IgM is present only in children**, confirming that for all coronaviruses the first infection takes place in early childhood (age <14 years). Furthermore, the repertoire of anti-CoV IgG in children consists of **predominantly low affinity IgG**, which will mature with high affinity anti-CoV IgG only after repeated infections.
In adults, the positivity for IgG for the various human coronaviruses tested was about 80% (87.5% 229E, 76.39% OC43, 71.88% HKU1, and 75.52% NL63) [100]

Paradoxically, these results suggest that lower levels of anti-SARS-CoV-2 IgG antibodies could, in some cases, explain the reduced severity of COVID-19 in individuals aged ≤20 years.
The potential lack of high-affinity, cross-reactive anti-SARS-CoV antibodies with the absence of associated ADEs may contribute to reduced viral loads as fewer host cells are infected and produce virus.

Second, the development of high-affinity class-switched IgG antibodies may occur during the immune response after about 7-14 days and may increase after multiple rounds of antigenic exposure with vaccine recalls or by recurrent infection.

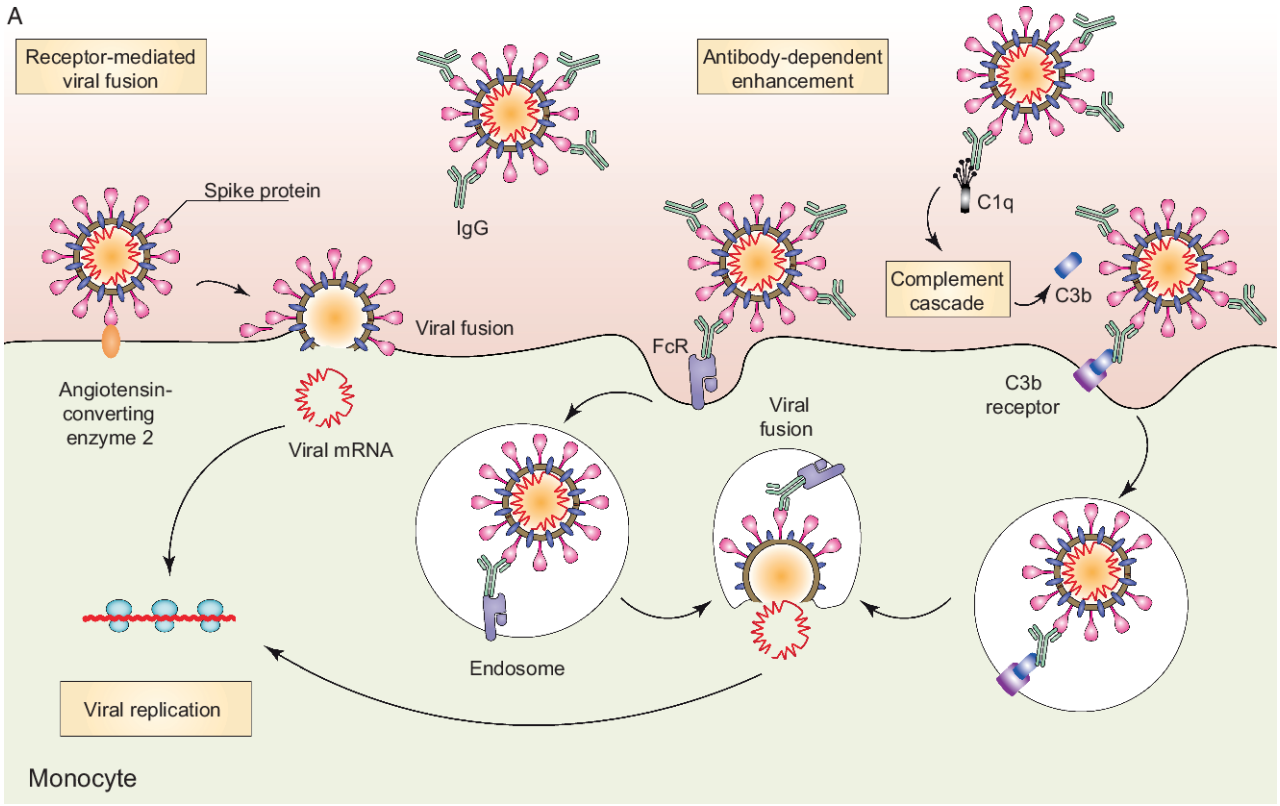
It follows that **the balance between neutralization and ADEs induced by IgG against SARS-CoV-2 differs in children and adults,** and neutralization is favored in children, probably because of the production of a different cytokine profile in response to infection. [101] (see IMMUNITARY RESPONSE, AGE AND GENDER DIFFERENCE: pediatric age)

[100] BMC Infect Dis 2013 Sep 16;13:433. doi: 10.1186/1471-2334-13-433.
First Infection by All Four Non-Severe Acute Respiratory Syndrome Human Coronaviruses Takes Place During Childhood
Weimin Zhou 1, Wen Wang, Huijuan Wang, Roujian Lu, Wenjie Tan
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3848659/

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/

[101] Pediatr Res 1997 Aug;42(2):237-40. doi: 10.1203/00006450-199708000-00018.
Cytokine Production Differs in Children and Adults
D Lilic 1, A J Cant, M Abinun, J E Calvert, G P Spickett
https://www.nature.com/articles/pr19972299

J Immunol 2008 Nov 1;181(9):6337-48. doi: 10.4049/jimmunol.181.9.6337.
Prior Immunization with Severe Acute Respiratory Syndrome (SARS)-associated Coronavirus (SARS-CoV) Nucleocapsid Protein Causes Severe Pneumonia in Mice Infected With SARS-CoV
Fumihiko Yasui et al
https://www.jimmunol.org/content/181/9/6337.long



Taken from https://doi.org/10.1017/cts.2020.39
Antibody-dependent potentiation: Mechanism. Normal viral fusion occurs with binding of the coronavirus spike protein to its receptor, angiotensin-converting enzyme 2 (ACE2) protein. This induces a conformational change in the S protein, exposing a membrane fusion domain, resulting in viral fusion and mRNA release into the cell. In ADE, antibody binding to protein S facilitates cellular binding via the FCR and induces a conformational change in the spike protein exposing the fusion domain. A similar process can occur if IgG binds to complement, with the virus:C3b:IgG complex captured through the C3b receptor.

The ADE from influenza vaccine [102]

Influenza vaccination is proposed for the prevention of seasonal infection, and as it is currently recommended in anticipation of the upcoming SARS-Cov-2 outbreak to improve the management of COVID-19 (it is believed that prevention of influenza will avoid overloading intensive care units when there is an epidemic peak for COVI-19) it is appropriate to examine the risks associated with the development of ADEs.

Vaccine-associated enhanced respiratory disease (VAERD) has been described in several respiratory infections in humans and animals.

HIV-negative children who received the formaldehyde-inactivated respiratory syncytial virus (RSV) vaccine followed by exposure to wild-type (wild) RSV manifested an even fatal worsening of respiratory disease (from studies conducted to understand the mechanism of ADE, it has been shown to be mediated by immune complexes and nullified in C3- and B-complement deficient mice, and evidence has been provided for

[102] Sci Transl Med 2013 Aug 28;5(200):200ra114. doi: 10.1126/scitranslmed.3006366.
Vaccine-induced anti-HA2 Antibodies Promote Virus Fusion and Enhance Influenza Virus Respiratory Disease
Surender Khurana 1, Crystal L Loving, Jody Manischewitz, Lisa R King, Phillip C Gauger, Jamie Henningson, Amy L Vincent, Hana Golding

Vaccine. Front Immunol. 2019 Mar 18;10:440. doi: 10.3389/fimmu.2019.00440.
Extra-Neutralizing FcR-Mediated Antibody Functions for a Universal Influenza
Boudreau CM, Alter G.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6436086/

complement activation in postmortem lung sections in children who died from RSV disease enhancement). [103]

Similarly, **atypical measles with severe complication has been reported in children vaccinated with formaldehyde** [104-inactivated] **vaccines**. The mechanisms underlying VAERD after respiratory infections is not completely understood and may vary with the disease and/or the manner in which the vaccine is prepared.

In a study done to evaluate VAERD associated with influenza infection in cases where the vaccine strain does not match the infecting strain, a swine animal model was used, as previous work by the same research group had shown that **pigs vaccinated with an inactivated viral vaccine containing a human-like H1N2 virus (WIV-H1N2) were not only unprotected against infection with swine H1N1 virus but also had more severe pneumonia after infection.**
The authors analyzed post-vaccination sera from WIV-H1N2 and showed that these antibodies had enhanced pH1N1 infection of MDCK cells by recognition of a hemagglutinin 2 (HA2) epitope. These antibodies were not able to block virus binding to receptors but even **promoted cell membrane fusion with pH1N2 by 250 to 300% compared with negative controls**. Their analysis showed a **positive correlation between the percentage of viral fusion and the percentage of macroscopic lung lesions in pigs vaccinated with WIV-H1N2**.

These data **confirm what was already found in an ISS study conducted in 2018** [105] in which a cohort of 64854 elderly subjects was analyzed and 53.0% of subjects were administered the flu vaccine in the 2016-17 season. The frequency of vaccination increased with increasing age, from less than 40% in subjects aged 65-69 years to nearly 70% in those ≥80 years, and was more likely among women than men. Co-morbidity was, on average, higher among vaccinated subjects than among others (thus older people with more risk conditions were more likely to adhere to the medical recommendation).
Elderly people who were vaccinated against pneumococcus were also vaccinated against influenza much more frequently than others.

As can be seen from the table below, the effectiveness of vaccination was virtually zero, with even a **statistically significant increase in hospitalizations and deaths compared to the unvaccinated among the group of vaccinated who received the tetravalent vaccine.** In fact, tetravalent vaccination had 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%).

	Outcome		
	ED visit	Hospitalization	Death
	HR[1] (95% CI)	HR[1] (95% CI)	HR[1] (95% CI)
Influenza vaccination (any *vs* no vaccination)	1.13 (0.91-1.40)	1.11 (0.93-1.33)	1.05 (0.70-1.58)
Influenza vaccination (intradermal *vs* no vaccination)	1.11 (0.95-1.48)	1.11 (0.92-1.34)	1.02 (0.67-1.54)
Influenza vaccination (tetravalent *vs* no vaccination)	0.81 (0.46-1.41)	1.47 (1.00-2.15)	1.12 (1.03-1.54)

Taken from http://old.iss.it/binary/publ/cont/ANN_18_01_13.pdf

[103] J Exp Med 2002 Sep 16;196(6):859-65. doi: 10.1084/jem.20020781.
A Role for Immune Complexes in Enhanced Respiratory Syncytial Virus Disease
Fernando P Polack et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2194058/

[104] JAMA 1967 Dec 18;202(12):1075-80. doi: 10.1001/jama.202.12.1075.
Altered Reactivity to Measles Virus. Atypical Measles in Children Previously Immunized With Inactivated Measles Virus Vaccines
V A Fulginiti, J J Eller, A W Downie, C H Kempe
https://jamanetwork.com/journals/jama/article-abstract/336928

[105] Ann Ist Super Health Jan-Mar 2018;54(1):67-71. doi: 10.4415/ANN_18_01_13.
Influenza Vaccine Effectiveness in an Italian Elderly Population During the 2016-2017 Season
Francesca Valent 1, Tolinda Gallo 1
http://old.iss.it/binary/publ/cont/ANN_18_01_13.pdf

An important limitation of this study is that it considered only influenza and pneumonia as causes of death, without giving references regarding the total number of deaths and for what other causes. There are no data regarding the incidence of postvaccinal adverse reactions.
It follows that based on these data, the influenza vaccine, and particularly the tetravalent**,** in addition to being ineffective, even has a statistically significant and relevant association with the risk of hospitalizations and deaths.

In Italy, influenza A (H3N2) was found in 88% of positive samples collected in the 2016-17 season, and **several clusters characterized by amino acid substitutions in hemagglutinin HA**, particularly the N121K substitutions, were **identified** in this viral population, T135K, and I140M, which are believed to be responsible for the decline in vaccine efficacy for the 2016-17 season observed among some Northern European elderly populations, but may certainly have led to the formation of cross-reactive non-neutralizing antibodies and thus triggered the vaccine ADE.

This means that in the coming season there is a risk that the elderly who will be vaccinated with the flu vaccine will not only be unprotected against the infection but will potentially be susceptible to developing the even fatal respiratory complications.

To this evidence should be added the findings of a recent study, carried out on U.S. Department of Defense military personnel, comparing a vaccinated (6541 people) and unvaccinated (2928 people) population with the flu vaccine, in which it was shown that **vaccination increased the risk of coronavirus co-infection precisely by 36 percent**. [106]
This phenomenon, known as **viral interference**, can be explained on the basis of the similarities between the proteins expressed by coronaviruses, including SARS-Cov-2, and influenza virus: in fact, it is known that **the hemagglutinin-esterase protein is similar in the two viruses** and that the spike protein of coronaviruses shares common features with the viral membrane fusion protein class 1 of influenza viruses. [107]

*Thus**, influenza vaccination may lead to the formation of nonneutralizing and cross-reactive antibodies to coronaviruses and SARS-Cov-2, which may increase both the risk of SARS-Cov-2 infection and the induction of the enhancement of respiratory complications in case of influenza vaccination and subsequent SARS-Cov-2 infection.*

For further study, we recommend reading the book Flu Vaccination: what the scientific evidence says.
Can indiscriminately vaccinating the elderly, pregnant women, children, and health care workers be more harmful than helpful?
Alberto Donzelli, Daniele Agostini, Paolo Bellavite, Adriano Cattaneo, Piergiorgio Duca, Eugenio Serravalle

https://fioritieditore.com/wp-content/.../2020/06/Donzelli.pdf

Other comparative studies d e m o n s t r a t i n g increased parainfluenza virus infections in those vaccinated with influenza vaccine are reviewed in the following popular article:
Pentagon Study: Flu Shot Raises Risk of Catching Coronavirus
June 14, 2020 By Robert F. Kennedy, Jr., Chairman, Children's Health Defense
https://operationdisclosure1.blogspot.com/2020/06/pentagon-study-flu-shot-raises-risk-of.html?m=1

[106] Vaccine. 2020 Jan 10;38(2):350-354. doi: 10.1016/j.vaccine.2019.10.005.
Influenza vaccination and respiratory virus interference among Department of Defense personnel during the 2017-2018 influenza season.
Wolff GG1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7126676/pdf/main.pdf

[107] Proc Natl Acad Sci U S A. 2008 Jul 1;105(26):9065-9. doi: 10.1073/pnas.0800502105.
Structure of coronavirus hemagglutinin-esterase offers insight into corona and influenza virus evolution.
Zeng Q1, Langereis MA, van Vliet AL, Huizinga EG, de Groot RJ.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2449365/

Intravenous immunoglobulin infusion therapy: the mechanism of action

In this context, it is useful to discuss the use of hyperimmune serum and the mechanism by which it acts in case of viral infection.

Intravenous immunoglobulin (IVIG) is a blood product consisting of aggregated IgG fractions obtained from about 3,000 to 60,000 plasma samples from blood donors.
Since its discovery 60 years ago, IVIG has been conventionally used as a therapeutic treatment for immunocompromised individuals suffering from immunodeficiency diseases such as hypogammaglobulinemia.

Hyperimmune plasma, on the other hand, is derived from individuals with high antibody titers to specific pathogens (convalescent people cured of disease) and has been used successfully in the treatment of infections, such as cytomegalovirus and H1N1 influenza. [108]

Comparison of Intravenous Immunoglobulin (IVIG) vs. Hyperimmune Sera.

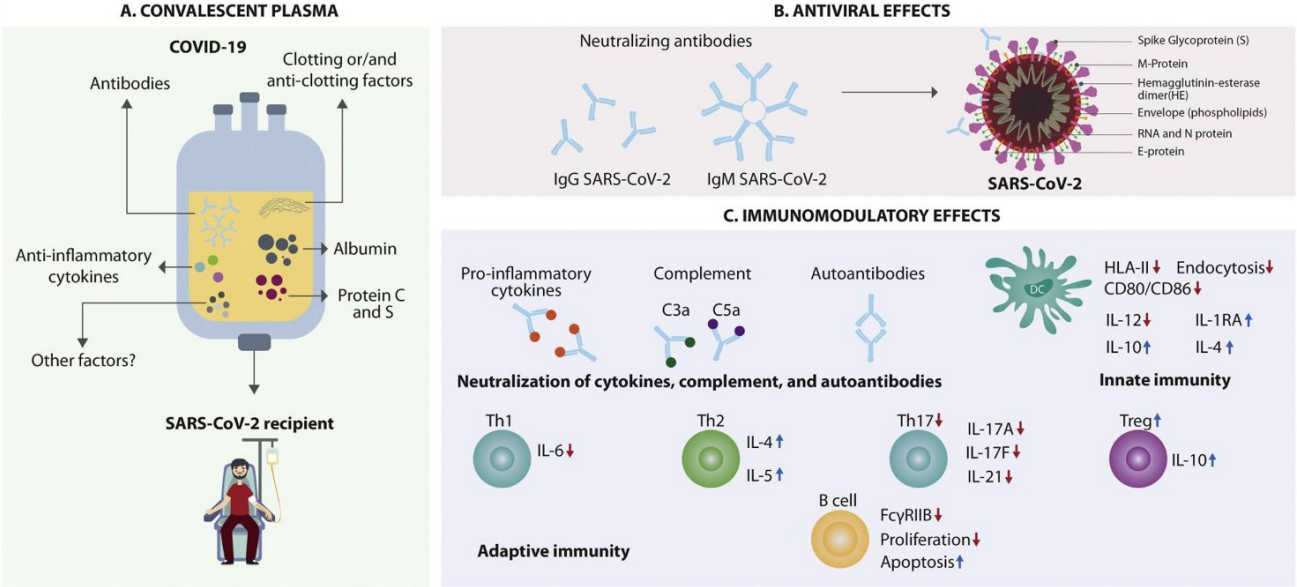
	Intravenous immunoglobulin (IVIG)	Hyperimmune sera
Preparation	- Pooled human plasma	- Pooled human plasma
Donors	- General population	- Individuals seropositive for specific pathogen(s) with sufficient neutralizing antibody titer(s)
Usage	- Ig replacement in primary and secondary immunodeficiency - Immune modulation	- Treatment of specific pathogen(s)
Benefits	- Provides widespread protection against common infections - Treatment of hyper-inflammatory states - Large donor pool - Commercial availability	- Targeted therapy in specific infection(s), especially novel infections without herd immunity
Limitations	- Absent or variable specific neutralizing antibody titer(s) against novel pathogen(s)	- Limited donor availability, must be previously exposed - Variable antibody titer among donors, limited timeframe for donation - May aggravate disease
Rationale for use in COVID-19	- May provide immunomodulatory effect in hyperinflammation state (limited/inconclusive data) - Competitively bind Fcγ receptor to prevent antibody-dependent enhancement triggered by virus-antibody immune complexes[19]	- Has demonstrated effectiveness in SARS and MERS corona virus infections[16,17,18]

[108] Nguyen AA, Habiballah SB, Platt CD, Geha RS, Chou JS, McDonald DR. Immunoglobulins in the treatment of COVID-19 infection: Proceed with caution! Clin Immunol. 2020;216:108459. doi:10.1016/j.clim.2020.108459 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/

Immunocompromised individuals are highly susceptible to pathogenic infections, so IVIG preparations must have undergone viral inactivation, depletion of blood clotting factors, and IgG aggregates is crucial before infusion to induce protection against pathogens and avoid any deleterious effects that might be caused by the infusion.

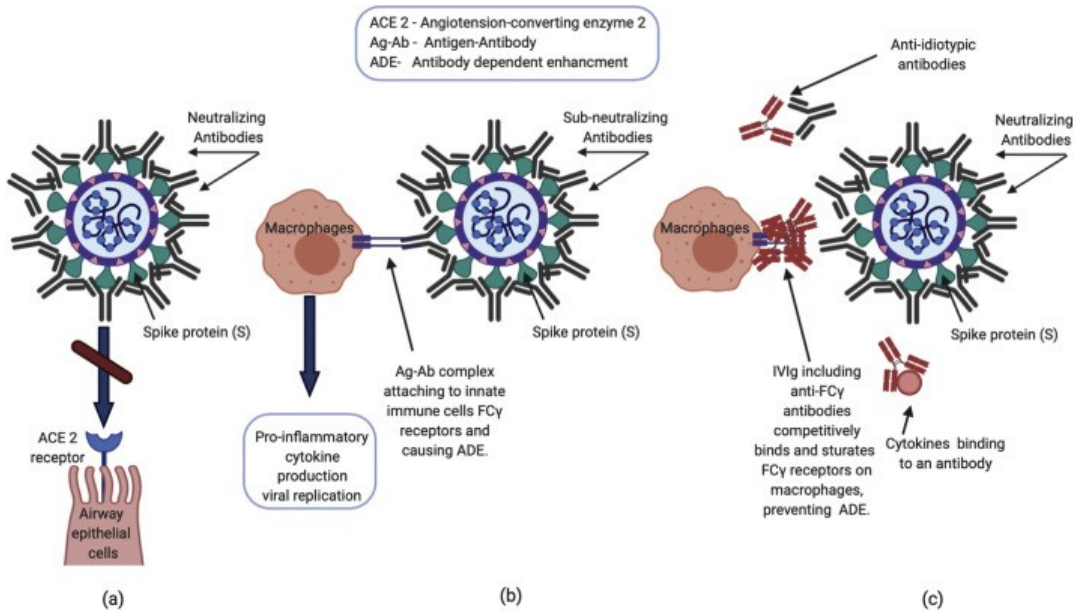
To date, despite the efficacy of IVIG treatment in several autoimmune and inflammatory diseases, the mechanism by which this occurs remains poorly defined. [109]



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198427/
Schematic representation of the components of convalescent plasma and its mechanisms of action. A. Main components of convalescent plasma. B. Antiviral effects of NAbs. IgG and IgM are the main isotypes, although IgA may also be important, particularly in viral mucosal infections. Other non-NAbs may exert a protective effect. The humoral immune response is mainly directed toward the spike protein (S). C. Anti-inflammatory effects of CP include autoantibody network and control of a hyperactive immune system (e.g., cytokine storm, Th1/Th17 ratio, complement activation, and regulation of a hypercoagulative state) (see text for details). N: Nucleoprotein; M: membrane; E: envelope

The possible mechanisms underlying the efficacy of IVIG revolve mainly around its ability to modify the immune response.

109 Front Immunol. 2019 Jun 11;10:1090. doi: 10.3389/fimmu.2019.01090.
High-Dose Intravenous Immunoglobulin in Skin Autoimmune Disease
Hoffmann JHO, Enk AH.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6579842/



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/

Proposed mechanisms of neutralizing antibodies and IVIG in COVID-19 infection. (a) Neutralizing antibodies prevent the SARS-CoV2 spike protein from binding to the ACE2 receptor, inhibiting viral entry into the cell**. (b**) Immune complexes consisting of viral antigens and sub-neutralizing antiviral antibodies can activate Fcγ receptors on innate immune cells (e.g., macrophages) in the lung, triggering an exaggerated inflammatory response leading to acute lung injury through antibody-dependent enhancement (ADE) . In addition, antibody-associated virus can be internalized through Fcγ receptors, enhancing viral replication. **(c)** Proposed mechanisms by which IVIG exerts anti-inflammatory action include saturation of Fcγ receptor binding, anti-idiotypic binding to anti-viral antibodies, and proinflammatory cytokine binding.

One possible mechanism is that high-dose IVIG infusion ***prevents FcγR activation through saturation of the binding of these receptors with infused IgG, as well as through increased expression of inhibitory FcγRIIb.***[110]

[110] Nat Rev Immunol 2013 Mar;13(3):176-89. doi: 10.1038/nri3401. Epub 2013 Feb 15.
Intravenous Immunoglobulin Therapy: How Does IgG Modulate the Immune System?
Inessa Schwab 1, Falk Nimmerjahn

Rojas M, et al.
Convalescent plasma in Covid-19: Possible mechanisms of action
Autoimmun Rev. 2020;102554. doi:10.1016/j.autrev.2020.102554
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7198427/

Fischer JC, et al.
The role of passive immunization in the age of SARS-CoV-2: an update.
Eur J Med Res. 2020;25(1):16. doi:10.1186/s40001-020-00414-5
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7220618/

Brown BL, McCullough J.
Treatment for emerging viruses: Convalescent plasma and COVID-19.
Transfus Apher Sci. 2020;102790. doi:10.1016/j.transci.2020.102790
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194745/

Bloch EM, et al.
Deployment of convalescent plasma for the prevention and treatment of COVID-19.
J Clin Invest. 2020;130(6):2757-2765. doi:10.1172/JCI138745
https://www.jci.org/articles/view/138745

Sullivan HC, Roback JD.
Convalescent Plasma: Therapeutic Hope or Hopeless Strategy in the SARS-CoV-2 Pandemic
Transfus Med Rev. 2020;S0887-7963(20)30025-0. doi:10.1016/j.tmrv.2020.04.001
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7179481/

Knudson CM, Jackson JB.
COVID-19 convalescent plasma: phase 2.
Transfusion. 2020;10.1111/trf.15842. doi:10.1111/trf.15842

Regarding the **limitations of using IVIG and hyperimmune plasma**, a recent study of secondary DENV infection in an immunocompetent mouse animal model showed that IVIG-treated mice had **increased mortality, exacerbation of disease severity, and elevated virus replication in a dose-dependent manner**.
Further examination found sub-neutralizing titers of anti-DENV antibodies in the serum of IVIG-treated mice as a contributing factor to aggravation [111].

It follows that administration of IVIG should be carefully evaluated by detection and purification from nonneutralizing antibodies in serum in case of infections in which ADE as disease potentiation is possible. [112]

IN BRIEF

Augmentation of disease (ADE) is an immunopathological phenomenon affecting various types of viral infections, including SARS-Cov-2 and influenza.

ADE occurs following infection by SARS-Cov-2 of cells of the innate immune system (macrophages). The virus enters these cells as an immune complex formed by binding with poorly effective antibodies (nonneutralizing and in low amounts) from previous human coronavirus infections or vaccinations (influenza).
Once inside, instead of being processed for presentation to other cells of the immune system, it manages to block the antiviral response and stimulate a strong inflammatory response (cytokine storm) responsible for multi-organ damage.

From the perspective of the mechanism of damage induction, severe/fatal complications associated with SARS-Cov-2 infection can be considered a consequence of ADE.

The ADE explains why the elderly are at greater risk than healthy children and adults, as they possess a greater amount of nonneutralizing antibodies from coronavirus infections or from infrequent vaccinations, and have an underperforming immune system in fighting infections.

Pregnant women, and infants under one year of age, are also susceptible to disease enhancement in case of reinfection.

Hyperimmune serum and IVIGs are effective in treating COVId-19 patients because the transfused antibodies are able to block viral immune complexes from entering immune system cells.

ADE is an important risk factor for vaccination against COVID-19 and influenza because of the high variability of viruses that may predispose to the production of nonneutralizing antibodies.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267265/

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;55:102768. doi:10.1016/j.ebiom.2020.102768
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7161485/

[111] Med Microbiol Immunol 2014 Aug;203(4):231-50. doi: 10.1007/s00430-014-0334-5.
Subversion of Early Innate Antiviral Responses During Antibody-Dependent Enhancement of Dengue Virus Infection Induces Severe Disease in Immunocompetent Mice
Vivian V Costa et al
https://link.springer.com/article/10.1007/s00430-014-0334-5

[112] Shoenfeld Y.
Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning.
Autoimmun Rev. 2020;19(6):102538. doi:10.1016/j.autrev.2020.102538
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7131471/

INDIVIDUAL FACTORS

One of the factors associated with adverse outcome of COVID-19 is advanced age; in fact, children who contract SARS-CoV2 infection usually do not develop severe symptoms or complications.
This actually does not explain much because children are prone to viral infections even with severe manifestations.

As already seen, more than 75% of children are exposed to seasonal coronaviruses before age 4 and demonstrate seroconversion. However, antibody titers decline over time, most noticeably in individuals over 60 years of age [110].* [113]

This may, on the one hand, make the immune response against SARS-CoV2 in the elderly less effective because of **limited cross-reactivity between antibodies against seasonal coronaviruses and anti-SARS-Cov2 antibodies**, and on the other hand, it may contribute to the **potentiation of inflammation and complications because**, as a result of immunologic recall, there is the increase in seasonal antibody titers against coronaviruses as detected in sera of patients convalescing from SARS [111] with potentiation of immunopathology.

As mentioned earlier, antibody-bound virions can enter susceptible cells, such as macrophages, through binding to the Fcγ receptor in the process known as antibody enhancement (ADE) [112].
In other viral infections (e.g., dengue fever), ADE allows infection of immune cells and reduces type I IFN-dependent antiviral responses while promoting pro-inflammatory expression of IL-6 and TNF-α [113,114]. In addition, massive production of booster antibodies in individuals with a history of seasonal coronavirus exposure but declining titers, such as the elderly, may result in **deposition of the immune complex in tissues** and promote inflammation and damage, including immune complex vasculitis [110].[114]

As already seen, ACE2 acts as a transmembrane cell receptor for SARS-CoV2 allowing cellular infection [117].
Variability in ACE2 expression influences susceptibility to disease among tissues (e.g., respiratory epithelium vs. immune cells), but potentially also among individuals (men vs. women, children vs. adults) thus determining diversity in disease progression and outcomes.

---

[113] see literature attached to article superscripted in text in square brackets Clin Immunol. 2020 Apr 27;215:108448. doi: 10.1016/j.clim.2020.108448.
COVID-19: Immunology and treatment options.
Felsenstein S1, Herbert JA2, McNamara PS2, Hedrich CM3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185015/pdf/main.pdf

[114] J. Inf. Secur. 2015;71(5):599-602.
Antibody against nucleocapsid protein predicts susceptibility to human coronavirus infection.
Gao X., Zhou H., Wu C., Xiao Y., Ren L., Paranhos-Baccala G.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7127429/

J. Infect. Dis. 2005;191(12):2033-2037.
Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43.
Che X.Y., Qiu L.W., Liao Z.Y., Wang Y.D., Wen K., Pan Y.X.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7109809/

Virus Res. 2008;133(1):20-32.
Animal models and vaccines for SARS-CoV infection.
Roberts A., Lamirande E.W., Vogel L., Jackson J.P., Paddock C.D., Guarner J.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6357155/

Sci Rep. 2016;6:29201
Antibody-Dependent Enhancement of Dengue Virus Infection in Primary Human Macrophages. Balancing Higher Fusion against Antiviral Responses.
Flipse J., Diosa-Toro M.A., Hoornweg T.E., van de Pol D.P., Urcuqui-Inchima S., Smit J.M.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4933910/

J. Virol. 2005;79(12):7819–7826.
Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis.
Cheung C.Y., Poon L.L., Ng I.H., Luk W., Sia S.F., Wu M.H.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1143636/

ACE2 expression is higher in children and young women, and its expression decreases with age and is lower in individuals with chronic disease, including diabetes and hypertension, and inversely correlated with risk of severe disease and adverse outcomes [118].

This is because ACE2 indeed facilitates viral entry into cells, but it also plays a role in controlling infection and inflammation as it catalyzes angiotensin processing.
2 in angiotensin-1-7, which helps limit tissue inflammation by p r o m o t i n g repair mechanisms.

With this hypothesis, one could explain why increased ACE2 expression in children and young adults, particularly young women, makes them relatively protected from COVID-19 and associated complications.[115]

Taken together, **the** new coronaviruses, such as SARS-CoV2, **are able to suppress early T1IFN responses, a factor that contributes to uncontrolled virus replication resulting in delayed and increased cytokine responses in later stages.**

Early and sufficient control of virus replication and pathogen clearance may be impaired in at-risk individuals, such as the elderly, patients with diabetes or metabolic syndrome, etc. [74,75].

Healthy children and young people, on the other hand, can effectively control viral load in the early stages of infection (i.e., through the innate response) and less frequently develop severe illness and life-threatening complications.
However**, early production of antibodies (due to activation of the adaptive response) may result in infection by the virus in immune cells and increased viral replication**, leading to immune-mediated pathology in young patients without obvious risk factors [100]. [116]

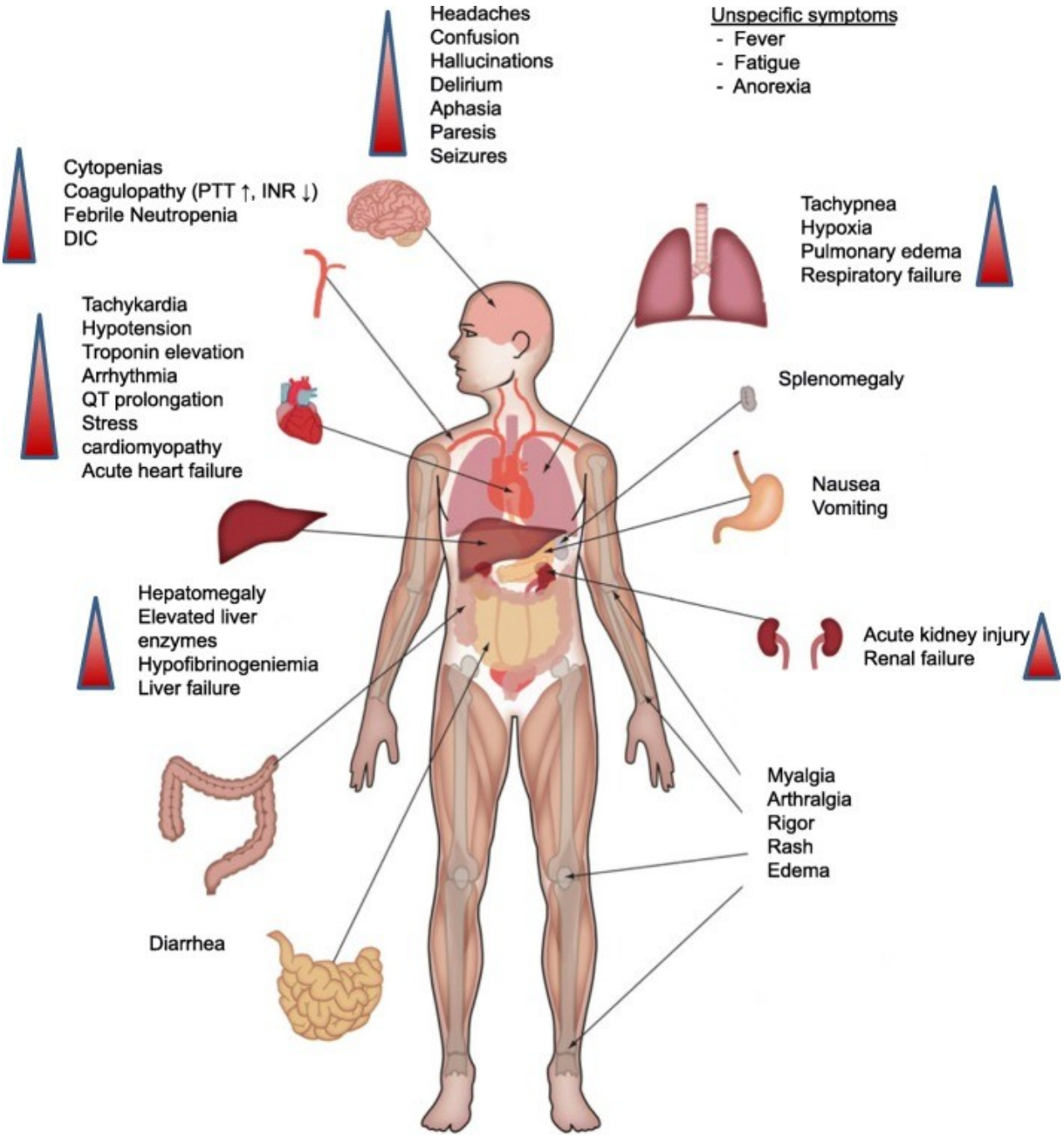
It follows that the balance, timing, and effectiveness of activation of the innate and adaptive response of the immune system are critical to resolving viral infection without complications.

[115] Clin. Immunol. 2020;214:108420.
COVID-19 - Considerations for the paediatric rheumatologist
Hedrich C.M.

Preprints 2020, 2020030191 https://www.preprints.org/manuscript/202003.0191/v1
Individual Variation of the SARS-CoV2 Receptor ACE2 Gene Expression and Regulation.
Chen J.J.Q.X., Liu K., Yu Z., Tao W., Gong W., Han J.D.J

[116] Lancet. 2020;395(10223):497–506.
Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China.
Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y.

Viruses. 2020;12(4)
Virology, Epidemiology, Pathogenesis, and Control of COVID-19.
Jin Y., Yang H., Ji W., Wu W., Chen S., Zhang W.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6003181/pdf/40425_2018_Article_343.pdf
Caption: Clinical presentation of **CRS** (cytokine release syndrome).
Starting with fever and nonspecific symptoms, CRS could impact most organ systems. Mild cases may present as flu-like illness. Grade III to IV shows signs of cardiovascular, pulmonary and renal danger. Neurotoxicity may occur simultaneously or with delay.
Abbreviations: DIC: disseminated intravascular coagulation; INR: international normalized ratio; PTT: partial thromboplastin time

COVID-19 in old age

From what has been discussed so far, the complication that arises about a week after the first symptoms is not caused directly by the viral infection but by the excessive immune response, which is not followed by an effective repair of the damage, which is why people with more severely disabling diseases and the over-65 age group are affected more severely. [117]

From the context of basic knowledge about COVID-19 and the interaction of the host immune system, the possible explanation of the course of COVID-19 infections is as follows:

> *healthy adults, unlike older and weakened individuals, have intact innate immunity along with competent cell-mediated and humoral immunity; the net result is that their immune system can limit infection from progression and lead to recovery within 2 to 3 weeks of symptom onset.*

In the elderly with co-morbidity and impaired innate and humoral immunity, the virus continues to replicate, resulting in a strong inflammatory reaction by cell-mediated immunity with attack of all previously infected tissues and very severe systemic damage.

[117] https://www.epicentro.iss.it/coronavirus/bollettino/Bollettino-sorveglianza-integrata-COVID-19_16-aprile-2020.pdf

Proc Biol Sci. 2015 Dec 22;282(1821):20143085. doi: 10.1098/rspb.2014.3085.
Evolution of the immune system in humans from infancy to old age.
Simon AK1, Hollander GA2, McMichael A3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707740/

Electron J Gen Med. 2020;17(4):em202. https://doi.org/10.29333/ejgm/7850
The Possible Immunological Pathways for the Variable Immunopathogenesis of COVID-19 Infections among Healthy Adults, Elderly and Children.
Abdulamir AS, Hafidh RR.
https://www.ejgm.co.uk/download/the-possible-immunological-pathways-for-the-variable-immunopathogenesis-of-covid-19-infections-among-7850.pdf

Ann N Y Acad Sci. 2000 Jun;908:244-54.
Inflamm-aging. An evolutionary perspective on immunosenescence.
Franceschi C1, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G.
https://pubmed.ncbi.nlm.nih.gov/10911963/

FEBS Lett. 2005 Apr 11;579(10):2035-9.
Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity.
De Martinis M1, Franceschi C, Monti D, Ginaldi L.
https://febs.onlinelibrary.wiley.com/doi/full/10.1016/j.febslet.2005.02.055

Endocr Metab Immune Disord Drug Targets. 2020 Apr 6. doi: 10.2174/1871530320666200406123734.
Peripheral Immunosenescence and Central Neuroinflammation: A Dangerous Liaison. A Dietary Approach.
Magrone T1, Magrone M1, Russo MA2, Jirillo E1.
https://pubmed.ncbi.nlm.nih.gov/32250234/

Eur Heart J. 2020 Feb 1. pii: ehz961. doi: 10.1093/eurheartj/ehz961.
Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease.
Liberale L1,2, Montecucco F3,4, Tardif JC5, Libby P6, Camici GG1,7,8.
https://pubmed.ncbi.nlm.nih.gov/32006431/

Clin Exp Immunol. 2017 Jan;187(1):16-25. doi: 10.1111/cei.12881.
Innate immune responses in the aging lung.
Boe DM1, Boule LA1, Kovacs EJ1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5167032/.

COVID-19 pediatric

As mentioned above, the infection does not appear to cause serious or fatal complications in the pediatric age group [118] According to the Centers for Disease Control and Prevention (CDC) report, fewer children were admitted to the hospital and intensive care unit (ICU) (5.7% -20% and 0.58% -2.0%, respectively) than adults aged 18-64 years (10% -33% and 1.4% -4.5%, respectively).

However, **infants had higher hospitalization rates (15% -62%) than older children (1-17 years) (4.1% -14%) and adults**.

There were 3 deaths (0.1%) in children compared with the overall mortality of 2.27%. The Chinese case series of 171 laboratory-confirmed children also reported one death in a 10-month-old child who suffered intussusception and multi-organ failure.

In another Chinese series of 728 laboratory-confirmed children, the percentage of "severe and critical" cases was 8.2%, 2.1%, 0.6%, 1.1% and 5.1% for the age groups of <1, 1-5, 6- 10, 11-15 and> 15 y, respectively.

> *These results suggest that* ***although children in general are less affected and have milder disease than adults, infants have more severe disease than older children.*** [119]

Physicians have reported an **increase in temporary vasculitis** (acro-ischemic lesions) in the lower limbs in asymptomatic children [120] and cases of **Kawasaki disease**, a particularly severe form of vasculitis that is underestimated precisely because of the absence of symptoms of infection in children [121].

---

[118] Indian J Pediatr. 2020 May 14:1-10. doi: 10.1007/s12098-020-03322-y
Pathophysiology of COVID-19: Why Children Do Better than Adults?
Dhochak N, Singhal T, Kabra SK, Lodha R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7221011/

Acta Biomed 2020 May 11;91(2):214-215. doi: 10.23750/abm.v91i2.9563.
SARS-CoV-2 Infection in Children in Parma.
Icilio Dodi et al
https://www.mattioli1885journals.com/index.php/actabiomedica/article/view/9563/8770

[119] N Engl J Med 2020 Apr 23;382(17):1663-1665. doi: 10.1056/NEJMc2005073.
SARS-CoV-2 Infection in Children
Xiaoxia Lu et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7121177/

MMWR Morb Mortal Wkly Rep. 2020; 10.15585/mmwr.mm6914e4
CDC COVID-19 Response Team. Coronavirus disease 2019 in children - United States, February 12-April 2, 2020.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7147903/

Pediatrics. 2020:e20200702. 10.1542/peds.2020-0702
Epidemiology of COVID-19 among children in China.
Yuanyuan Dong 1 2 3, Xi Mo 1 3, Yabin Hu 1, Xin Qi 4, Fan Jiang 1, Zhongyi Jiang 1 2, Shilu Tong
https://pediatrics.aappublications.org/content/145/6/e20200702.long

[120] https://www.fip-ifp.org/wp-content/uploads/2020/04/acroischemia-ENG.pdf

Pediatr Dermatol. 2020;10.1111/pde.14210. doi:10.1111/pde.14210
Chilblains-like lesions in children following suspected Covid-19 infection
Colonna C, Monzani NA, Rocchi A, Gianotti R, Boggio F, Gelmetti C.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7283832/pdf/PDE-9999-na.pdf

Pain Ther. 2020;1-3. doi:10.1007/s40122-020-00174-4
Images in Practice: Painful Cutaneous Vasculitis in a SARS-Cov-2 IgG-Positive Child
Papa A, Salzano AM, Di Dato MT, Varrassi G.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7241586/

[121] J Pediatr. 2020 Apr 23. pii: S0022-3476(20)30556-4. doi: 10.1016/j.jpeds.2020.04.052.

Table 1

Comparison of clinical characteristics among pediatric and adult COVID-19 case series

Characteristics	Pediatric		Adult	
	CDC report [6]	Lu X et al. 2020 [7]	CDC report [6]	Guan WJ et al. 2020 [8]
Number of patients	2572*	171	113,985*	1099
Age group, years	<18	<16	18–64	All ages (99.1% above 15 y)
Region	USA	China	USA	China
Male	1408 (53)	104 (60.8)	75,450 (53)#	637 (58.1)
Age, years	11 (median)			47 (35, 58)@
Fever	163 (56)	71 (41.5)	7794 (71)	975 (88.7)
Cough	158 (54)	83 (48.5)	8775 (80)	745 (67.8)
Fatigue		13 (7.6)		419 (38.1)
Myalgia	66 (23)		6713 (61)	164 (14.9)
Headache	81 (28)		6335 (58)	150 (13.6)
Shortness of breath	39 (13)	¥	4674 (43)	205 (18.7)
Sore throat	71 (24)		3795 (35)	153 (13.9)
Rhinorrhea	21 (7.2)	13 (7.6)	757 (6.9)	
Diarrhea	37 (13)	15 (8.8)	3353 (31)	42 (3.8)
Nausea/Vomiting	31 (11)		1746 (16)	55 (5.0)
Abdominal pain	17 (5.8)		1329 (12)	

Data are summarized as number (%), unless specified. @data summarized as median (IQR). *Denominator for estimation of symptom frequency was 291 (age < 18 y) and 10,944 (age 18–64 y), because details of symptoms were available for these patients only; #Includes all patients 18 y and above; ¥49 (28.7%) children had tachypnea on examination in hospital. *CDC* Centers for Disease Control and Prevention

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7221011/
Data are summarized as number (%) unless otherwise stated. @data summarized as median (IQR). * Denominator for estimating symptom frequency was 291 (age <18 years) and 10,944 (age 18-64 years), as symptom details were only available for these patients; # Includes all patients age 18 years and older; ¥ 49 children (28.7%) had tachypnea on hospital examination. CDC Centers for Disease Control and Prevention.

Missed or Delayed Diagnosis of Kawasaki Disease During the 2019 Novel Coronavirus Disease (COVID-19) Pandemic.
Harahsheh AS et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7196408/pdf/main.pdf

Cardiol Young. 2020;1-5. doi:10.1017/S1047951120001432
Covid-19 and Kawasaki syndrome: should we really be surprised?
Loomba RS, Villarreal E, Flores S.
https://www.cambridge.org/core/services/aop-cambridge-core/content/view/2AD153D73A34F46B580097685BD403D2/S1047951120001432a.pdf/covid19_and_kawasaki_syndrome_should_we_really_be_surprised.pdf

Lancet. 2020;10.1016/S0140-6736(20)31129-6. doi:10.1016/S0140-6736(20)31129-6
Kawasaki-like disease: emerging complication during the COVID-19 pandemic
Viner RM, Whittaker E.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7220168/

Lancet. 2020;10.1016/S0140-6736(20)31103-X. doi:10.1016/S0140-6736(20)31103-X
An outbreak of severe Kawasaki-like disease at the Italian epicenter of the SARS-CoV-2 epidemic: an observational cohort study
Lucio Verdoni et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7220177/

J Pediatric Infect Dis Soc. 2020;piaa061. doi:10.1093/jpids/piaa061
COVID-19 Associated Pediatric Multi-System Inflammatory Syndrome
M P Deza Leon et al
https://academic.oup.com/jpids/advance-article/doi/10.1093/jpids/piaa061/5842067

Acta Biomed. 2020;91(2):177-183. doi:10.23750/abm.v91i2.9655
COVID19: potential cardiovascular issues in pediatric patients.
Deborah Bertoncelli et al
https://pubmed.ncbi.nlm.nih.gov/32420942/

COVID-19 in pregnancy

Regarding pregnancy from the most recent literature review [122] in the combined data from the eight consecutive case series reviewed, including 211 (71.5%) laboratory-confirmed cases and 84 (28.5%) clinically diagnosed COVID-19, maternal age ranged from 20 to 44 years and gestational age at admission from 5 to 41 weeks.

The most common symptoms at presentation were fever, cough, dyspnea/shortness of breath, fatigue, and myalgia.
The rate of severe pneumonia reported among the case series ranged from 0 to 14 percent, and most required admission to the intensive care unit.
Almost all cases in the series had positive results on chest computed tomography.

Cases tested by RT-PCR in vaginal mucus samples (6 cases) and breast milk (22 cases) were negative for SARS- CoV-2.
Four cases of abortion were reported. In the series of consecutive cases, 219/295 women had given birth at the time of reporting, and most of them performed cesarean section. Gestational age at delivery ranged from 28 to 41 weeks.
Apgar scores at 1 and 5 minutes ranged from 7 to 10 and 7 to 10, respectively.
Only eight infants had a birth weight <2500 g, and nearly one-third of the cases were transferred to the neonatal intensive care unit. There was one case each of neonatal asphyxia and neonatal death.

In 155 infants tested by RT-PCR in the throat swab, all but three cases were negative for SARS-CoV-2. Seven maternal deaths, four intrauterine fetal deaths (one with twin pregnancy) and two neonatal deaths (twin pregnancy) were reported in a nonconsecutive series of nine cases with severe COVID-19.
From the case-control reports, two maternal deaths, one neonatal death, and two cases of neonatal SARS-CoV-2 infection were detected.

Currently, there is no evidence that SARS-CoV-2 undergoes intrauterine or transplacental transmission from infected pregnant women to their fetuses, while the possibility of transmission by direct contact during lactation [123] remains plausible, and all neonatal samples tested, including placentas in some cases, were negative on RT-PCR for SARS-CoV-2.

[122] Ultrasound Obstet Gynecol 2020 May 19. doi: 10.1002/uog.22088.
Effects of Coronavirus Disease 2019 (COVID-19) on Maternal, Perinatal and Neonatal Outcomes: A Systematic Review
J Juan 1, M M Gil 2 3, Z Rong 4, Y Zhang 5 6, H Yang 1, L C Poon 7 8
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7276742/

Di Mascio D, et al.
Outcome of Coronavirus spectrum infections (SARS, MERS, COVID 1 -19) during pregnancy: a systematic review and meta-analysis
Am J Obstet Gynecol MFM. 2020;2(2):100107. doi:10.1016/j.ajogmf.2020.100107
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7104131/
[123] Ultrasound Obstet Gynecol 2020 May;55(5):586-592. doi: 10.1002/uog.22014.
Coronavirus in Pregnancy and Delivery: Rapid Review.
E Mullins 1, D Evans 2 3, R M Viner 3 4, P O'Brien 5 6, E Morris 6 7
https://obgyn.onlinelibrary.wiley.com/doi/full/10.1002/uog.22014

Am J Obstet Gynecol 2020 May;222(5):415-426. doi: 10.1016/j.ajog.2020.02.017.
Coronavirus Disease 2019 (COVID-19) and Pregnancy: What Obstetricians Need to Know
Sonja A Rasmussen 1, John C Smulian 2, John A Lednicky 3, Tony S Wen 2, Denise J Jamieson
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7093856/

Am J Obstet Gynecol. 2020 Apr 23. pii: S0002-9378(20)30462-2. doi: 10.1016/j.ajog.2020.04.014.
Coronavirus disease 2019 (COVID-19) in pregnant women: A report based on 116 cases.
Yan J1, Guo J et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7177142/

IMMUNE RESPONSE, AGE, AND GENDER DIFFERENCE

Pediatric age [124]

The immune system in infants has a decreased innate response with increased susceptibility to viral infections (respiratory syncytial virus, herpes simplex virus and cytomegalovirus), Mycobacterium tuberculosis and Salmonella sp.

T cells in newborns promote tolerance toward self proteins and exhibit a Th2-shifted profile with a poor response toward foreign antigens, due to the need to be able to create a symbiosis with the microbiota and physiological virome and to avoid tissue damage in a period of rapid growth of the organism. [125]

---

[124] The Neonatal Immune System: A Unique Host-Microbial Interface.
Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-403-7 Bliss, J. M., Wynn, J. L., eds. (2018).

Proc Biol Sci. 2015 Dec 22;282(1821):20143085. doi: 10.1098/rspb.2014.3085.
Evolution of the immune system in humans from infancy to old age.
Simon AK, Hollander GA, McMichael A.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707740/pdf/rspb20143085.pdf

Acta Paediatr 2012 Feb;101(2):120-7. doi: 10.1111/j.1651-2227.2011.02494.x.
The Developing Immune System - From Foetus to Toddler
Sofia Ygberg 1, Anna Nilsson
https://pubmed.ncbi.nlm.nih.gov/22003882/

Nat Rev Immunol 2007 May;7(5):379-90. doi: 10.1038/nri2075.
Innate Immunity of the Newborn: Basic Mechanisms and Clinical Correlates
Ofer Levy 1

Nat Rev Immunol. 2017 Aug;17(8):495-507. doi: 10.1038/nri.2017.54.
Unique aspects of the perinatal immune system.
Zhang X1, Zhivaki D2, Lo-Man R2.
https://www.nature.com/articles/nri2075

Curr Opin Infect Dis. 2013 Jun;26(3):213-8. doi: 10.1097/QCO.0b013e32835fb8bf.
Early life response to infection.
Ghazal P1, Dickinson P, Smith CL.
https://pubmed.ncbi.nlm.nih.gov/23449137/

[125] Trends Immunol 35:299-310 (2014)
Ontogeny of early life immunity.
Dowling DJ, Levy O
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4109609/

Int Rev Immunol 19:173-193 (2000) Cellular
immune responses in neonates Fadel S,
Sarzotti M
https://pubmed.ncbi.nlm.nih.gov/10763708/

Immunol Res 22:177-190 (2000)
T cell immunity in neonates.
Garcia AM, Fadel SA, Cao S, Sarzotti M
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4407563/

Clin Exp Immunol 141:10-18 (2005)
T cell-mediated immune responses in human newborns: ready to learn?
Marchant A, Goldman M
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1809424/

Immunol 5:393 (2014)
Unbalanced neonatal CD4(+) T cell immunity.
Debock I, Flamand V Front
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4145351/

Immunity 46:350-363 (2017)
Protecting the newborn and young infant from infectious diseases: lessons from immune ontogeny
Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O.

At birth, B1 cells constitute 40% of peripheral blood B cells, and this frequency remains high for a few months. [126] The efficiency of the adaptive immune system required to respond to T- cell-dependent antigens early is significantly limited in infants compared with older children and adults.

Together with reduced innate immunity, weak Th1 and antibody responses explain why neonatal mortality can be high under conditions of increased pathogen exposure [127] and why vaccinations are poorly effective.

In particular:[128]
The fetal and neonatal immune system is associated with physiological needs on three levels:

- **Protection** against **infections**, including viral and bacterial pathogens present at the maternal-fetal interface [1,2]
- **Prevention of** potentially harmful **pro-inflammatory/(Th1)-polarized responses** that may induce allo-immune reactions, i.e., attack by the mother's immune system toward the fetus [3]
- **mediation of the transition from the** normally pathogen-free **intrauterine environment to the** antigen-rich **external environment**, including primary colonization of the skin [4] and intestinal tract [5] by microorganisms.

The fetus is an allograft on the mother and is at risk of rejection, so there is a **strong shift toward T helper 2** (Th2) **cells** in fetal immune responses, a trend that is maintained even in the neonatal period.

Because of the reduced production of Th1 cell-associated cytokines, the neonatal immune system was initially thought to be immature or depressed; in fact, neonatal monocytes and antigen-presenting cells (APCs) show reduced production of TNF, IL-12, and IFNγ, but retain production of IL-6, IL-10, and IL-23, an indication that the immune system is efficient. [12-14]

Birth initiates an acute inflammatory response characterized by increased serum levels of **IL-6** and increased IL-6-inducible hepatocyte products such as **C-reactive protein** (CRP) and lipopolysaccharide binding protein (**LPS**).
It is hypothesized that this response may serve to eliminate harmful microbes and their products that might pass through mucosal barriers during birth or in the initial colonization phase.[81, 12]

---

https://www.cell.com/action/showPdf?pii=S1074-7613%2817%2930090-0

Immunity 37:771-783 (2012)
Innate immune function by Toll-like receptors: distinct responses in newborns and the elderly.
Kollmann TR, Levy O, Montgomery RR, Goriely S
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3538030/

[126] Immunol Today. 1992;13(6):215-218. doi:10.1016/0167-5699(92)90157-3
Developmental and maturational changes in human blood lymphocyte subpopulations.
Hannet I, Erkeller-Yuksel F, Lydyard P, Deneys V, DeBruyère M.
https://pubmed.ncbi.nlm.nih.gov/1627249/
[127] Expert Rev Clin Immunol. 2014 Sep;10(9):1171-84. doi: 10.1586/1744666X.2014.942288.
Immune responses in neonates.
Basha S, Surendran N, Pichichero M.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4407563/

[128] Literature reference in square brackets
Nat Rev Immunol 7, 379-390 (2007). https://doi.org/10.1038/nri2075
Innate immunity of the newborn: basic mechanisms and clinical correlates.
Levy, O.
https://www.nature.com/articles/nri2075

The physiological significance of the profile of cytokines produced in the newborn can be observed by studying the potentially negative consequences of excessive production of pro-inflammatory cytokines, such as TNF, such as reduced intrauterine growth [117] and spontaneous abortion [10].

In addition, new evidence indicates that TLR-mediated induction of pro-inflammatory cytokines by microglia (the macrophages of the CNS) may contribute to CNS damage in premature infants that can culminate in cerebral palsy [118,119], thus providing further confirmation of the function of the cytokines produced.

Shortly after birth, following initial microbial colonization, **the neonatal intestinal tract inhibits its inflammatory responses to endotoxins to avoid excessive and potentially harmful reactions to the common Gram-negative bacterial flora**.
Neonatal intestinal immunity can be significantly modified by breastfeeding, and it is hypothesized that **the immunomodulatory effect mediated by breast milk is to induce subclinical infections in the intestine that gradually stimulate immunological memory to pathogens** [43].

The neonatal respiratory tract manifests age-dependent maturation of Toll-like receptor (TLR)-mediated responses and expression of antimicrobial proteins and peptides (APPs).
As the main gateway for environmental adjuvants (aeroadjuvants: TLR agonist microbial products) and antigens (aeroallergens), **the respiratory tract could mediate the maturation of Th1-type cellular responses** in **neonates and infants** following repeated low-dose exposure to environmental TLR agonists, as supported by hygiene theory.

> ***Hygiene theory*** *argues that exposure to microbial components, including Toll-like receptor (TLR) agonists during the neonatal, infancy, and pre-adolescent developmental stages, serves to bias the immune system's response toward T helper 1 (TH1) cells versus Th2 cells, thereby reducing the likelihood of allergy and/or atopy. Consistent with this hypothesis, there are findings from epidemiological studies showing an inverse association between the incidence of infections and autoimmunity/allergies.* [18,63]

Immune characteristics in infants:

- Levels of multiple soluble plasma proteins that play a role in innate immunity **are lower** in infants than in adults.
- Neonatal plasma concentrations of complement components **are decreased** by 10 to 70 percent compared with those in adults [8].
- A deficiency in complement proteins could contribute to the inability of newborns to limit the replication of many bacterial strains in the blood [67], and since complement components also play a role in adaptive immunity [68], this could contribute to **impaired neonatal responses** [69].
- Neonatal neutrophils show **qualitative defects** in reduced expression of integrin and selectin, some antimicrobial proteins, and phagocyte oxidase induction. In addition, newborns experience a **quantitative deficit of** neutrophils during stress conditions.[88,90]

Solid responses to certain microbial stimuli, including single-stranded viral RNAs that activate cells through TLR8s, are exceptions to the generally impaired production of pro-inflammatory and Th1-polarizing cytokines. [75,100]
Mycobacterium bovis bacillus Calmette - Guérin (BCG) is a vaccine administered at birth that can induce a strong cytokine response that induces Th1 cell polarization, including IFNγ expression by $^{CD4+}$ T cells in response to a mycobacterial purified protein derivative (PPD) [130].

These examples indicate that stimuli with certain characteristics, including the ability to effectively activate certain pathways mediated by TLRs and/or the complement system, are able to overcome the infant's immune tolerance and induce a robust Th1 response.

The hyporesponsiveness to pediatric vaccines

> ***Hyporesponsiveness** (immune tolerance) is defined as the **individual's inability to develop an immune response after vaccination booster at least equal to, or greater in magnitude than, the response that was induced after primary vaccination**.*

Hyporesponsiveness can occur when vaccination starts very early in life and has been observed, for example, after administration of DTP, DTaP, or DT vaccine to infants, **but also after repeated doses of bacterial polysaccharide** (PS) **conjugate vaccines**, particularly against serogroup C meningococcus (MencC) **and other polysaccharide-glycoconjugate vaccines** (e.g. anti-pneumococcal, anti-Haemophylus influenzae b,) **regardless of the age at which vaccination was given**. [129]

[129] Curr Opin Infect Dis 24:190-195 (2011)
Neonatal immunization: where do we stand?
Wood N, Siegrist CA
https://pubmed.ncbi.nlm.nih.gov/21415741/

Eur J Immunol 39:36-46 (2009)
Safety and efficacy of neonatal vaccination.
Demirjian A, Levy O
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2739303/

J Pediatr 153:305-307 (2008)
Blame vaccine interference, not neonatal immunization, for suboptimal responses after neonatal diphtheria, tetanus, and acellular pertussis immunization.
Siegrist CA
https://www.jpeds.com/article/S0022-3476(08)00309-0/fulltext

J Comp Pathol 137(Suppl 1): (S4-S92007)
The challenges of vaccine responses in early life: selected examples.
Siegrist CA
https://pubmed.ncbi.nlm.nih.gov/17559867/

Nat Immunol 12:189-194 (2011)
Challenges in infant immunity: implications for responses to infection and vaccines.
PrabhuDas M, Adkins B, Gans H, King C, Levy O, Ramilo O, Siegrist CA
https://www.nature.com/articles/ni0311-189

Am. J. Public Health 49(5), 644-655 (1959).
Multiple antigen for immunization against polyomyelitis, diphtheria, pertussis, and tetanus. II. Response of infants and young children to primary immunization and eighteen-month boosters. Barrett CD, Timm EA, Molner JG, Wilner BI, Fahey MF, McLean IW.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1372843/pdf/amjphnation00321-0066.pdf

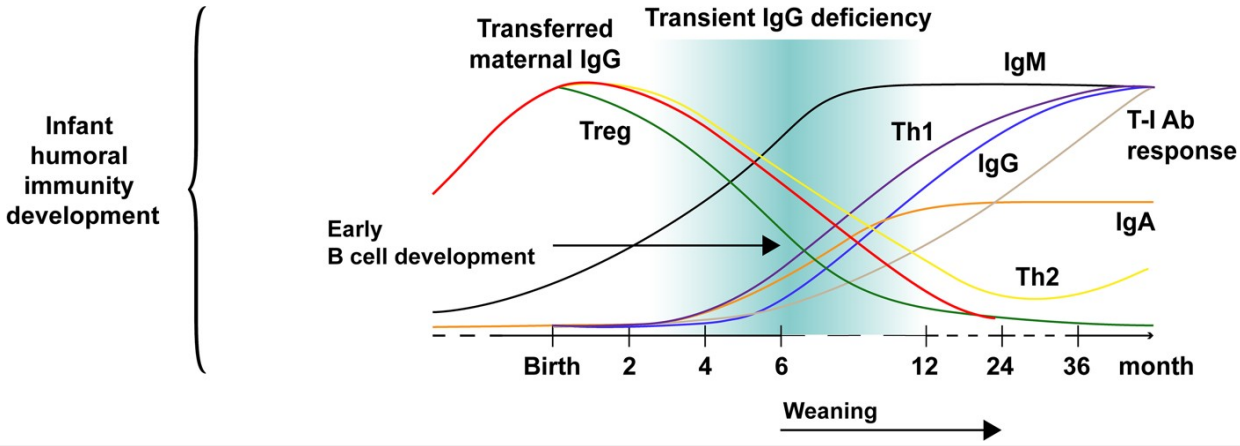
J. Pediatr. 153(3), 327-332 (2008).
Poor immune responses to a birth dose of diphtheria, tetanus, and acellular pertussis vaccine.
Halasa NB, O'Shea A, Shi JR, LaFleur BJ, Edwards KM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3773719/

J. Pediatr. 126(2), 198-205 (1995).
Effect of neonatal immunization with diphtheria and tetanus toxoids on antibody responses to Haemophilus influenzae type b conjugate vaccines.
Lieberman JM, Greenberg DP, Wong VK et al.
https://www.jpeds.com/article/S0022-3476(95)70545-7/fulltext

J. Infect. Dis. 201(10), 1570-1579 (2010).
Nasopharyngeal carriage of Streptococcus pneumoniae shortly before vaccination with a pneumococcal conjugate vaccine causes serotype-specific hyporesponsiveness in early infancy
Dagan R, Givon-Lavi N, Greenberg D, Fritzell B, Siegrist CA.
https://academic.oup.com/jid/article/201/10/1570/993471

As discussed above, given the limited exposure to antigens in utero, infants are especially dependent on their innate immunity to stimulate adaptive immune responses.

The unique characteristics of newborn infants' innate immunity are therefore critical in explaining why they have suboptimal vaccine responses compared with older children. [130]



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5179050/pdf/ppat.1005997.pdf [131]
The curves represent various levels of T- and B-cell response in infants, with the upper limit equal to 100% of adult levels.

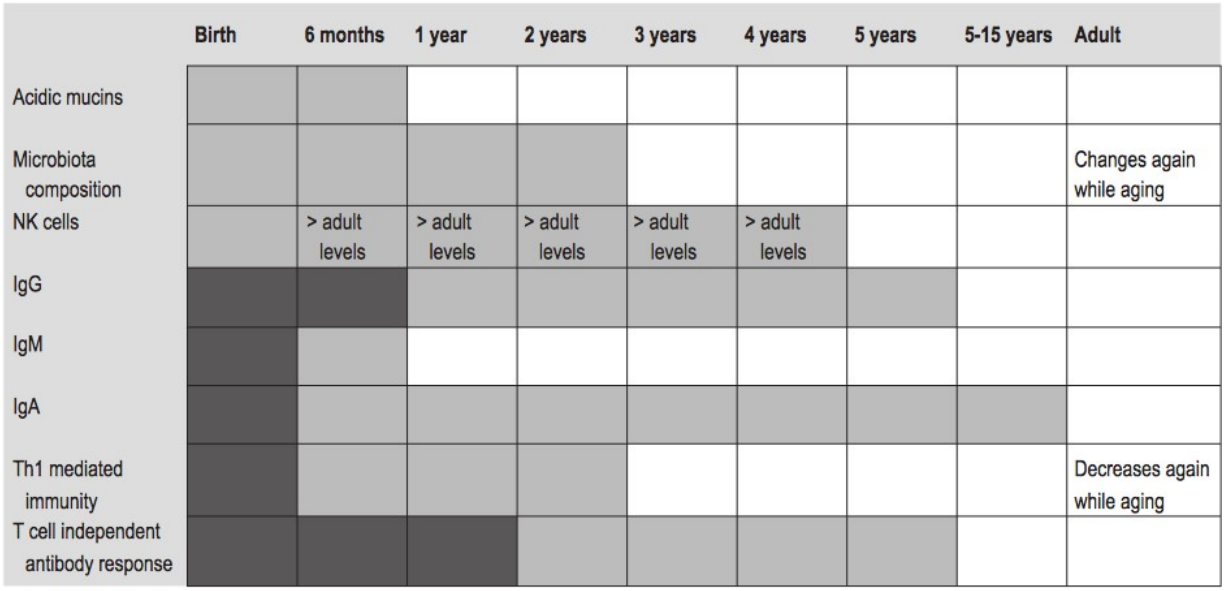
For summary purposes, it is recalled that:
*- **IgM** are immunoglobulins produced upon first contact with antigen; they circulate only in the blood and protect the blood against disease.*
*- **IgG** are the most numerous plasma immunoglobulins; they are produced when IgM concentrations begin to fall and especially when there is in recall, that is, a memory immune response for a second contact with an antigen (foreign to the body); they are found in both the blood and extravascular spaces and are the only ones capable of crossing the placental barrier; they protect against bacteria, viruses, and toxins*
*- **IgA** are the immunoglobulins that defend mucous membranes (respiratory, digestive, genito-urinary) and are therefore found in all mucosal secretions.*
*- **IgE**, like IgA, are immunoglobulins found primarily in mucosal secretions, especially of the respiratory and gastrointestinal tracts; they play a role in defense against parasites. They are underrepresented in the blood, but increase in allergic or parasitic diseases.*

J Pediatr. Infect. Dis. 26(8), 716-722 (2007).
Reconsideration of the use of meningococcal polysaccharide vaccine.
Granoff DM, Pollard AJ
https://pubmed.ncbi.nlm.nih.gov/17848884/

Expert Review of Vaccines, (2011) 10:3, 307-322, DOI: 10.1586/erv.11.8
Hyporesponsiveness and its clinical implications after vaccination with polysaccharide or glycoconjugate vaccines
Jan Poolman, Ray Borrow
https://www.tandfonline.com/doi/full/10.1586/erv.11.8

[130] Semin Immunopathol. 2017 Nov;39(6):627-642. doi: 10.1007/s00281-017-0654-9
Vaccine responses in newborns.
Saso A, Kampmann B.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5711983/

[131] PLoS Pathog. 2016 Dec 22;12(12): e1005997. doi: 10.1371/journal.ppat.1005997.
The Impact of the Gut Microbiota on Humoral Immunity to Pathogens and Vaccination in Early Infancy.
Nguyen QN, Himes JE, Martinez DR, Permar SR.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5179050/



Taken from https://www.wageningenacademic.com/doi/pdf/10.3920/BM2010.0027[132]
Immune system development. Infants have a limited capacity to initiate immune responses, and innate and adaptive immune responses are impaired. The kinetics of immune system maturation varies among different components (dark gray = immature; light gray = developing; white = levels in adults).

Looking at the two figures above, it can be seen that from the sixth month of intrauterine life, the baby begins to produce relatively low levels of immunoglobulin M (**IgM**), while at the time of birth (at 9 months after conception) there is a peak in the baby's blood relative to **maternal antibodies.**
The level of these antibodies is very high and is formed almost exclusively by G-type immunoglobulins (**IgG**).

Immediately after birth, maternal antibodies drop gradually from the third month of extrauterine life, while the level of IgG antibodies produced by the baby, which starts at birth and rises slowly over time, is still relatively low.
As for **type A antibodies**, after birth and for several months their level remains very low.
It follows that infants are more prone to diseases affecting the mucous membranes because their defenses are still deficient due to low IgA production.

*It is important to remember that there is **transient hypogammaglobulinemia** in the newborn that begins at the age of 3-6 months and usually lasts 6-18 months.*
It is caused by a transient deficit in antibody production due to a physiological delay in the onset of antibody synthesis and can be diagnosed by a normal blood test: total immunoglobulins are less than 400 mg/dl.

*The time window in which transient hypogammaglobulinemia occurs is also called the **"window of vulnerability"** because the infant could be exposed to infectious diseases and especially the risk of complications without adequate support from the immune system.*

During this period, any attack on the immune system by toxic (environmental contaminants, in food, drugs, vaccines, ect) and infectious maternal substances during gestation and postnatally can result in a

[132] Benef Microbes 2010 Nov;1(4):367-82. doi: 10.3920/BM2010.0027.
Early Life: Gut Microbiota and Immune Development in Infancy
R Martin 1, A J Nauta, K Ben Amor, L M J Knippels, J Knol, J Garssen
https://www.wageningenacademic.com/doi/pdf/10.3920/BM2010.0027

increased risk of subsequently developing chronic infectious diseases, cancer, allergy, autoimmunity, and neurological, reproductive, and endocrine system disorders. [133]

Most antibody responses, including those to bacterial proteins, bacterial polysaccharides, and polysaccharide-protein conjugate vaccines, depend on T-cell activity.
However, neonatal B cells express low levels of receptors that interact with T cells, limiting their ability to respond [134].

In addition, low levels of the receptor for complement fragment C3d (CD21) prevent responses to polysaccharide-complement [135] complexes.
Together, these features lead to **weak humoral immune responses** with incomplete immunoglobulin class switching [136] although memory B cells [137] are generated.

B cells of infants and children younger than 2 months show **reduced somatic hypermutation** compared with adults, limiting [138] antibody affinity maturity.

---

[133] J Toxicol Environ Health B Crit Rev. 2008;11(8):660-680. doi:10.1080/10937400802370923
Potential for early-life immune insult including developmental immunotoxicity in autism and autism spectrum disorders: focus on critical windows of immune vulnerability.
Dietert RR, Dietert JM.
https://pubmed.ncbi.nlm.nih.gov/18821424/

Environ Health Perspect. 2000 Jun;108 Suppl 3(Suppl 3):483-90. doi: 10.1289/ehp.00108s3483
Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary
Dietert RR, Etzel RA, Chen D, Halonen M, Holladay SD, Jarabek AM, Landreth K, Peden DB, Pinkerton K, Smialowicz RJ, Zoetis T.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1637823/

Cell. 2018;174(5):1051-1053. doi:10.1016/j.cell.2018.08.001
Neonate-omics: Charting the Unknown Immune Response in Early Life.
Jennewein MF, Butler AL, Alter G.
https://www.cell.com/action/showPdf?pii=S0092-8674%2818%2930978-4

Nat Rev Immunol. 2017 Jan;17(1):21-29. doi: 10.1038/nri.2016.125.
Human immune system variation.
Brodin P, Davis MM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5328245/

Front Immunol. 2017 Aug 11;8:957. doi: 10.3389/fimmu.2017.00957. Postnatal
Innate Immune Development: From Birth to Adulthood.
Georgountzou A, Papadopoulos NG.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5554489/

[134] Blood 2007, 110, 2948-2954. 10.1182/blood-2007-01-069245
Decreased expression of tumor necrosis factor family receptors involved in humoral immune responses in preterm neonates.
Kaur K, Chowdhury S, Greenspan NS, Schreiber JR
https://ashpublications.org/blood/article/110/8/2948/24000/Decreased-expression-of-tumor-necrosis-factor

[135] Infect. Immun. 1991; 59, 1839-1845.
Pneumococcal polysaccharides complexed with C3d bind to human B lymphocytes via complement receptor type 2.
Griffioen AW, Rijkers GT, Janssens-Korpela P, Zegers BJ
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC257924/pdf/iai00041-0263.pdf

[136] Clin Exp. Immunol. 2010, 160, 331-339. 10.1111/j.1365-2249.2010.04104.x
Antigen-dependent immunotherapy of non-obese diabetic mice with immature dendritic cells.
Haase C, Yu L, Eisenbarth G, Markholst H.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2883103/

[137] J. Exp. Med. 2005. 201, 993-1005.
Complement receptors regulate differentiation of bone marrow plasma cell precursors expressing transcription factors Blimp-1 and XBP-1.
Gatto D, Pfister T, Jegerlehner A, Martin SW, Kopf M, Bachmann MF.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2213108/

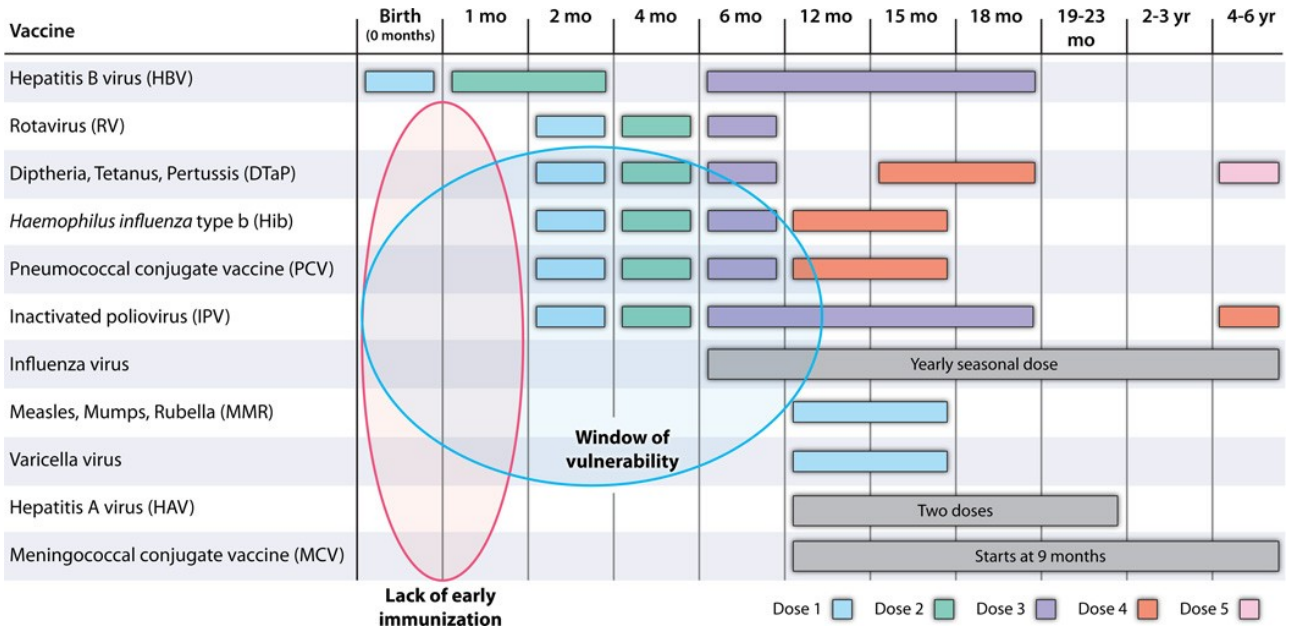
[138] Clin. Exp. Immunol. 1998. 114, 33-39. 10.1046/j.1365-2249.1998.00694.x
Somatic mutation of immunoglobulin VH6 genes in human infants.
Ridings J, Dinan L, Williams R, Roberton D, Zola H.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1905087/

Finally, there is a **failure of bone marrow stromal cells at an** early age to support long-term plasma survival and plasma cell differentiation, such that the IgG antibodies that are formed decline rapidly after immunization, unlike in children and adults [139].

Therefore, the efficiency of the adaptive immune system in early response to T-cell-dependent antigens is significantly reduced in infants.
This physiological behavior is particularly relevant to vaccination programs.

Together with reduced innate immunity, weak Th1 and antibody responses largely explain why neonatal mortality can be high under conditions of increased pathogen exposure.

As can be seen in the diagram below, ***most pediatric vaccinations are administered precisely during this time interval when the immune system is unable to respond effectively to vaccine antigenic stimuli*** *and as will be seen later is even predisposed to inhibition of the vaccination response and the* ***potential risk of post-vaccinal ADE due to the possible presence of maternal antibodies****.*



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4108897/ [140]

[139] J. Immunol. 2006. 176, 165-172. 10.4049/jimmunol.176.1.165
Reduced ability of neonatal and early-life bone marrow stromal cells to support plasmablast survival.
Pihlgren M, Friedli M, Tougne C, Rochat AF, Lambert PH, Siegrist CA.
https://www.jimmunol.org/content/176/1/165.long

[140] Sci Transl Med. 2011 Jul 6;3(90):90ps27 doi: 10.1126/scitranslmed.3001880.
Development of newborn and infant vaccines
Sanchez-Schmitz G, Levy O.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4108897/

Maternal factors that can potentially influence the outcome of neonatal immunization (i.e., vaccine antibody production) are of three types:

- **Maternal-fetal transfer of antibodies,**
- **maternal-fetal transfer of pathogenic organisms**
- **Chronic maternal infections.** [141]

Maternal antibody transfer from mother to offspring can occur during pregnancy (from maternal blood through transplacental transfer, mediated by FcR receptors) and within 24 hours of birth (from colostrum through the small intestine).
In general, the placenta poses a barrier that can, at least partially, control and hinder the transmission of harmful substances from the mother to the fetus, and the presence of a specific and active transport mechanism that transfers specific antibodies against maternal pathogens to the fetus is necessary.

In this regard, as already mentioned, **the neonatal Fc receptor (FcRn) expressed in placental syncytiotrophoblasts plays a key role.**[142]

---

[141] Front Immunol. 2020 Mar 31;11:555. doi: 10.3389/fimmu.2020.00555
Vertically Transferred Immunity in Neonates: Mothers, Mechanisms and Mediators.
Albrecht M, Arck PC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7136470/

Front Immunol. 2014 Sep 16;5:446. doi: 10.3389/fimmu.2014.00446. Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies.
Niewiesk S.
https://pubmed.ncbi.nlm.nih.gov/25278941/

Vaccine 2015 Nov 25;33(47):6469-72. doi: 10.1016/j.vaccine.2015.07.085.
Maternal Antibodies and Infant Immune Responses to Vaccines
Kathryn M Edwards 1
https://www.sciencedirect.com/science/article/pii/S0264410X15010634?via%3Dihub

Cell Rep. 2019;28(7):1773-1784.e5. doi:10.1016/j.celrep.2019.07.047
Maternal Antibodies Inhibit Neonatal and Infant Responses to Vaccination by Shaping the Early-Life B Cell Repertoire within Germinal Centers.
Maria Vono et al
https://www.sciencedirect.com/science/article/pii/S2211124719309519

Saso A, Kampmann B.
Vaccine responses in newborns.
Semin Immunopathol. 2017;39(6):627-642. doi:10.1007/s00281-017-0654-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5711983/

J Comp Pathol. 2007;137(Suppl 1):S27-S31. doi: 10.1016/j.jcpa.2007.04.008.
Immune responsiveness in the neonatal period.
Morein B, Blomqvist G, Hu K.
https://pubmed.ncbi.nlm.nih.gov/17548093/

Vaccine. 2003;21:3406-3412. doi: 10.1016/S0264-410X(03)00342-6.
Mechanisms by which maternal antibodies influence infant vaccine responses: review of hypotheses and definition of main determinants.
Siegrist CA.
https://pubmed.ncbi.nlm.nih.gov/12850349/

[142] Front Immunol 2019 Jul 10;10:1540. doi: 10.3389/fimmu.2019.01540.
The Neonatal Fc Receptor (FcRn): A Misnomer?
Michal Pyzik 1, Kine M K Sand 1 2, Jonathan J Hubbard 1 3, Jan Terje Andersen 4 5, Inger Sandlie 2, Richard S Blumberg
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6636548/

Kiskova T, Mytsko Y, Schepelmann M, et al. Expression of the neonatal Fc-receptor in placental-fetal endothelium and in cells of the placental immune system. Placenta. 2019;78:36-43. doi:10.1016/j.placenta.2019.02.012
https://pubmed.ncbi.nlm.nih.gov/30955709/

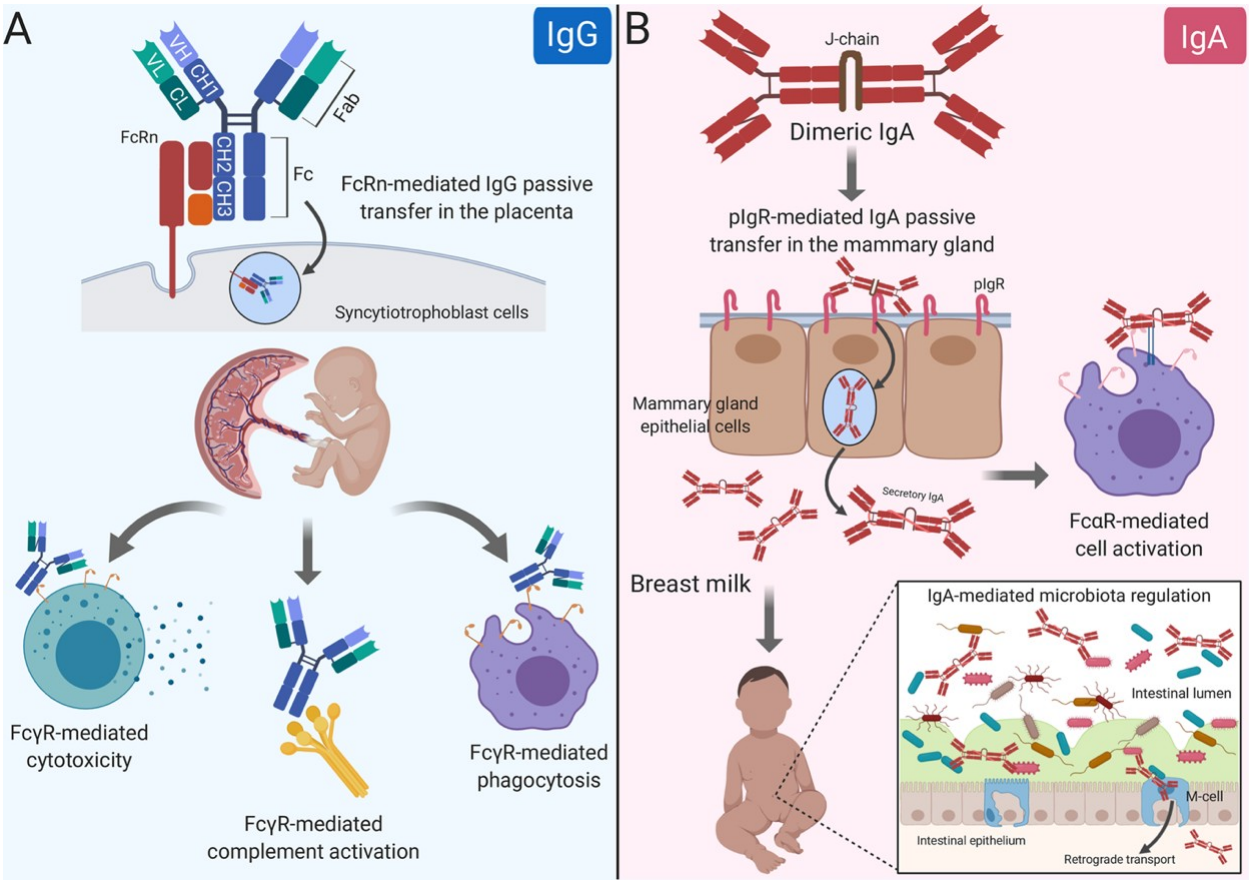
Front Immunol. 2019 Mar 19;10:464. doi: 10.3389/fimmu.2019.00464.
The Human FcγRII (CD32) Family of Leukocyte FcRs in Health and
Disease. Anania JC, Chenoweth AM, Wines BD, Hogarth PM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6433993/.

The vast majority of maternal antibodies are of the IgG isotype.
These passively acquired antibodies enter the bloodstream of the offspring and act as a protective shield throughout the body in the same way as actively produced antibodies.

Sometimes IgA antibodies contained in breast milk are also called maternal antibodies, however, there are important differences in the action of passively transferred IgG and IgA antibodies.

After birth, ***IgG antibodies are present in the infant's bloodstream in finite amounts that decrease over time and are responsible for suppressing vaccine-induced immune responses.***

In contrast, ***IgA antibodies are continuously provided by the mother through breast milk*** *and protect the gastrointestinal tract from pathogens without affecting the immune response.*



Taken from https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1008303
Passive maternal antibody transfer and functional activity in the newborn. **(A)** Passive transfer of IgG in the placenta influences FcγR-mediated cell cytotoxicity, phagocytosis and complement activation in the developing fetus/newborn. **(B**) Passive transfer of IgA from mammary gland causes FcαR- and IgA-mediated cell activation and microbiota regulation, respectively; Fab, antigen-binding fragment; Fc, crystallizable fragment; FcαR, Fc alpha receptor; FcRn, neonatal Fc receptor; FcγR, Fc gamma receptor; IgA, immunoglobulin A; IgG, immunoglobulin G; J-chain, splicing chain; pIgR, polymeric immunoglobulin receptor.

Maternal antibodies are very effective in protecting infants and children from most infectious diseases. The most striking example is the **protection of children with agammaglobulinemia** (deficiency in antibody production) against bacterial infection up to 6 months of age.

Other documented examples of the ability of maternal antibodies to fully or partially protect against infectious diseases include respiratory syncytial virus (RSV) infection and influenza virus [143].

Over time, maternal antibody titers decline because antibodies are metabolized; in humans, maternal antibodies decline within a period of 6-12 months , [144] with kinetics of decline correlating with the amount of maternal antibody present in the infant after birth, and higher titers persist for a longer period.

It is this stage of maternal antibody depletion that presents a **window of opportunity** for pathogens to infect the infant. However, even low and unprotective titers of maternal antibodies are still capable of inhibiting vaccination against infectious diseases in humans and animals.

Interestingly, in the studies conducted, **all types of vaccines (attenuated, inactivated, subunit vaccines) were found to be inhibited**. [145]

Table 1

Inhibition of seroconversion of human vaccines by maternal antibodies.

Infectious agent	Type of vaccine	Reference
Tetanus	Combination protein vaccine	(25)
Pneumococcus	Combination protein vaccine	(25, 26)
Hib	Combination protein vaccine	(25, 27)
Pertussis	Combination protein vaccine	(25)
	Acellular and whole-cell vaccine	(28)
Measles virus	Live-attenuated	(29–31)
Mumps virus	Live-attenuated	(32)
Hepatitis A virus	Inactivated virus	(33)
Hepatitis B virus	Protein vaccine	(34)
Rotavirus	Live-attenuated	(35)
Poliovirus	Inactivated virus	(36, 37)
	Live-attenuated vaccine	(38)
Influenza virus	Cold recombinant influenza and trivalent inactivated virus	(39)

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4165321/

[143] Medicine (Baltimore) (2006) 85:193-202. doi:10.1097/01.md.0000229482.27398.ad
X-linked agammaglobulinemia: report on a United States registry of 201 patients.
Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al.
https://journals.lww.com/md-journal/Fulltext/2006/07000/X_Linked_Agammaglobulinemia __Report_on_to_United.1.aspx

PLoS One (2009) 4:e8088. doi:10.1371/journal.pone.0008088
The level and duration of RSV-specific maternal IgG in infants in Kilifi Kenya.
Ochola R, Sande C, Fegan G, Scott PD, Medley GF, Cane PA, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2779853/

Clin Infect Dis (2010) 51:1355-61. doi:10.1086/657309
Influenza vaccine given to pregnant women reduces hospitalization due to influenza in their infants
Benowitz I, Esposito DB, Gracey KD, Shapiro ED, Vazquez M
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3106242/

[144] J Trop Pediatr (2003) 49:302-5. doi:10.1093/tropej/49.5.302
The duration of maternal measles antibodies in children
Kilic A, Altinkaynak S, Ertekin V, Inandi T.

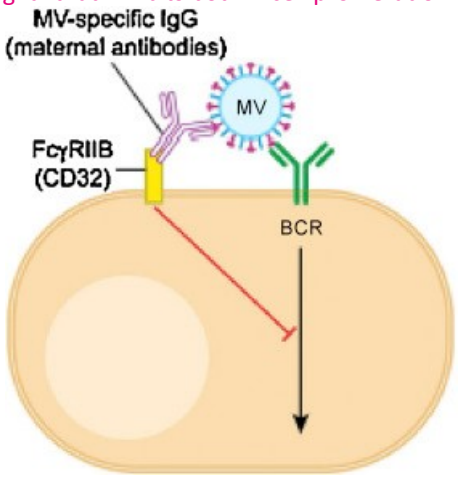
[145] Niewiesk S.
Maternal antibodies: clinical significance, mechanism of interference with immune responses, and possible vaccination strategies.
Front Immunol. 2014;5:446. doi:10.3389/fimmu.2014.00446
https://academic.oup.com/tropej/article/49/5/302/1690003

This table shows a list of examples of studies documenting inhibition or reduction of seroconversion after immunization of attenuated or inactivated vaccines. Jones et al. document a greater inhibitory effect by maternal antibodies on tetanus and penumococcal vaccines than on Hib and pertussis vaccines. Most studies report that higher levels of maternal antibodies inhibit antibody development more severely than lower titers. Two studies indicate that increased dose helps or improves responses after immunization in the presence of maternal antibodies

One of the most interesting mechanisms hypothesized for the role of maternal antibody in the inhibition of pediatric vaccine responses is the effective **blockade of B-cell activation by the cross-linkage between the B-cell receptor (BCR), which recognizes the vaccine antigen, and the FcγRIIB** receptor* that binds the maternal IgG molecule on the B-cell surface.

> * *Recall again that the three human FcγR classes include FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16).* ***FcγRI*** *is the only high-affinity receptor that can bind to monomeric IgG molecules. All other FcγRs are low-affinity receptors and therefore require multiple interactions to produce sufficiently high avidity binding by IgG immune complexes to transduce relevant signals. Of these,* ***FcγRIIa and FcγRIIc are activating receptors, while FcγRIIb is the only known inhibitory FcγR****.* [146]

This cross-binding causes a negative signal that inhibits both B-cell proliferation and antibody secretion.



Taken from https://www.sciencedirect.com/science/article/pii/S0264410X15010634?via%3Dihub#tbl0005
A frequent example involves measles (MV). When maternal-specific IgG binds to vaccine MV, the constant region of IgG binds to the receptor for the constant region (Fc) of IgG (which is FcγRIIB) present on the B lymphocyte membrane. FcγRIIB is the only Fc receptor of B cells and does not bind to IgM or IgA immunoglobulins. After the cross linkage between BCR and FcγRIIB is formed, the inhibitory signal from FcγRIIB blocks BCR activation, as shown by the red line.

In evolutionary terms, **this mechanism evolved to avoid a hyper-reactive B-cell response in order to conserve resources**.
If IgG antibodies are already present in an organism after infection or vaccination of the mother during pregnancy, there is no need to produce more antibodies.
In essence, maternal antibodies signal that more antibodies do not need to be produced.

In contrast to antibody production after an active immune response, passively transferred maternal antibodies decrease, and the child is left without an adequate B-cell and antibody response, during the window of vulnerability as seen above.

Keep in mind that during the very early stages of life, FcRn mediates the passive transfer of IgG from mother to offspring both before and after birth.

[146] Immunol Rev. 2015 Nov;268(1):340-64. doi: 10.1111/imr.12367
Fc receptors in antibody-dependent enhancement of viral infections
Taylor A, Foo SS, Bruzzone R, Dinh LV, King NJ, Mahalingam S.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7165974/

As already seen, IgG in milk is bound to the apical surface of the intestinal epithelium.
The resulting FcRn-IgG complexes are internalized in the cells of the intestinal epithelium, and IgG is released from FcRn into the blood or tissue fluids.

During life, FcRn contributes to effective humoral immunity by recycling IgG and extending its half-life in the circulation. In adults, FcRn regulates the persistence of IgG and albumin in serum, as well as the movement of IgG and any bound cargo, between different body compartments, facilitating the efficient initiation of immune responses toward pathogens.

Therefore, FcRn continues to play the role of an immunological sensor throughout adult life, particularly in regions such as the intestine that are exposed to a large number of infectious antigens. [147]

Maternal-fetal immunopathology

Maternal antibodies

As already widely discussed, pre-existing antibodies can facilitate viral infections.
In dengue, nonneutralizing antibodies induced by natural infection with one of the four dengue viruses (DENV) can enhance infection with a different virus by intrinsic antibody-dependent enhancement (iADE).
In addition, nonprotective antibodies induced by respiratory syncytial virus (RSV) and measles inactivated by formaldehyde have been shown to cause disastrous worsening of the disease during subsequent natural infection, [148]
In that they are immunogens that fail to stimulate TLR4, and led to failure of affinity maturation.

Low avidity, nonneutralizing but complement-fixing antibodies induced by administration of inactivated vaccines have been able to produce pathological immune complexes following respiratory tract viral infections.

---

[147] Semin Immunopathol. 2009 Jul;31(2):223-36. doi: 10.1007/s00281-009-0160-9.
Immune and non-immune functions of the (not so) neonatal Fc receptor, FcRn.
Baker K, Qiao SW, Kuo T, Kobayashi K, Yoshida M, Lencer WI, Blumberg RS.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3898171/

https://www.proteinatlas.org/ENSG00000104870-FCGRT

J Biol Chem. 2014 Mar 14;289(11):7812-24. doi: 10.1074/jbc.M113.537563.
Structural insights into neonatal Fc receptor-based recycling mechanisms.
Oganesyan V, Damschroder MM, Cook KE, Li Q, Gao C, Wu H, Dall'Acqua WF.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3953293/

J Exp Med. 1994 Dec 1;180(6):2377-81. doi: 10.1084/jem.180.6.2377.
A major histocompatibility complex class I-like Fc receptor cloned from human placenta: possible role in transfer of immunoglobulin G from mother to fetus.
Story CM, Mikulska JE, Simister NE.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2191771/

Eur J Immunogenet 2000 Aug;27(4):231-40. doi: 10.1046/j.1365-2370.2000.00225.x.
Cloning and Analysis of the Gene Encoding the Human Neonatal Fc Receptor
J E Mikulska 1, L Pablo, J Canel, N E Simister
https://pubmed.ncbi.nlm.nih.gov/10998088/

[148] JAMA 202:1075-1080. 1967.
Altered reactivity to measles virus. Atypical measles in children previously immunized with inactivated measles virus vaccines
Fulginiti, F. A., J. J. Eller, A. W. Downie, and C. H. Kempe
https://pubmed.ncbi.nlm.nih.gov/6072745/

Am. J. Epidemiol. 89:422-434. 1969
Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine.
Kim, H. W., J. G. Canchola, C. D. Brandt, G. Pyles, K. Chanock, K. Jensen, and R. H. Parrott. .
https://academic.oup.com/aje/article-abstract/89/4/422/198849?redirectedFrom=fulltext

These antibodies were responsible for the pathogenesis of disease potentiation, as they failed to neutralize RSV and promoted its replication. Inactivation of RSV by methods other than the use of formaldehyde equally sensitized experimental animals to [149] disease potentiation, confirming that **this mechanism depends on the binding of the virus-antibody complex to the FcRI receptor of macrophages and not on the way the vaccines are prepared**.

Maternal nonneutralizing antibodies can be either of natural origin (i.e., from infections contracted naturally before pregnancy by the mother), or of vaccine origin if the mother was vaccinated during pregnancy, and **have been implicated in the ADE that occurs in children less than 1 year old born to immune mothers who are exposed to infections by a second serotype**.[150]

*In addition to the above mechanisms regarding the functionality of the infant's immune system, it should be kept in mind that cells of innate immunity and other nonimmune cells are already capable of responding to reinfection by the acquisition of a nonspecific memory termed **trained immunity or memory of innate immunity**; positive features of the memory of innate immunity include an enhanced ability to respond to secondary infections.*

The activation state of innate immunity declines rapidly after infection is resolved, however, it often does not return to basal levels after infection, but remains enhanced through mid- and long-term reprogramming effects.

Trained immunity can be considered the opposite of immune tolerance, the state in which innate immunity is suppressed during and after severe infections such as sepsis.
A deleterious effect of trained immunity is the persistence of a chronic hyperinflammatory state and the risk of autoimmunity, while that of immune tolerance is an increased risk of complications from infectious diseases. [151]

---

[149] Clinical and Vaccine Immunology Nov 2010, 17 (12) 1829-1835; DOI: 10.1128/CVI.00316-10
How Innate Immune Mechanisms Contribute to Antibody-Enhanced Viral Infections
Sukathida Ubol, Scott B. Halstead
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3008185/

[150] PLoS Pathog. 2019 Apr 22;15(4):e1007721. doi: 10.1371/journal.ppat.1007721
Detection of post-vaccination enhanced dengue virus infection in macaques: An improved model for early assessment of dengue vaccines.
Borges MB, Marchevsky et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6497418/

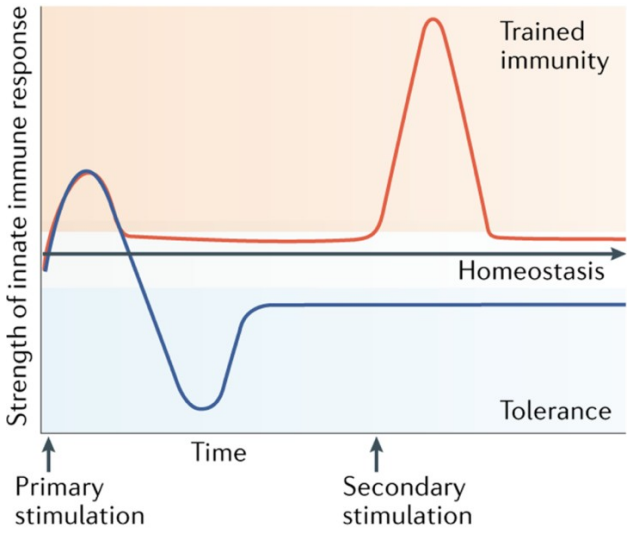
Virology. 2016 Apr;491:79-88. doi: 10.1016/j.virol.2016.01.015.
Heterologous challenge in the presence of maternally-derived antibodies results in vaccine-associated enhanced respiratory disease in weaned piglets.
Rajao DS, Sandbulte MR, Gauger PC, Kitikoon P, Platt R, Roth JA, Perez DR, Loving CL, Vincent AL.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4825855/

[151] Front Microbiol. 2019 Jan 14;9:3225. doi: 10.3389/fmicb.2018.03225.
Trained Immunity Carried by Non-immune Cells.
Hamada A1, Tower C1, Drancourt M1, Ghigo E1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6340064/

Neonatology. 2014;105(2):136-41. doi: 10.1159/000356035.
A prime time for trained immunity: innate immune memory in newborns and infants.
Levy O1, Wynn JL.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3946366/

Nat Rev Immunol. 2020 Mar 4. doi: 10.1038/s41577-020-0285-6.
Defining trained immunity and its role in health and disease.
Netea MG et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7186935/

Hajishengallis G, Li X, Mitroulis I, Chavakis T.
Trained Innate Immunity and Its Implications for Mucosal Immunity and Inflammation.
Adv Exp Med Biol. 2019;1197:11-26. doi:10.1007/978-3-030-28524-1_2
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6986364/



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7186935/
memory of innate immunity and tolerance: two opposing functional programs of innate immunity.
Infections or sterile triggers in tissues induce inflammation and activation of immune system mechanisms. In conjunction with a proinflammatory response, anti-inflammatory mechanisms are induced to prevent potentiation of inflammation and tissue damage and to limit the inflammatory response over time. Training immunity involves epigenetic and metabolic reprogramming of innate immune cells, enabling qualitatively and quantitatively adapted innate immune cell responses to subsequent heterologous stimulation. Malfunctioning responses to training immunity can contribute to disease progression by causing a chronic hyperinflammatory state, as opposed to the persistent state of immune tolerance, a mechanism that dampens the host inflammatory response to maintain homeostasis and prevent tissue damage and organ failure, resulting in the risk of secondary infections and other diseases related to reduced immune system activity.

As the child grows, the immune repertoire is also shaped by intercurrent infections and vaccinations.

Pathogenic infections may be symptomatic, however, for many viruses, such a s influenza, the infection may be subclinical, but still sufficient to stimulate or boost immune responses.
In general, the protection offered by the immune response, whether by antibodies or T cells, is very powerful.

Most childhood infections occur only once and protection is permanent.

Interestingly, a mother is able to transfer sufficient antibodies to protect her baby when she was infected even ***20-30 years earlier***.

Transmission of protective antibody protection from a mother to her child is extremely important, especially in settings where 15 percent or more of infants and children die from infection. [152]

Netea MG, et al.

Trained immunity: A program of innate immune memory in health and disease.
Science. 2016;352(6284):aaf1098. doi:10.1126/science.aaf1098
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5087274/

Netea MG.
Training innate immunity: the changing concept of immunological memory in innate host defense.
Eur J Clin Invest. 2013;43(8):881-884. doi:10.1111/eci.12132
https://onlinelibrary.wiley.com/doi/full/10.1111/eci.12132

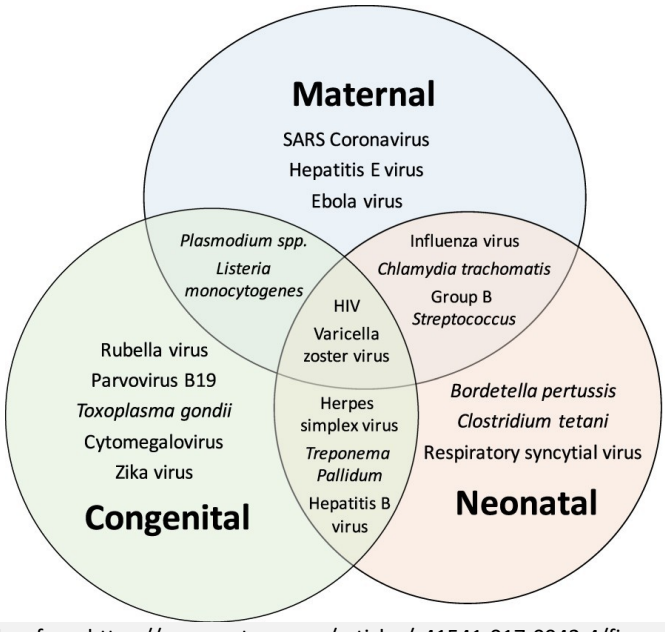
[152] Nat Rev Immunol. 2007;7(5):379-390. doi:10.1038/nri2075.
Innate immunity of the newborn: basic mechanisms and clinical correlates.
Levy O
https://www.nature.com/articles/nri2075

Paradoxically, **a mother who has avoided a dangerous childhood infection may actually put her child at risk by not being able to transfer specific protective antibodies**.

> ***Mass vaccinations have greatly reduced the transmission of protective antibodies*** *and created a* ***much larger window of vulnerability in infants****; this unnatural, man-made phenomenon can in fact be considered a very serious adverse vaccine reaction, the consequences of which in industrialized countries may be mitigated by the fact that infectious diseases are a negligible risk factor compared to other diseases, but may have a major impact in developing countries where, on the other hand, infant mortality from infectious diseases is still very high.* [153]

Based on this evidence, i.e., that mothers no longer provide adequate protection in the early months of the infant's life through maternal-fetal transmission of antibodies, **vaccination in pregnancy** was introduced on the assumption, not true from what has been discussed so far, that mother-transmitted vaccine antibodies are protective for the infant and that vaccination reduces the risk in the mother of contracting the disease.

A description of infectious diseases that can cause complications in the mother, fetus, or infant and the theoretical goals and timing of vaccination are shown in the figure below



Pathogen Category	Goal of Vaccination	Optimal Timing of Vaccination
Maternal	Prevent maternal infection/disease	PRIOR to or DURING pregnancy
Congenital	Prevent fetal infection/disease	PRIOR to pregnancy
Neonatal	Prevent neonatal infection/disease	DURING pregnancy

Taken from https://www.nature.com/articles/s41541-017-0042-4/figures/1 [154]

Nat Med 2019 Apr;25(4):591-596. doi: 10.1038/s41591-019-0392-8.
The Repertoire of Maternal Anti-Viral Antibodies in Human Newborns
Christian Pou et al
https://www.nature.com/articles/s41591-019-0392-8

Am J Reprod Immunol. 2018 Sep;80(3):e12972. doi: 10.1111/aji.12972.
Expression of FcRn receptor in placental tissue and its relationship with IgG levels in term and preterm newborns.
Lozano NA, Lozano A, Marini V, Saranz RJ, Blumberg RS, Baker K, Agresta MF, Ponzio MF.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6153031/

[153] https://www.who.int/gho/child_health/mortality/neonatal_infant_text/en/

https://en.wikipedia.org/wiki/Infant_mortality

[154] npj Vaccines 3, 6 (2018). https://doi.org/10.1038/s41541-017-0042-4
Pregnancy and infection: using disease pathogenesis to inform vaccine strategy.
Vermillion, M.S., Klein, S.L.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5794984/

Infectious microbes that cause maternal, congenital, or postnatal complications. Infectious microbes are classified according to the mechanism of transmission and disease, and the population at greatest risk of serious outcomes during or after pregnancy. Infection with some pathogens (e.g., SARS coronavirus, hepatitis E virus, and Ebola virus) during pregnancy causes serious illness in pregnant women but is not transmitted to offspring. Other infectious microbes (e.g., Toxoplasma gondii, rubella virus, parvovirus B19, cytomegalovirus, and Zika virus) infect and cause mild or asymptomatic disease in pregnant women, but can be transmitted vertically to the fetus and cause congenital complications. Another category of microbes (e.g., Bordetella pertussis, Clostridium tetani, and respiratory syncytial virus) pose the greatest risk to infants after birth. Many infectious microbes (e.g., Listeria monocytogenes, Plasmodium spp., HIV, VZV, influenza viruses, Chlamydia trachomatis, GBS, Treponema pallidum, and herpes virus) can cause overlapping syndromes depending on the time of infection during pregnancy.

In pregnancy at the time of implantation, a local inflammatory response creates the stable placental site.

Maternal immunological states then actively adapt and change with the growth and development of the fetus at different gestational stages:

- ***From a pro-inflammatory state*** *(beneficial for implantation and placentation of the embryo)* ***in the first trimester to***
- ***An anti-inflammatory state*** *(useful for fetal growth)* ***in the second trimester,***
- ***and finally reaching a second pro-inflammatory state*** *(preparatory for the onset of delivery)* ***in the third trimester.*** [155]

*Recently, it has been suggested that during full-term pregnancy immunological events in the peripheral blood proceed according to a precise timing, called the "**immune clock.**"* [156]

Based on the knowledge that pregnant women in their first and third trimesters have a pro-inflammatory state, it should be kept in mind that the cytokine storm induced by infections (such as COVID-19 [157]) and vaccinations [158] **can further enhance the inflammatory state** with very serious consequences in both mother and fetus [159] , and later in the newborn [160] .

[155] Mor G, Aldo P, Alvero AB.
The unique immunological and microbial aspects of pregnancy.
Nat Rev Immunol. 2017;17(8):469-482. doi:10.1038/nri.2017.64
https://www.nature.com/articles/nri.2017.64

[156] Aghaeepour N, et al.
An immune clock of human pregnancy.
Sci Immunol. 2017;2(15):eaan2946. doi:10.1126/sciimmunol.aan2946
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5701281/

[157] Liu H, Wang LL, Zhao SJ, Kwak-Kim J, Mor G, Liao AH.
Why are pregnant women susceptible to COVID-19? An immunological viewpoint.
J Reprod Immunol. 2020;139:103122. doi:10.1016/j.jri.2020.103122
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7156163/

[158] Hervé C, Laupèze B, Del Giudice G, Didierlaurent AM, Tavares Da Silva F.
The how's and what's of vaccine reactogenicity.
NPJ Vaccines. 2019;4:39. doi:10.1038/s41541-019-0132-6
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6760227/

[159] Garcia-Flores V, et al.
Inflammation-Induced Adverse Pregnancy and Neonatal Outcomes Can Be Improved by the Immunomodulatory Peptide Exendin-4.
Front Immunol. 2018;9:1291. doi:10.3389/fimmu.2018.01291
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6015905/

[160] Schepanski S, Buss C, Hanganu-Opatz IL, Arck PC.
Prenatal Immune and Endocrine Modulators of Offspring's Brain Development and Cognitive Functions Later in Life.
Front Immunol. 2018;9:2186. doi:10.3389/fimmu.2018.02186
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6168638/

There is also evidence that the mother changes the balance of her T-cell responses to Th2 rather than Th1.[161]

> *Therefore, pregnant women may show* ***remissions of autoimmune diseases****, but become more* ***susceptible to serious complications of influenza and other infections,*** [162] *and for the same reason,* ***serologic response to vaccines (e.g., inactivated influenza vaccine*** [163]***) is particularly reduced****.*

This immune modulation, which is necessary for the well-being of the fetus, can sometimes be particularly harmful to the mother.

Vaccinations in pregnancy and during puerperium: [164]

[161] Am J Reprod Immunol. 2010 Jun; 63(6):425-33. doi: 10.1111/j.1600-0897.2010.00836.x
The immune system in pregnancy: a unique complexity.
Mor G, Cardenas I
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3025805/

Annu Rev Immunol. 2013;31:387-411. doi:10.1146/annurev-immunol-032712-100003
Immunology of the maternal-fetal interface.
Erlebacher A.
https://www.annualreviews.org/doi/pdf/10.1146/annurev-immunol-032712-100003

Nat Immunol. 2004;5(3):266-271. doi:10.1038/ni1037
Regulatory T cells mediate maternal tolerance to the fetus.
Aluvihare VR, Kallikourdis M, Betz AG.
https://www.nature.com/articles/ni1037

J Clin Invest. 2014;124(5):1872-1879. doi:10.1172/JCI68107
Uterine NK cells: active regulators at the maternal-fetal interface
Moffett A, Colucci F.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4001528/

[162] Semin. Immunopathol. 2007. 29, 185-191. 10.1007/s00281-007-0072-5
The remission of rheumatoid arthritis during pregnancy
Ostensen M, Villiger PM.
https://pubmed.ncbi.nlm.nih.gov/17621703/

Influenza Other Respir. Viruses 2013. 7, 1033-1039. 10.1111/irv.12055
Influenza in pregnancy.
Memoli MJ, Harvey H, Morens DM, Taubenberger JK
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3582707/

[163] PLoS One. 2013;8(4):e56700. doi:10.1371/journal.pone.0056700
Altered response to A(H1N1)pnd09 vaccination in pregnant women: a single blinded randomized controlled trial.
Bischoff AL et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3630160/

J Infect Dis. 2012;206(11):1670-1673. doi:10.1093/infdis/jis592
Pregnancy modifies the antibody response to trivalent influenza immunization.
Schlaudecker EP, McNeal MM, Dodd CN, Ranz JB, Steinhoff MC.
https://academic.oup.com/jid/article/206/11/1670/897117

[164] Recommended Vaccinations for Women of Childbearing Age and Pregnancy - CORRECTION ERROR
http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2018&codLeg=66751&parte=1%20&serie=null

Vaccinazioni in gravidanza		
Raccomandate	dTpa	*Terzo trimestre di gravidanza,* idealmente intorno alla 28esima settimana, e ad ogni gravidanza, indipendentemente dall'anamnesi positiva per malattia o pregressa vaccinazione
	influenza inattivato	*Donne che all'inizio o nel corso della stagione epidemica dell'influenza si trovino nel secondo o terzo trimestre di gravidanza*
Controindicate	Vaccini vivi attenuati (MPR, Varicella, zoster), BCG[1], encefalite giapponese[2]	Vaccini MPR, Varicella, zoster se somministrati non comportano indicazioni all'interruzione volontaria di gravidanza.
Non raccomandate per dati non disponibili	HPV, Tifo orale (se necessario, preferire la formulazione a subunità iniettabile), pneumococco	Se somministrate non comportano indicazioni all'interruzione volontaria di gravidanza.
Possibili se beneficio maggiore del rischio	Epatite A, epatite B, IPV[3], meningococco, TBE[4], rabbia, colera, febbre gialla[5]	Se somministrate non comportano indicazioni all'interruzione volontaria di gravidanza.
Vaccinazioni nel puerperio		
Raccomandate	MPR e varicella	Se la donna non è stata vaccinata e se anamnesticamente negativa anche solo a una delle malattie elencate
	dTpa	Se la donna non è stata vaccinata durante la gravidanza. In tale evenienza è altresì opportuna la vaccinazione dei contatti stretti.

As can be seen, DTaP and flu vaccine are offered in the third month of pregnancy, while MRPV and DTaP in the puerperium.

The risks to the fetus and unborn child and to the mother have been discussed above in detail and are related to disease enhancement in the event of postvaccinal infection and adverse reactions to the vaccine-induced cytokine storm inflammatory response.

Microchimerism and immune tolerance [165]

Beginning at the beginning of pregnancy, fetal cells are found in maternal blood and tissues, and gradually increase until term. Similarly, maternal cells are found in fetal tissues starting from the second trimester of pregnancy. This phenomenon is known as "**microchimerism**."

Perhaps the most striking finding is the **long-term persistence of these genetically different fetal cells in mothers many years after pregnancy and the finding in adulthood of maternal cells in offspring** transmitted during postnatal development.

Recent discoveries suggest that these microchemical cells are not incidental accessories of pregnancy, but are instead intentionally retained in the mother to promote both ongoing gestation and the success of future pregnancies by expanding immunological tolerance, while in the newborn they appear to have the function of

[165] Bioessays. 2015;37(10):1106-1118. doi:10.1002/bies.201500059
Fetal microchimerism and maternal health: a review and evolutionary analysis of cooperation and conflict beyond the womb
Boddy AM, Fortunato A, Wilson Sayres M, Aktipis A
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4712643/

Expert Rev Mol Med. 2009;11:e33. doi:10.1017/S1462399409001264
Fetal microchimerism: the cellular and immunological legacy of pregnancy.
Lissauer DM, Piper KP, Moss PA, Kilby MD.
https://pubmed.ncbi.nlm.nih.gov/19909558/

Trends Immunol. 2012;33(8):421-427. doi:10.1016/j.it.2012.03.002
The otherness of self: microchimerism in health and disease.
Nelson JL.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3516290/

increase defense against infectious microorganisms and to suppress aberrant stimuli that could cause allergies or autoimmunity. [166]

On the other hand, microchimerism when associated with a maternal inflammatory state can lead to chronic inflammation and autoimmunity in both mother and infant over time. [167]

*Expanded immune tolerance to genetically foreign antigens expressed by microchimeric cells ("**microchiome**": diversity of microchimeric cells in an individual) expands the way in which the immunological identity of individuals is defined beyond classical models of binary "self" vs. "non-self" discrimination to include a **repertoire of "self-extended" antigens from progenitors**, which are transmitted vertically from mothers from generation to generation (mother-daughter transmission but also to grandchildren through daughter)*

Overview of the potential beneficial and harmful effects of fetal and maternal microchimeric cells

	Potential beneficial roles	Potential harmful effects
Fetal microchimeric cells in mothers	• Priming the expansion of maternal T_{reg} cell populations, and other suppressive adaptive immune components, with specificity that averts maternal–fetal conflict during pregnancy[25–27,35,128] • Amelioration of organ-specific autoimmunity during pregnancy and after parturition[15,16] • Replacement of injured cells in diseased tissues[96–104] • Promoting postpartum care of the offspring[66]	• Target of alloimmune inflammatory response in recipients[62,84,85] • Fetal-derived adaptive immune cells promote graft-versus host like attack of recipient cells and tissues[62]
Maternal microchimeric cells in offspring	• Priming the expansion of fetal T_{reg} cell populations, and other suppressive adaptive immune components, with NIMA specificity that avert maternal–fetal conflict during pregnancy[11,14,60] • Replacement of injured cells in diseased tissues[107–110] • Replacement of deficient or underdeveloped fetal immune cells[114–116] • Promoting immune cell development in offspring[11,47,117]	• Target of alloimmune inflammatory response in recipients[62,129] • Maternal adaptive immune cells promote graft-versus host like attack of recipient cells and tissues[105,106,130]

NIMA, non-inherited maternal antigen; T_{reg} cell, regulatory T cell.

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5532073/

[166] Nat Rev Immunol. 2017 Aug;17(8):483-494. doi: 10.1038/nri.2017.38
Immunological implications of pregnancy-induced microchimerism.
Kinder JM, Stelzer IA, Arck PC, Way SS
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5532073/

167 Best Pract Res Clin Obstet Gynaecol. 2016;31:121-130. doi:10.1016/j.bpobgyn.2015.08.005
Maternal microchimerism in health and disease.
Stevens AM.
https://pubmed.ncbi.nlm.nih.gov/26612343/

Microbiota and immune system in pregnancy and the newborn

It is important to remember that many of the bacteria that colonize the gut and other mucosal sites are essential for healthy living, as they are involved in the digestion of food, the assimilation of vital nutrients, and also have a major impact on the development of the immune system. [168]
Approximately 20 percent of all lymphocytes, [169] which monitor potentially dangerous sources of infection, **reside in the gut.**

---

[168] J Dev Orig Health Dis. 2018;9(6):590-597. doi:10.1017/S2040174418000119
The developing gut microbiota and its consequences for health.
Butel MJ, Waligora-Dupriet AJ, Wydau-Dematteis S.
https://www.cambridge.org/core/journals/journal-of-developmental-origins-of-health-and-disease/article/developing-gut-microbiota-and-its-consequences-for-health/2B7BC1C8128692852F42DBCB883A9462/core-reader

Front Microbiol. 2020 Apr 3;11:439. doi: 10.3389/fmicb.2020.00439.
Evolution of the Gut Microbiome in Early Childhood: A Cross-Sectional Study of Chinese Children.
Niu J, Xu L, Qian Y, Sun Z, Yu D, Huang J, Zhou X, Wang Y, Zhang T, Ren R, Li Z, Yu J, Gao X.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7169428/

Ital J Pediatr. 2020 Feb 5;46(1):16. doi: 10.1186/s13052-020-0781-0.
The infant gut microbiome as a microbial organ influencing host well-being.
Turroni F, Milani C, Duranti S, Lugli GA, Bernasconi S, Margolles A, Di Pierro F, van Sinderen D, Ventura M.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7003403/

Nat Rev Immunol. 2009 May; 9(5):313-23. doi: 10.1038/nri2515.
The gut microbiota shapes intestinal immune responses during health and
disease. Round JL, Mazmanian SK
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4095778/

Lin L, Zhang J.
Role of intestinal microbiota and metabolites on gut homeostasis and human diseases.
BMC Immunol. 2017 Jan 6;18(1):2. doi: 10.1186/s12865-016-0187-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5219689/
Pan WH, Sommer F, et al
Exposure to the gut microbiota drives distinct methylome and transcriptome changes in intestinal epithelial cells during postnatal development.
Genome Med. 2018 Apr 13;10(1):27. doi: 10.1186/s13073-018-0534-5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5899322/

Rooks MG, Garrett WS.
Gut microbiota, metabolites and host immunity.
Nat Rev Immunol. 2016 May 27;16(6):341-52. doi: 10.1038/nri.2016.42.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5541232/

Microbiol Mol Biol Rev. 2017 Nov 8;81(4):e00036-17. doi: 10.1128/MMBR.00036-17.
The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota.
Milani C, et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5706746/

Mil Med Res. 2017 Apr 27;4:14. doi: 10.1186/s40779-017-0122-9.
Interaction between the gut microbiome and mucosal immune system.
Shi N, Li N, Duan X, Niu H.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5408367/

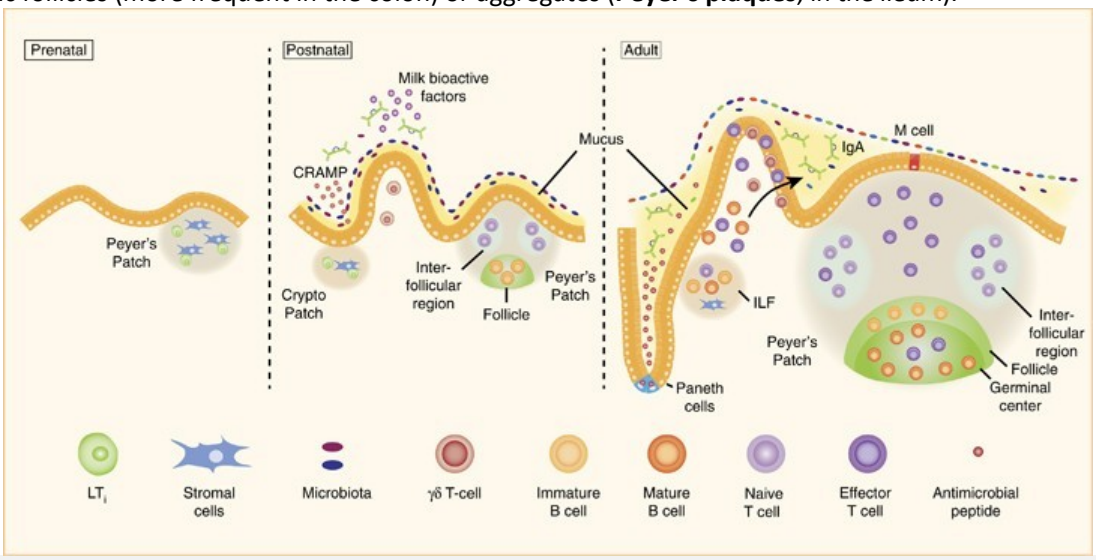
Gerontology. 2018;64(6):513-520. doi: 10.1159/000490615
The Gut Microbiota and Healthy Aging: A Mini-Review.
Kim S, Jazwinski SM
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6191326/

[169] Trends Immunol. 2007 Dec; 28(12):514-8.
Do most lymphocytes in humans really reside in the gut?
Ganusov VV, De Boer RJ
https://pubmed.ncbi.nlm.nih.gov/17964854/

Clin Immunol. 2015 Aug;159(2):122-127. doi: 10.1016/j.clim.2015.05.014.
Immune-microbiota interactions in health and disease.
Palm NW, de Zoete MR, Flavell RA.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4943041/

Front Immunol. 2020 Apr 3;11:575. doi: 10.3389/fimmu.2020.00575.
Intestinal Flora and Disease Mutually Shape the Regional Immune System in the Intestinal Tract.
Zhou B, Yuan Y, Zhang S, Guo C, Li X, Li G, Xiong W, Zeng Z.

Gut bacteria influence the development of Th17 cells, Treg cells and memory T cells. [170]
A key part of the barrier effect against foreign antigens is played by the **gut-associated lymphoid tissue (**GALT**)**, consisting mainly of T lymphocytes, which make up about one-sixth of the villus cells, B lymphocytes, dendritic cells, and plasma cells (secreting mainly Ig A) found in the connective tissue of the lamina propria, and isolated lymphatic follicles (more frequent in the colon) or aggregates (**Peyer's plaques**, in the ileum).[171]



Taken from https://www.nature.com/articles/mi201681

---

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7147503/

[170] Nature. 2014 Jun 5;510(7503):152-6. doi: 10.1038/nature13279.
Focused specificity of intestinal TH17 cells toward commensal bacterial antigens.
Yang Y et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4128479/

Cell. 2009 Oct 30;139(3):485-98. doi: 10.1016/j.cell.2009.09.033.
Induction of intestinal Th17 cells by segmented filamentous bacteria.
Ivanov II et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2796826/

J Exp Med. 2014 Jun 30;211(7):1273-80. doi: 10.1084/jem.20130555.
Proteome-wide analysis of HIV-specific naive and memory CD4(+) T cells in unexposed blood donors.
Campion SL, Brodie TM, Fischer W, Korber BT, Rossetti A, Goonetilleke N, McMichael AJ, Sallusto F.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4076590/

J Autoimmun. 2010 May;34(3):J220-5. doi: 10.1016/j.jaut.2009.11.007.
Coordination of tolerogenic immune responses by the commensal microbiota.
Round JL, O'Connell RM, Mazmanian SK.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155383/.

Immunity. 2013 Feb 21;38(2):373-83. doi: 10.1016/j.immuni.2012.10.021.
Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults.
Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3626102/

Med Sci (Basel). 2018 Jul 17;6(3):56. doi: 10.3390/medsci6030056.
Gut Microbiota and Mucosal Immunity in the Neonate.
Dzidic M, Boix-Amorós A, Selma-Royo M, Mira A, Collado MC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6163169/

Clin Dev Immunol. 2013;2013:270301. doi: 10.1155/2013/270301.
Neonatal immune adaptation of the gut and its role during infections.
Tourneur E, Chassin C.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3659470/

[171] Torow N, Marsland BJ, Hornef MW, Gollwitzer ES.
Neonatal mucosal immunology.
Mucosal Immunol. 2017;10(1):5-17. doi:10.1038/mi.2016.81
https://www.nature.com/articles/mi201681

Development of the intestinal mucosal immune system

Before birth, Peyer's plaques develop through an exchange between lymphoid tissue inducer (LTi) and stroma cells within the sterile environment of the uterus. The mucosa of the small intestine is populated by the fetal wave of T γδ lymphocytes. At birth, the architecture of murine intestinal tissue is immature and undergoes numerous developmental changes until it reaches the adult state. The neonatal mucosa is characterized by a lack of crypts and Paneth cells residing in the crypt, a major source of antimicrobial substances in adult tissue.

Neonatal enterocytes, however, express cathelicidin-related antimicrobial peptide (CRAMP). Birth initiates colonization of the intestinal mucosa, and microbial density in the newborn rapidly reaches plateau levels. The neonatal microbiota is dominated by lactobacilli, streptococci, and bifidobacteria, whereas bacteriodetes phylum species become prevalent only in adults. Microbial diversity in the postnatal gut is reduced about 3-fold compared with the adult situation. Despite the presence of calyciform cells, the expression of mucins muc2, muc3, and muc5ac is reduced in the newborn resulting in a thinner mucus layer. αβ T (including thymus-derived regulatory T lymphocytes (tTregs) and B lymphocytes begin to populate the gut shortly after birth, however, they show a discrete residence profile only in Peyer's plaques and remain naïve throughout the neonatal phase. The presence of germinal centers and activated lymphocytes is noted in Peyer's plaques. M-cell maturation, which are the main antigen uptake pathways in the adult, also occurs after the second week of life. The effector sites of the lamina propria and intraepithelial compartment are populated only by lymphocytes after weaning. After birth, cryptopatch formation is noted, and their further maturation into isolated lymphoid follicles (ILFs) depends on the presence of a microbiota. During the postnatal phase, many bioactive factors are provided by breast milk including cytokines , growth factors, as well as secretory immunoglobulin A (SIgA). Endogenous SIgA is produced only by plasma cells and in adult mucosal tissue.

At birth, almost all T cells have the glycoprotein CD45RA, which is typical of naïve T cells that have never encountered foreign antigens.

During childhood, Th17 and Th2 cells gradually increase to match the number of naïve T cells. [172]

Although some of the memory T cells can be stimulated by infection with specific pathogens and by vaccination, many are stimulated by the microbiome, not only in the gut but also in the respiratory tract and skin.

These memory T cells are able to respond to subsequent infections through cross-reactions even for pathogens that the person has never encountered.[173]

It is important to keep in mind that important changes in maternal microbiomes occur during pregnancy. The translocation of bacteria from the maternal oral and intestinal microbiomes during pregnancy, in addition to the ascension of bacteria from the vaginal microbiome, may explain the presence of nonpathogenic bacteria in intrauterine locations.

Bacteria of maternal origin detected in neonatal meconium suggest prenatal transfer of bacteria from mother to baby.

The proposed mechanisms for maternal transfer of bacteria to the fetus in utero are as follows:

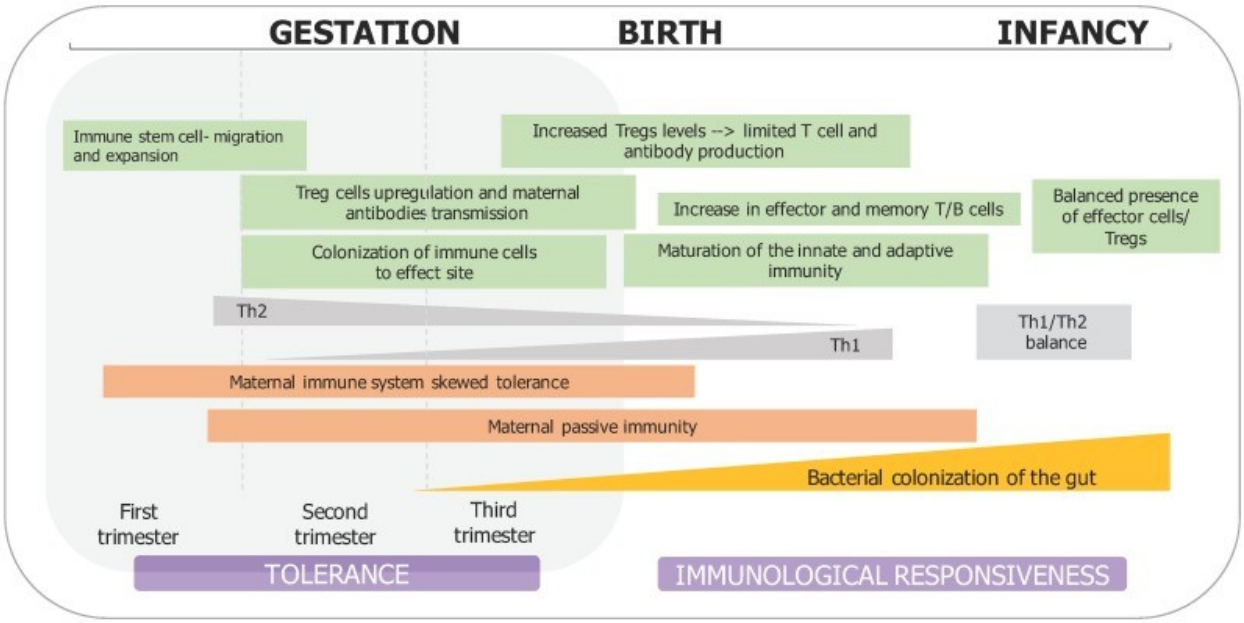
Intestine: the lumen of the maternal distal intestine is lined with enterocytes, which under normal conditions form a cellular and mucosal barrier to intestinal microbes.
Diet, stress, antibiotic exposure, disease, and pregnancy itself can alter the thickness of the mucosal layer and the integrity of the enterocyte barrier.
Gaps in this layer (intestinal permeability) allow bacteria to cross the intestinal barrier and enter the bloodstream or lymphatic vessels and translocate to other sites in the body.

[172] J Allergy Clin Immunol. 2003;112(5):973-980. doi:10.1016/j.jaci.2003.07.003
Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study
Shearer WT et al
https://www.jacionline.org/article/S0091-6749(03)02030-X/fulltext

[173] J Exp Med. 1994 Jun 1;179(6):1933-43. doi: 10.1084/jem.179.6.1933.
Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses.
Selin LK, Nahill SR, Welsh RM. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2191532/

Oral: Dental injury or surgery and oral conditions that cause inflammation (gingivitis) allow oral bacteria contained in salivary and subgingival microbiome communities to be exposed to the circulatory system.

Placenta: bacteria already present in the endometrial lining or urogenital regions can be incorporated into the developing placental decidua. Bacteria transferred into the bloodstream from other maternal microbiomes to the placenta can populate the decidua, fetal membranes and villi and transfer to the developing fetus in utero through amniotic fluid and cord blood. [174]



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6163169/
Main events occurring in the immune system and gut development. During pregnancy, T helper cells of the maternal immune system are shifted to T helper (Th) 2 type immunity to maintain tolerance of the developing fetus. The mother contributes tolerogenic mediators across the placenta (including antibodies and growth factors), thereby instructing the development of the fetal immune system. However, during the first weeks and months of life, infants subsequently increase Th1 activity, thereby restoring the balance of helper T cells. Without this shift, the persistence of Th2 could be associated with atopic diseases including asthma. While Th cells play a key role in directing immune responses, primarily in the neonatal period, regulatory T cells suppress the activation and development of naïve T cells toward Th types, thereby maintaining mucosal homeostasis during both pregnancy and infancy

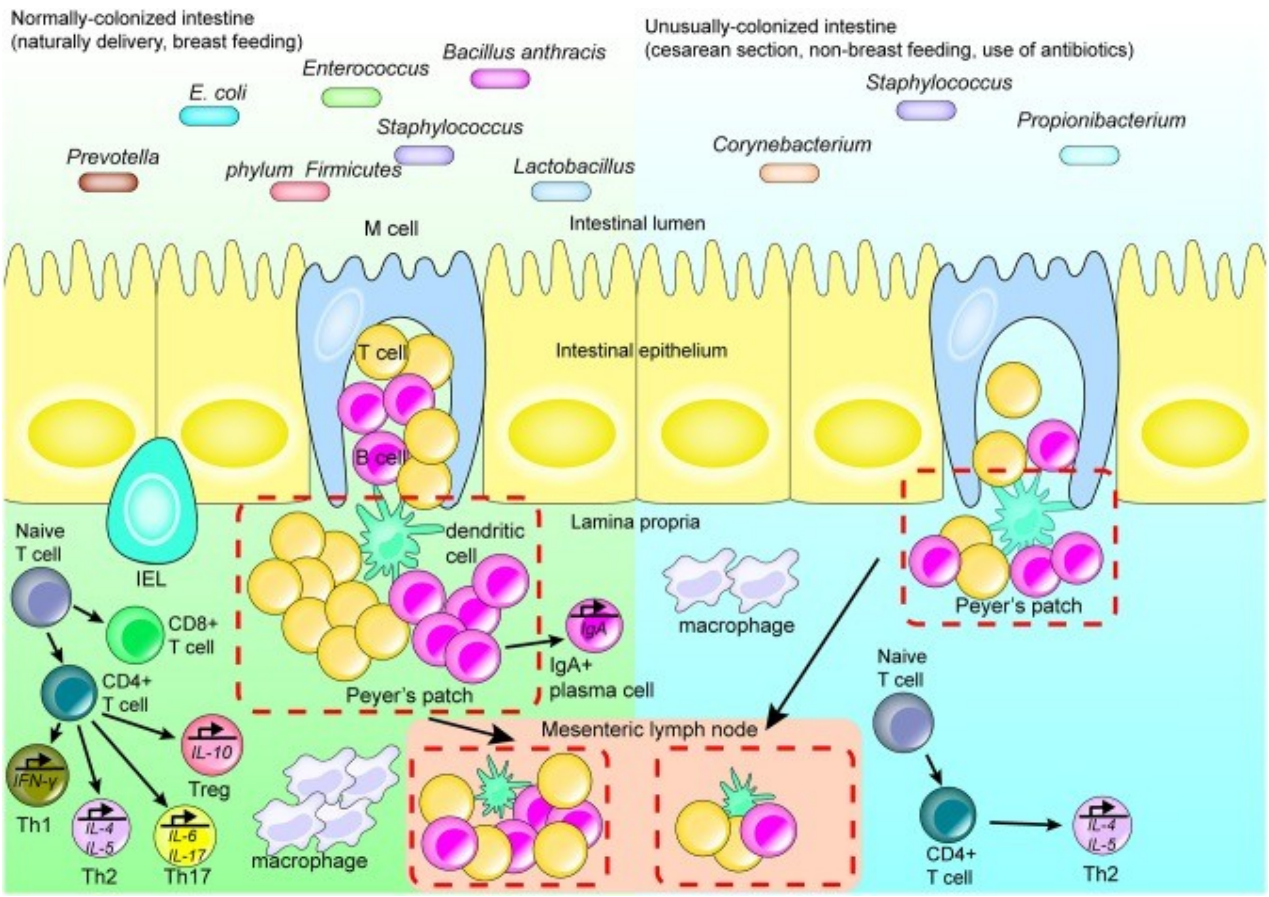
The key role of these events in the microbiota in early life is demonstrated by in vivo studies in mouse models in which conventional animals were compared with germ-free counterparts.

These investigations clearly demonstrated the serious health consequences caused by the absence of any microbiota-host interaction [175].
In addition, several authors have studied the effect of recolonization of germ-free animals at different ages on the restoration of parameters altered by lack of microbial exposure.
Interestingly, **it is necessary to recolonize animals in early childhood, as** opposed to adulthood, to restore the altered phenotypes found in germ-free models.

[174] Pediatr Obes. 2017;12 Suppl 1(Suppl 1):3-17. doi:10.1111/ijpo.12217
The prenatal gut microbiome: are we colonized with bacteria in utero?
Walker RW, Clement JC, Peter I, Loos RJF.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5583026/
[175] J Microbiol Biotechnol 2015, 25:1583-1588. doi:10.4014/jmb.1501.01039
Use of germ-free animal models in microbiota-related research.
Al-Asmakh M, Zadjali F.
https://pubmed.ncbi.nlm.nih.gov/26032361/

For example, animals lacking a microbiota (germ-free) have been shown to exhibit increased levels of certain immune cells in the mucous membranes, a phenomenon that is restored (to normal levels) when these animals are recolonized during early childhood, but this reversal does not occur when recolonization is carried out in adulthood [176]

Thus, an altered early colonization pattern may pose a risk with immediate consequences for the child's health and development, but may also present a risk for long-term effects.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7147503/

The gut microbiota influences the development of the regional immune system. Many factors influence the structure and development of the regional immune system by changing the type and amount of gut microbiota. Cesarean section, formula feeding, and antibiotic use after birth can lead to an abnormally colonized gut compared with vaginally delivered and breastfed fetuses. In a normal healthy intestinal tract with abundant microbial flora, isolated lymphoid follicles and Peyer's plaques are unchanged. Upon stimulation, antigen-presenting cells migrate to the mesenteric lymph node and promote T-cell differentiation. IgA-producing and secreting plasma cells perform a protective function of the mucosa, while macrophages migrate to the lamina propria of the intestinal tract and perform their normal function. When the abundance and species diversity of intestinal flora are relatively low, the intestinal immune system is not adequately stimulated and only isolated lymphoid follicles form.

The human body activates many processes that enable it to detect and respond to both commensal bacteria and pathogens, differentiating responses toward the different structures on their cell walls.

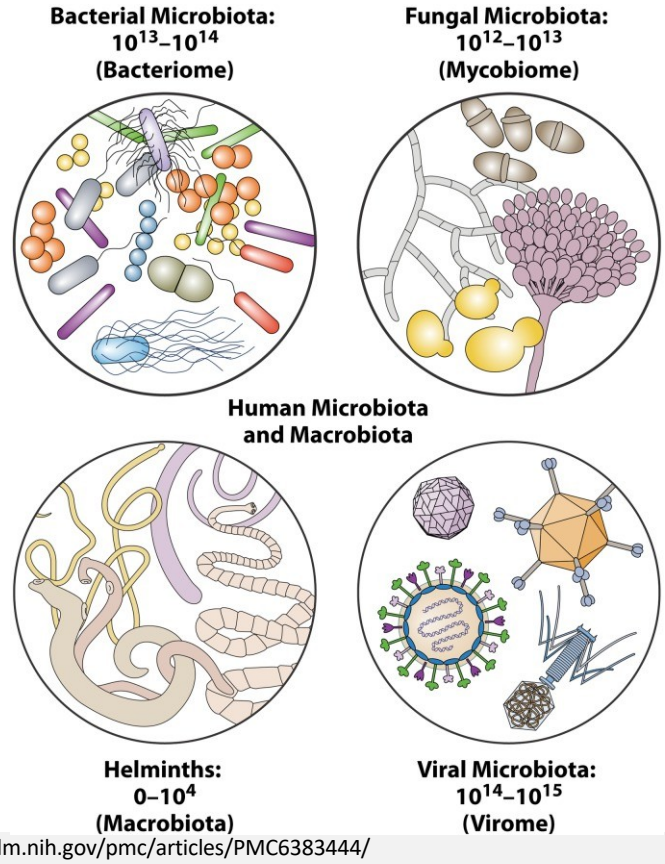
[176] Science. 2012 Apr 27;336(6080):489-93. doi: 10.1126/science.1219328
Microbial exposure during early life has persistent effects on natural killer T cell function.
Olszak T et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3437652/

Early in life, the developing immune system is trained to develop immunological tolerance, and an imbalance in inhibitory and stimulatory responses at this time has potential effects on autoimmune diseases that can occur in later life.

Although much of the research work has focused on a few aspects, particularly the bacterial component of the microbiome most frequently of the gastrointestinal tract, **humans and other animals can be colonized by a wide range of organisms covering all areas of life, and including in addition to bacteria and archaea, unicellular eukaryotes such as fungi, multicellular eukaryotes such as helminths, and viruses**.

Because they share the same host niches, they can compete, synergize, and antagonize each other, potentially affecting their host.
It should also be noted that the gut microbiota is not a single homogeneous community but instead shows **significant three-dimensional organization**.
First, the intestine is composed of several **distinct environments**, namely the stomach, small intestine (divided into duodenum, jejunum, and ileum), and large intestine (colon), each of which has different properties and houses its own community.
[177]

To date, the vast majority of research has focused on the colon because of the ease of obtaining fecal samples and the fact that it contains by far the highest density and number of bacteria. [178]
Second, even within a given compartment, bacteria may differ along the transverse axis, with different populations found in the lumen than in the mucosa.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6383444/
Hints on the main components of the human microbiota, summarized at all sites in the body, including the gastrointestinal tract, oral cavity, vaginal mucosa and skin. **(Top left)** Bacteria are the most abundant and include members of the phyla Firmicutes

[177] Cell Host Microbe. 2017 Apr 12;21(4):433-442. doi: 10.1016/j.chom.2017.03.010.
The Gut Microbiome: Connecting Spatial Organization to Function.
Tropini C, Earle KA, Huang KC, Sonnenburg JL
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5576359/

[178] PLoS Biol. 2016 Aug 19;14(8):e1002533. doi: 10.1371/journal.pbio.1002533.
Revised Estimates for the Number of Human and Bacteria Cells in the Body.
Sender R, Fuchs S, Milo R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4991899/

(Clostridium, Lactobacillus and Enterococcus), Bacteroidetes (Bacteroides and Prevotella), Proteobacteria (Escherichia and Acinetobacter), Actinobacteria (Bifidobacterium), and Akucomicia (Akifomic). **(Top right)** Based on metagenomics, fungi associated with humans are significantly more numerous than bacteria; they are mainly members of the phylum Ascomycota (Candida, Saccharomyces, Aspergillus, and Malassezia), but some Basidiomycota are detectable. Humans can also be infected by non-fungal eukaryotic pathogens, which are not shown here. **(Bottom right)** Viruses in the human microbiota are primarily bacteriophages and probably outnumber the bacterial population at least 10-fold. The viroma is largely composed of Caudovirales (Siphoviridae, Myoviridae and Podoviridae) and Microviridae, along with some eukaryotic host viruses. **(Bottom left)** Helminths are now typically absent from humans in high-income nations, but they still parasitize billions worldwide at various levels of severity. They include trematodes (flatworms), nematodes (nematodes) and cestodes (tapeworms).

The other key component in the development of the microbiota and the microbiota-immune system axis is the **gut viroma**, defined as the portion of the gut microbiome consisting of viruses that colonize eukaryotic host cells (eukaryotic viroma), bacteria (bacterial viroma), and archaea (archaeal viroma).
It also includes all genetic elements derived from viruses that are integrated into host chromosomes (HERVs - endogenous viral elements).[179]

*The human gut is estimated to host more than 10^{12} **bacterial cells**, which in turn are in an estimated ratio to their infectious or associated viral counterparts (bacteriophages or phages) of 10 (viruses) to 1 (bacteria).*

Colonization of bacterial populations by phages is believed to play an important role in shaping the structure of the gut bacterial community.[180]
A recent study determined that viruses that colonize bacteria are found in abundance in the infant intestine. [181]

A hypothesis of virus-bacteria interactions has been proposed, in which both parties are responsible for modulating their relative composition and impacting the health status of the host.
This dynamic relationship is exemplified by their progression from early childhood to adulthood (see figure below).

Immediately after birth and up to 2 or 3 years of age, the infant gut microbiota appears to be extremely plastic, undergoing a process of rapid expansion and diversification.

Similar to the bacterial microbiota, viroma also appears to be highly dynamic during the development of the infant microbiota, with the highest diversity in bacteriophages observed during the first few months after birth.

Subsequently, the viroma of the phage undergoes a mechanism of contraction and loss of diversity.
Interestingly, **viroma contraction occurs during the same period when the infant microbiota adopts an adult-like composition**, indicating that the resulting reduction in the number of colonizers facilitates the establishment of a diverse bacterial community in the gastrointestinal tract [182].

[179] Cell. 2014 Mar 27;157(1):142-50. doi: 10.1016/j.cell.2014.02.032.
The virome in mammalian physiology and disease.
Virgin HW.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3977141/

Microbiol Mol Biol Rev. 2019 Jan 9;83(1):e00044-18. doi: 10.1128/MMBR.00044-18.
Cross-Domain and Viral Interactions in the Microbiome.
Rowan-Nash AD, Korry BJ, Mylonakis E, Belenky P.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6383444/

[180] Nat Rev Gastroenterol Hepatol. 2015;12(1):6. doi:10.1038/nrgastro.2014.220
Gut microbiota: a 'friendly' gut virus?
Ray K.
https://www.nature.com/articles/nrgastro.2014.220

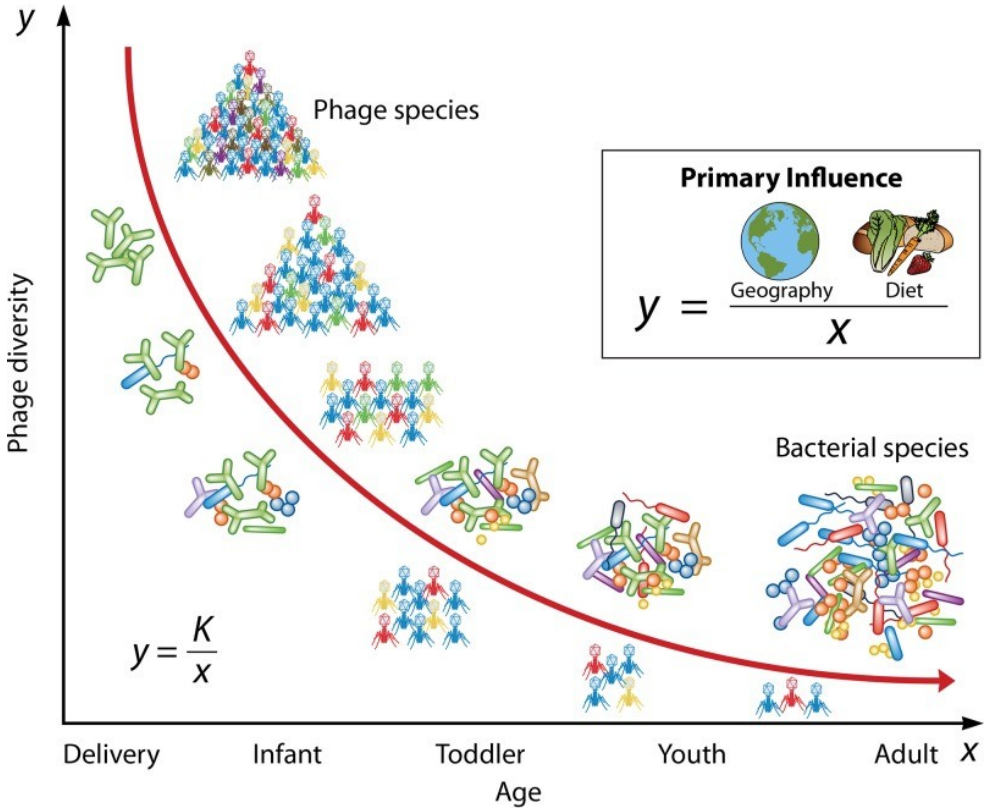
[181] Nat Med. 2015 Oct;21(10):1228-34. doi: 10.1038/nm.3950.
Early life dynamics of the human gut virome and bacterial microbiome in infants.
Lim ES, Zhou Y, Zhao G, Bauer IK, Droit L, Ndao IM, Warner BB, Tarr PI, Wang D, Holtz LR.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4710368/

[182] Trends Microbiol 2016. 24:801–810. doi:10.1016/j.tim.2016.06.001

In contrast, it is observed **that eukaryotic virome is poorly diversified during the first few days of life, and then goes through an increase in diversity over a period of 24 months**. This suggests that eukaryotic viruses are mainly obtained from environmental sources.

The composition of viroma in the intestine can be influenced by several factors, among which geography and diet seem to have the greatest influence.

Interestingly, individuals following the same dietary habits have a tendency to harbor a similar virome, probably reflecting a **dietary-dependent microbiota**, allowing the proliferation of phages that infect the more dominant members of this microbiota. [183]
This becomes even more relevant in the context of early childhood, a crucial period when host immune maturation and various metabolic developments take place.

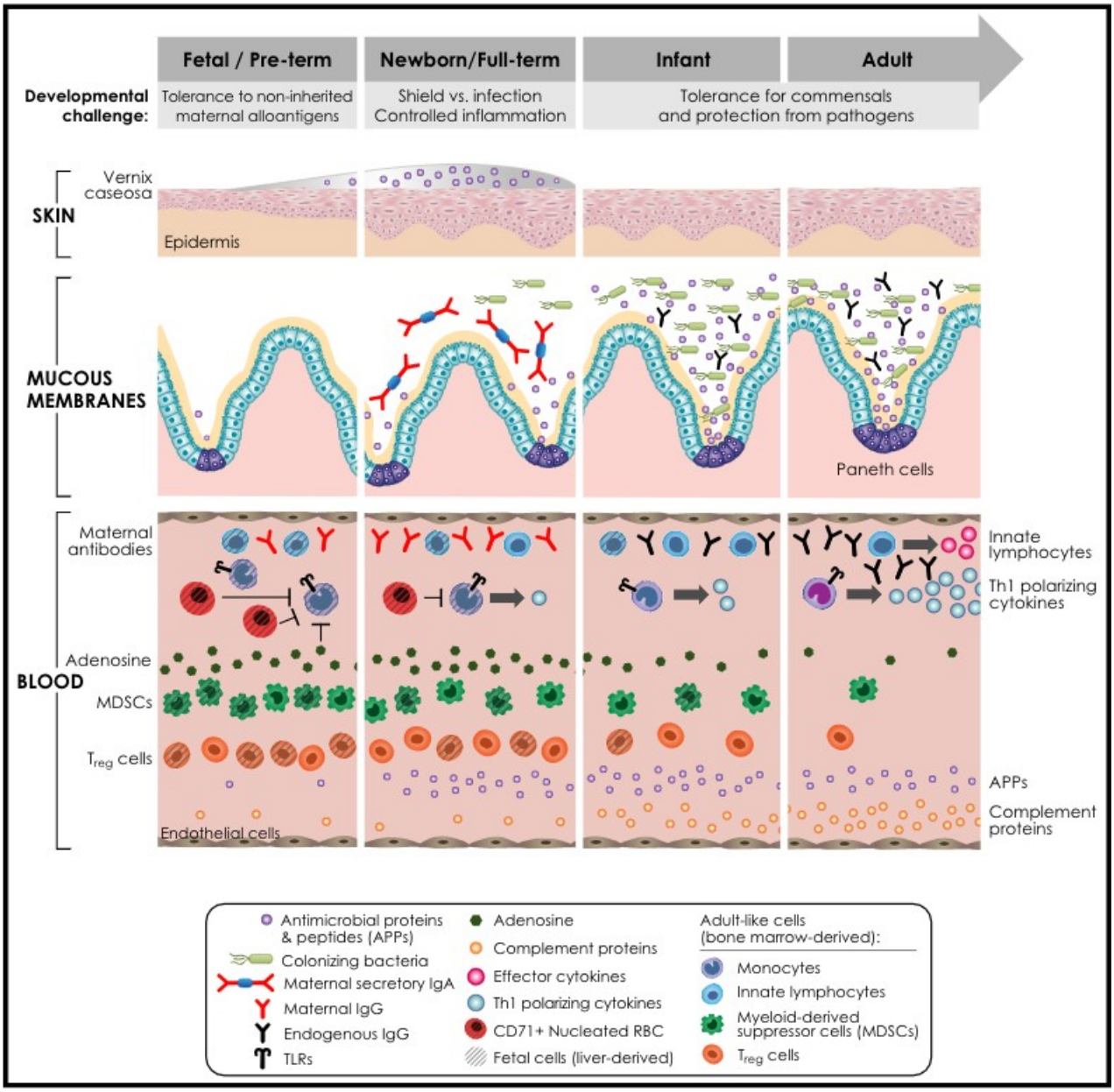


Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5706746/
Contribution of phages to the development of the gut microbiota during human aging. The putative factors affecting virome biodiversity from infancy to adulthood are represented schematically as factors in the curve formula. Phage and bacterial loads are represented schematically to express the concept that while the phage load decreases during aging, the gut microbial population increases in complexity and abundance. The number of bacterial or phage particles schematically represents the number of species and the complexity of the population.

To conclude, a figure on ontogeny (biological development of an organism) of fetal, neonatal and infant host defense is given: [184]

The bacterial microbiome and virome milestones of infant development.
Lim ES, Wang D, Holtz LR.
https://pubmed.ncbi.nlm.nih.gov/27353648/
[183] Genome Res. 2011 Oct;21(10):1616-25. doi: 10.1101/gr.122705.111.
The human gut virome: inter-individual variation and dynamic response to diet.
Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, Lewis JD, Bushman FD.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3202279/

[184] Immunity. 2017;46(3):350-363. doi:10.1016/j.immuni.2017.03.009
Protecting the Newborn and Young Infant from Infectious Diseases: Lessons from Immune Ontogeny.



Tratta da https://www.cell.com/action/showPdf?pii=S1074-7613%2817%2930090-0
Host protective barrier functions include physical, chemical, and functional components of the epithelial membrane of the skin and mucous membranes of the fetus, newborn (birth to 28 days of age), and infant (1 month to 1 year of age).

(A) **Skin:** while physical and chemical barriers are reduced early in life, especially in preterm infants, caseous varnish (vernix) and skin epithelia of term infants effectively express APP (antimicrobial proteins and peptides
-proteins and antimicrobial peptides)

(B) **Mucosal membranes: in** parallel with and induced by an increasingly complex microbiota, the epithelium of the neonatal intestinal mucosa changes structure rapidly with increasing crypts and crypt-based Paneth cells, as well as functionally with increasing APP expression

(C) **Blood:** The composition of neonatal blood consists of relatively low concentrations of complement and APP components and high concentrations of the immunosuppressive purine metabolite adenosine. Plasma also contains maternal antibodies transferred early in gestation and supplemented by postnatal factors derived from breast milk. Innate immunity is detectable by the end of the first month of gestation, with changes driven largely by increasing exposure to microbes

Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O.
https://www.cell.com/action/showPdf?pii=S1074-7613%2817%2930090-0

Environmental. Neonatal APCs like monocytes in blood express PRRs (e.g., TLRs) with distinct functional responses including cytokine production with limited Th1 bias for most stimuli. Age-dependent differences in interferon response factor (IRF) transcription factor activity and epigenetic changes contribute to cytokine ontogeny, while adaptive immunity develops from the 4th week of gestation.

Advanced age [185]

With advancing age, the immune system undergoes profound remodeling and decline, with great impact on health and survival. [186]
This **immune senescence** predisposes the elderly to a higher risk of acute viral and bacterial infections. Mortality rates of these infections are **three times higher** in elderly patients than in younger adults.

Infectious diseases are still the **fourth common cause of death** among the elderly in developing countries.
Aberrant immune responses in the elderly can exacerbate inflammation, possibly contributing to other diseases typical of old age: cancer, cardiovascular disease, stroke, Alzheimer's disease, and dementia. [187]
During a normal flu season, **about 90% of excess deaths occur in people older than 65 years**. In addition, poor immune responses explain the reduced effectiveness of vaccines. [188]

[185] Proc Biol Sci. 2015 Dec 22;282(1821):20143085. doi: 10.1098/rspb.2014.3085.
Evolution of the immune system in humans from infancy to old age.
Simon AK, Hollander GA, McMichael A.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707740/

The Immune System and Its Dysregulation with Aging. In: Harris J., Korolchuk V. (eds) Biochemistry and Cell Biology of Aging: Part II Clinical Science. Subcellular Biochemistry, vol 91. Springer, Singapore (2019)
Müller L., Di Benedetto S., Pawelec G.

[186] Transpl Int. 2009;22(11):1041-1050. doi:10.1111/j.1432-2277.2009.00927.x.
The aging of the immune system
Weiskopf D, Weinberger B, Grubeck-Loebenstein B.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1432-2277.2009.00927.x

Chin Med J (Engl). 2012;125(18):3325-3331.
Senescent remodeling of the immune system and its contribution to the predisposition of the elderly to infections.
Dewan SK, Zheng SB, Xia SJ, Bill K.
https://pubmed.ncbi.nlm.nih.gov/22964331/

Ann Allergy Asthma Immunol. 2010;104(3):183-210. doi:10.1016/j.anai.2009.11.009
Innate and adaptive immunosenescence
Agarwal S, Busse PJ
https://pubmed.ncbi.nlm.nih.gov/20377107/

Clin Infect Dis. 2000;31(2):578-585. doi:10.1086/313947
Clinical relevance of age-related immune dysfunction.
Castle SC.
https://academic.oup.com/cid/article/31/2/578/299255

Biogerontology. 2006;7(5-6):471-481. doi:10.1007/s10522-006-9062-6
Immunological biomarkers of aging in man: changes in both innate and adaptive immunity are associated with health and longevity.
DelaRosa O, et al.
https://pubmed.ncbi.nlm.nih.gov/16957868/

[187] Clin Infect Dis. 2000;30(6):931-933. doi:10.1086/313792
Epidemiology and unique aspects of aging and infectious diseases.
Yoshikawa TT.
https://academic.oup.com/cid/article/30/6/931/433911

Ageing Res Rev. 2009;8(1):18-30. doi:10.1016/j.arr.2008.07.002
Molecular inflammation: underpinnings of aging and age-related diseases. Chung HY, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3782993/

[188] Sci Transl Med. 2013;5(171):171ra19. doi:10.1126/scitranslmed.3004794 [published correction appears in Sci Transl Med. 2013 Jul 10;5(193):193er8].

Immune senescence also causes reactivation of latent viruses, such as varicella-zoster virus, causing shingles and chronic neuralgia.

The increase with age in **pro-inflammatory pathobionts** may promote the reduction of microbiota species with immunomodulatory activity and support inflammatory disorders.

SYMBIONTs: essential bacteria that live with benefit of the host organism COMMENSALS:
bacteria that live together benefiting a host organism
PATOBIONTS: organisms that coexist with a host organism without causing harm or benefit under normal conditions, but can become pathogenic in situations of imbalance between species (dysbiosis)
PATHOGENS: external bacteria that by infecting a host organism trigger an infection

The aging immune system fails to maintain full tolerance to autoantigens, resulting in a ***higher incidence of autoimmune diseases****.*[189]

This is probably due to the ***lymphopenia**** *that occurs with age, leading to overproliferation of homeostatic lymphocytes, as well as decreased regulatory T-cell function and reduced clearance of apoptotic cells by macrophages.* [190]

The most critical aging-related change in the innate immune system is ***the increase in the pro-inflammatory cytokines*** *IL-1ß, IL-6, IL-18 and* TNFα, which *leads to the onset of a* ***low-grade inflammatory state*** *that likely contributes to atherosclerosis, dementia and cancer.* [191]

Lineage structure of the human antibody repertoire in response to influenza vaccination
Jiang N, He J, Weinstein JA, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3699344/

Clin Infect Dis. 2012;55(7):951-959. doi:10.1093/cid/cis574
Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains.
Treanor JJ, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3657521/

[189] Pharmacol Res. 2013;69(1):11-20. doi:10.1016/j.phrs.2012.10.005
Ageing and gut microbes: perspectives for health maintenance and longevity.
Biagi E, Candela M, Turroni S, Garagnani P, Franceschi C, Brigidi P.
https://pubmed.ncbi.nlm.nih.gov/23079287/

Arthritis Res Ther. 2003;5(5):225-234. doi:10.1186/ar974
Aging, autoimmunity and arthritis: T-cell senescence and contraction of T-cell repertoire diversity - catalysts of autoimmunity and chronic inflammation.
Goronzy JJ, Weyand CM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC193735/

[190] PLoS One. 2014;9(2):e89379. doi:10.1371/journal.pone.0089379
Premature CD4+ T cell aging and its contribution to lymphopenia-induced proliferation of memory cells in autoimmune-prone non-obese diabetic mice.
Sheu TT, Chiang BL, Yen JH, Lin WC.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3935863/.

Transpl Int. 2009;22(11):1041-1050. doi:10.1111/j.1432-2277.2009.00927.x.
The aging of the immune system
Weiskopf D, Weinberger B, Grubeck-Loebenstein B.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1432-2277.2009.00927.x

[191] Mech Ageing Dev. 2007;128(1):92-105. doi:10.1016/j.mad.2006.11.016
Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans.
Franceschi C, et al.
https://pubmed.ncbi.nlm.nih.gov/17116321/

Exp Gerontol. 2003;38(6):669-672. doi:10.1016/s0531-5565(03)00061-5
Increased circulating Interleukin-18 levels in centenarians with no signs of vascular disease: another paradox of longevity?

** **Lymphopenia-induced proliferation** (LIP), a mechanism for maintaining a constant number of circulating T cells, occurs in both normal aging and autoimmune disease.*
The incidence of most autoimmune diseases increases with age, and premature aging of CD4+ T cells has been reported in several autoimmune diseases.

Immuno-senescence and inflamm-aging [192]

"The **phenotype** o f **aging**," including immunosenescence is the result of an imbalance between inflammatory and anti-inflammatory mechanisms resulting in a state referred to as "**inflamm-aging.**"

Gangemi S, Basile G, Merendino RA, et al.
https://pubmed.ncbi.nlm.nih.gov/12814802/

Eur Heart J. 2020;ehz961. doi:10.1093/eurheartj/ehz961
Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease
Liberale L, Montecucco F, Tardif JC, Libby P, Camici GG.
https://pubmed.ncbi.nlm.nih.gov/32006431/

[192] Trends Cell Biol. 2018;28(6):436-453. doi:10.1016/j.tcb.2018.02.001
Hallmarks of Cellular Senescence
Hernandez-Segura A, Nehme J, Demaria M
https://pubmed.ncbi.nlm.nih.gov/29477613/

An Acad Bras Cienc. 2017;89(1):285-299. doi:10.1590/0001-3765201720160487
Immune System Dysfunction in the Elderly
Fuentes E, Fuentes M, Alarcón M, Palomo. I.
https://www.scielo.br/pdf/aabc/v89n1/0001-3765-aabc-89-01-00285.pdf

Clin Mol Allergy. 2017 Dec 14;15:21. doi: 10.1186/s12948-017-0077-0.
Immunosenescence in aging: between immune cells depletion and cytokines up-regulation.
Ventura MT, Casciaro M, Gangemi S, Buquicchio R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731094/

Clin Immunol. 2018 Nov;196:59-63. doi: 10.1016/j.clim.2018.04.002.
Immune senescence, epigenetics and autoimmunity.
Ray D, Yung R.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6548177/

Gerontology. 2019;65(5):495-504. doi:10.1159/000497375
Human Inflammaging.
Fülöp T, Larbi A, Witkowski JM.
https://pubmed.ncbi.nlm.nih.gov/31055573/.

Front Immunol. 2016;7:445. doi:10.3389/fimmu.2016.00445
Convergence of Innate and Adaptive Immunity during Human Aging.
Pereira BI, Akbar AN.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5095488/

Nat Immunol. 2018;19(1):10-19. doi:10.1038/s41590-017-0006-x [published correction appears in Nat Immunol. 2018 Oct;19(10):1146].
The twilight of immunity: emerging concepts in aging of the immune system.
Nikolich-Žugich J.
https://pubmed.ncbi.nlm.nih.gov/29242543/

Front Immunol. 2018;9:586. doi:10.3389/fimmu.2018.00586
Age and Age-Related Diseases: Role of Inflammation Triggers and Cytokines.
Rea IM, Gibson DS, McGilligan V, McNerlan SE, Alexander HD, Ross OA.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5900450/

Inflamma-ging is due to chronic antigenic stimulation that occurs throughout life and oxidative stress involving the production of oxygen free radicals and toxic products. [193]

Harmful agents are produced by the body as a consequence of ***normal*** *(unavoidable)* ***metabolic processes*** *(e.g., reactive oxygen species, ROS, from oxidative metabolism) or resulting from exposure to a* ***variety of physical factors*** *(e.g., UV rays from sun exposure) or* ***biological agents*** *(viruses, bacteria, parasites).*

Collectively they represent the ***environment****, and a clear distinction between internal and external environment is very difficult if not impossible*

The body is equipped with a panel of mechanisms that serve to counteract and neutralize the negative effects of such agents:

1. **at the molecular level**: mechanisms of DNA repair, production of heat shock proteins and other chaperones (families of proteins that assist in the folding and assembly of other proteins and enzyme complexes), protein and organelle turnover, and antioxidant and detoxification systems;

2. **at the cellular level:** apoptosis and autophagic cell death, phagocytosis and elimination of damaged and senescent cells, replacement of dead cells with stem cell-derived progenitors (cell and tissue renewal);

3. **Systemic level:** immune and inflammatory responses, stress response, neuroendocrine response**;**

4. **At the organism level:** behavioral/avoidance responses aimed at minimizing danger and harm.

All these types of responses contribute to survival in an integrated way, and the overall level of aging and ultimate longevity achieved by a species can be expected to be (more than) the sum of all these mechanisms (adaptation or remodeling capacity at the level of different species).

During evolution, a **process of positive selection** occurred in order to maximize the efficiency of these defense mechanisms as a unified whole, because they were essential for maintaining a healthy state and consequently for maximizing reproductive capacity and efficiency. [194]

[193] Arch Immunol Ther Exp (Warsz). 2016;64(2):111-126. doi:10.1007/s00005-015-0377-3
Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity.
Minciullo PL, et al.
https://pubmed.ncbi.nlm.nih.gov/26658771/

Curr Opin Clin Nutr Metab Care. 2013;16(1):14-20. doi:10.1097/MCO.0b013e32835ada13
Inflamm-ageing
Cevenini E, Monti D, Franceschi C
https://pubmed.ncbi.nlm.nih.gov/23132168/

[194] Mech Ageing Dev. 2007;128(1):92-105. doi:10.1016/j.mad.2006.11.016
Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans.
Franceschi C, et al.
https://pubmed.ncbi.nlm.nih.gov/17116321/

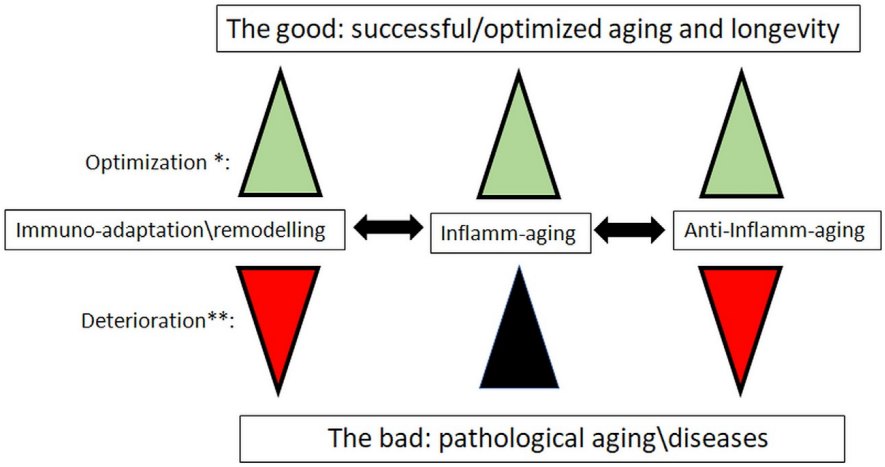
*Chronic, repetitive, lifelong antigenic stimulation by stimulating agents from inside and outside the body, abbreviated by recent terms in "**immune history**" and "**immunobiography**," shapes the entire immune system.* [195]

Antigenic stimulations, apart from those due to pathogens (bacteria, fungi, parasites, and viruses) and continuously forming neoplastic cells, also include the production and accumulation of cellular wastes, e.g., proteins and molecules with the incorrect conformation and nonfunctional.

*The accumulation of this garbage in aging cells, which eventually leads to inflammation, has recently been dubbed "**garb-aging.**"* [196]

The state of good health in the elderly depends not only on low-level pro-inflammatory mechanisms, but also on the presence of an efficient network that can neutralize with an effective anti-inflammatory response the antigenic insults received throughout life.

Therefore, **inflammation is not only important for immunosenescence mechanisms, but also for the issue of longevity**.



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5767595/pdf/fimmu-08-01960.pdf
The new paradigm for the role of inflammation and immunoadaptation/remodeling in the aging process.
* Optimization: all three processes are increasing in concert, balancing each other. ** Deterioration: influenza aging increases and is not balanced by the opposite processes of inflammatory aging and immune adaptation/remodeling, which are decreasing. We mean by anti-inflammatory aging all compensatory mechanisms of chronic inflammatory aging. The most important diseases that might have an aging component of inflammation are cancers, cardiovascular diseases, and neurodegenerative diseases.

[195] Front Immunol. 2017;8:982. doi:10.3389/fimmu.2017.00982
Immunobiography and the Heterogeneity of Immune Responses in the Elderly: A Focus on Inflammaging and Trained Immunity. Franceschi C, Salvioli S, Garagnani P, de Eguileor M, Monti D, Capri M.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5559470/

Front Immunol. 2018;8:1960. doi:10.3389/fimmu.2017.01960
Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes?
Fulop T, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5767595/

[196] Trends Endocrinol Metab. 2017;28(3):199-212. doi:10.1016/j.tem.2016.09.005
Inflammaging and 'Garb-aging'
Franceschi C, Garagnani P, Vitale G, Capri M, Salvioli S
https://pubmed.ncbi.nlm.nih.gov/27789101/

The ultracentenarians seem to cope with chronic subclinical inflammation through an **anti-inflammatory** response, thus called "**anti-inflammaging.**"

Circulating cytokines are involved in both inflammation and anti-inflammation, and are the expression of a system involving genes, polymorphisms, and environment. [197]

It has been reported that **frailty in** the **elderly is the result of an inflammatory state associated with the overproduction of certain lymphokines, including IL-6, called the geriatric cytokine.**

This factor, together with hormonal changes, nutritional deficiencies, and physical inactivity would lead to one of the most important components of frailty, which is **sarcopenia**, as well as **reduced bone mass**.

In this context, immunity appears to play an important role, both in regulating the mechanisms of aging and in the onset of diseases typical of aging (i.e., infectious diseases, autoimmunity, cancer, metabolic diseases, and neurodegenerative diseases).

The key to healthy aging therefore lies in the **ability to maintain a balanced response** to these immune messengers and a rapid and integrated return to inflammation resolution and immune homeostasis.[198]

---

[197] Mech Ageing Dev. 2007;128(1):92-105. doi:10.1016/j.mad.2006.11.016
Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans.
Franceschi C, et al.
https://pubmed.ncbi.nlm.nih.gov/17116321/

[198] J Gerontol A Biol Sci Med Sci. 2014;69 Suppl 1:S4-S9.
Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. doi:10.1093/gerona/glu057
Franceschi C, Campisi J.
https://academic.oup.com/biomedgerontology/article/69/Suppl_1/S4/587037

Mech Ageing Dev. 2003;124(4):487-493. doi:10.1016/s0047-6374(03)00025-3
Plasma cytokine profiles in elderly humans.
Forsey RJ, et al.
https://pubmed.ncbi.nlm.nih.gov/12714257/

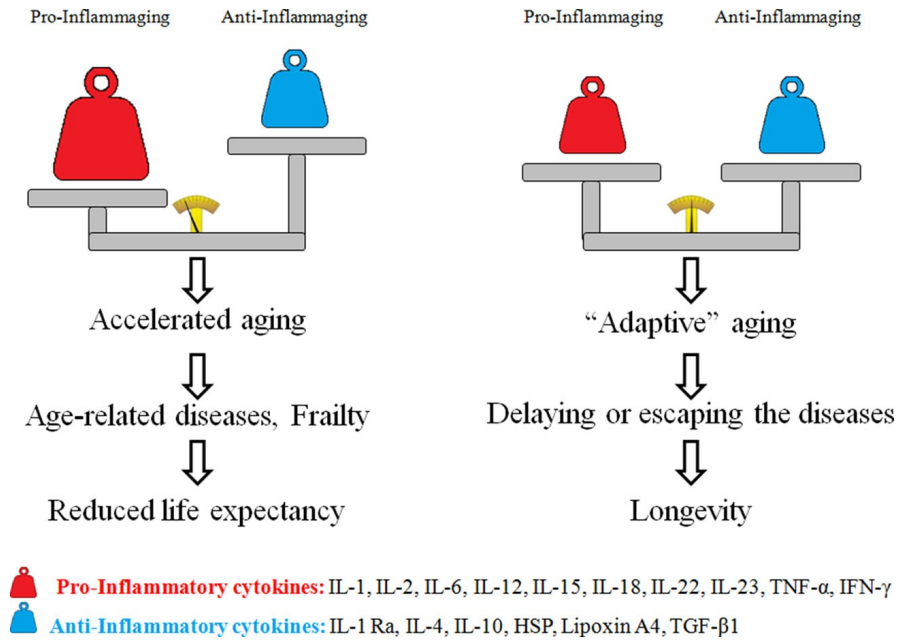
J Am Geriatr Soc. 1999;47(6):639-646. doi:10.1111/j.1532-5415.1999.tb01583.x
Serum IL-6 level and the development of disability in older persons.
Ferrucci L, et al.
https://pubmed.ncbi.nlm.nih.gov/10366160/

Life Sci. 1992;51(25):1953-1956. doi:10.1016/0024-3205(92)90112-3
Increase of plasma IL-6 concentration with age in healthy subjects.
Wei J, Xu H, Davies JL, Hemmings GP.
https://pubmed.ncbi.nlm.nih.gov/1453878/.

Ageing Res Rev. 2009;8(1):36-42. doi:10.1016/j.arr.2008.09.001
Effect of interleukin-6 polymorphisms on human longevity: a systematic review and meta-analysis.
Di Bona D, et al.
https://pubmed.ncbi.nlm.nih.gov/18930842/

J Nutr Health Aging. 2009;13(4):390-394. doi:10.1007/s12603-009-0051-8
Frailty: defining and measuring of a concept.
Pel-Littel RE, Schuurmans MJ, Emmelot-Vonk MH, Verhaar HJ.
https://pubmed.ncbi.nlm.nih.gov/19300888/

Biogerontology. 2010;11(5):527-536. doi:10.1007/s10522-010-9297-0
Frailty and muscle metabolism dysregulation in the
elderly. Evans WJ, et al
https://pubmed.ncbi.nlm.nih.gov/20683658/



Taken from https://cmb.i-learn.unito.it/pluginfile.php/5044/mod_resource/content/1/Minciullo_et_al.pdf
The "burden" of pro- and anti-inflammatory cytokines in aging and longevity. Increased pro-inflammatory cytokines promote frailty and age-related diseases and reduce life expectancy. The balance between pro-inflammaging and anti-inflammaging promotes adaptation to life conditions, enables avoidance of disease or delaying its onset, and leads to longevity

The aging process is closely related to significant alterations in the innate and adaptive immune system (Table 1).

In terms of **innate immunity**, aging can result in both quantitative and qualitative changes, including a

- **Reduced number of circulating monocytic and dendritic cells** (DCs),
- **Reduced phagocytic activities** of migratory macrophages or neutrophils and altered antigen-presenting abilities by DCs.

With respect to **T cells**, aging may result in the **reduction of the TCR repertoire** due to **thymic involution** during puberty and the **accumulation of senescent or exhausted T cells** that are functionally inert or dormant.

Numerous factors, including **chronic viral infection** and the release of damage-associated molecular profiles (DAMPs), may contribute to the age-dependent immune dysregulation that results in age-associated diseases such as atherosclerosis, Alzheimer's disease, and infectious diseases.

In addition, **vaccine efficacy** in the elderly is affected by age-related alterations, ranging from reduced numbers of circulating naïve B and T cells, limited diversity of BCR repertoires, and defective antibody response to novel antigens.[199]

The 2 features of aging innate immune system that are important to highlight are:

(1) its **pre-activated state in the absence of antigen recognition** (in the basal state), and
(2) **Immune paralysis in the presence of antigens** (in the activated state). [200]

[199] Exp Gerontol. 2018;105:4-9. doi:10.1016/j.exger.2017.10.024
Age and immunity: What is "immunosenescence"?
Pawelec G.
https://pubmed.ncbi.nlm.nih.gov/29111233/
[200] Biogerontology. 2016;17(1):147-157. doi:10.1007/s10522-015-9615-7
From inflamm-aging to immune-paralysis: a slippery slope during aging for immune-adaptation.
Fulop T, et al.
https://pubmed.ncbi.nlm.nih.gov/26472173/

This dichotomy is extremely important for understanding the consequences of lifelong immune changes.

The activated baseline state reflects what, as already discussed, is referred to as the **memory of innate immunity** (trained immunity).
Following an antigenic response, innate immune cells revert to a quiescent state through a series of **molecular processes** (at the level of epigenomes and transcriptomes) and **metabolic regulation** (alternating between aerobic oxidative phosphorylation and anaerobic glycolysis [Warburg effect]) but retain some **epigenetic/molecular changes** that constitute their memory.[201]
Subsequent stimulation of these cells that had already responded to pathogens elicits a different response in intensity and type than the first one because of innate memory.

The other feature is **immune paralysis**, understood as **underregulation of innate immune system responsiveness to antigenic stimulation**, or possibly a kind of **innate immune tolerance**.
This physiological condition may serve to protect the body from further self-induced inflammatory damage, albeit at the expense of effective elimination of pathogens or cellular wastes.
However, it should be emphasized that immune paralysis does not mean that the immune system is in a nonfunctional state.

> *Although there are functional alterations in older individuals compared with younger individuals, the simple assumption that immune cells in older people lose their protective power is incorrect.*
>
> *For example,* ***most elderly people are able to defend themselves against many types of infection even though the adaptive immune response is clearly less functional****.*

The decline of the immune system in aging is characterized by.

- A **shift from a naïve to a memory T cell phenotype**,
- A **cytokine profile of type 1 to type 2** [202],
- **Inefficient humoral immunity**
- An **increase in the degree of maturation of T cells**.

Other important features of senescent cells are:

- **telomere shortening** that accompanies each proliferation cycle, leading to the arrest of cell division or "replicative senescence."
- An **increase** in **mitochondrial dysfunction** and reactive oxygen species;
- a **senescence-associated secretory phenotype (SASP)**, defined as the secretion of pro-inflammatory cytokines, chemokines, and proteases by senescent cells.[203]

[201] Antioxid Redox Signal. 2018;29(11):1023-1040. doi:10.1089/ars.2017.7310
Epigenetics and Trained Immunity.
van der Heijden CDCC, Noz MP, Joosten LAB, Netea MG, Riksen NP, Keating ST.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6121175/

[202] Clin Exp Immunol. 2002;127(1):107-114. doi:10.1046/j.1365-2249.2002.01736.x
Is aging associated with a shift in the balance between Type 1 and Type 2 cytokines in humans?
Sandmand M, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1906284/

[203] Annu Rev Pathol. 2010;5:99-118. doi:10.1146/annurev-pathol-121808-102144
The senescence-associated secretory phenotype: the dark side of tumor suppression
Coppé JP, Desprez PY, Krtolica A, Campisi J
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4166495/

These characteristics impact active cells during mitosis leading to **depletion or division arrest** (e.g., hematopoietic stem cells-HSCs or T cells) and post-mitotic immune cells causing their **cellular dysfunction** (e.g., neutrophils).

Table 1

Summary of the immune changes associated with aging

Immunity	Cell	Aging-associated changes
Innate	Monocytes/macrophages	- Reduced phagocytic activity
		- Decreased MHC II expression
		- Decreased ROS and cytokine production
		- Altered TLR expression (decreased except for TLR5)
	DCs	- Decreased maturation and Ag presentation
		- Altered TLR expression and signaling
		- Impaired Ag uptake
		- Altered CD80 and CD86 expression
	Neutrophils	- Reduced chemotaxis
		- Decreased MHC II expression
		- Decreased ROS and cytokine production
		- Altered TLR expression
		- Decreased NET formation
Adaptive	B cells	- Limited diversity in BCR repertoire
		- Decreased numbers of naïve and circulating B cells
		- Reduced Ag-specific Ab production
		- Altered memory B cell homeostasis
	T cells	- Restricted diversity in TCR repertoire
		- Decreased numbers of naïve T cells
		- Increased numbers of senescent T cells
		- Increased numbers of exhausted T cells
		- Expansion of inflationary $CD8^+$ T cell populations caused by chronic viral infections (CMV, EBV)
		- Diminished effector T cell response to new Ag

BCR, B cell receptor; EBV, Epstein-Barr virus.

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6943173/

Response to infection and vaccination [204]

As discussed above, immunosenescence is characterized by a progressive deterioration of the immune system associated with aging.

Multiple components of the innate and adaptive immune system experience aging-related changes, such as altered numbers of circulating monocytic and dendritic cells, reduced phagocytic activities of neutrophils, limited diversity in the B/T cell repertoire, depletion or depletion of T cells, and chronic production of inflammatory cytokines (inflammatory).

> *The elderly are less likely to benefit from vaccinations as preventive measures against infectious diseases because of the* ***immune system's inability to activate an effective defense****.*
>
> *Therefore, aging is believed to reduce vaccine efficacy as a result of an age-associated decline in vaccination-induced immunogenicity.*

As already seen, influenza contributes significantly to worldwide morbidity and mortality among the elderly. More complications and a higher number of hospitalizations due to seasonal influenza are observed among people aged ≥65 years than among younger individuals, and up to 90% of influenza-related deaths occur in elderly groups. Therefore, the World Health Organization recommends annual immunization against seasonal influenza for people aged ≥65 years [205].

However, vaccine ineffectiveness remains a crucial issue to date unresolved, precisely because of immunosenescence. [206]

Regarding the risk of vaccine injury, particularly of disease enhancement following reinfection, please refer to the section: ADE FROM ANTINFLUENZA VACCINE.

---

[204] Maturitas. 2015;82(1):50-55. doi:10.1016/j.maturitas.2015.05.004
Immunosenescence: Implications for response to infection and vaccination in older people. Pera A, et al.
https://pubmed.ncbi.nlm.nih.gov/26044074/

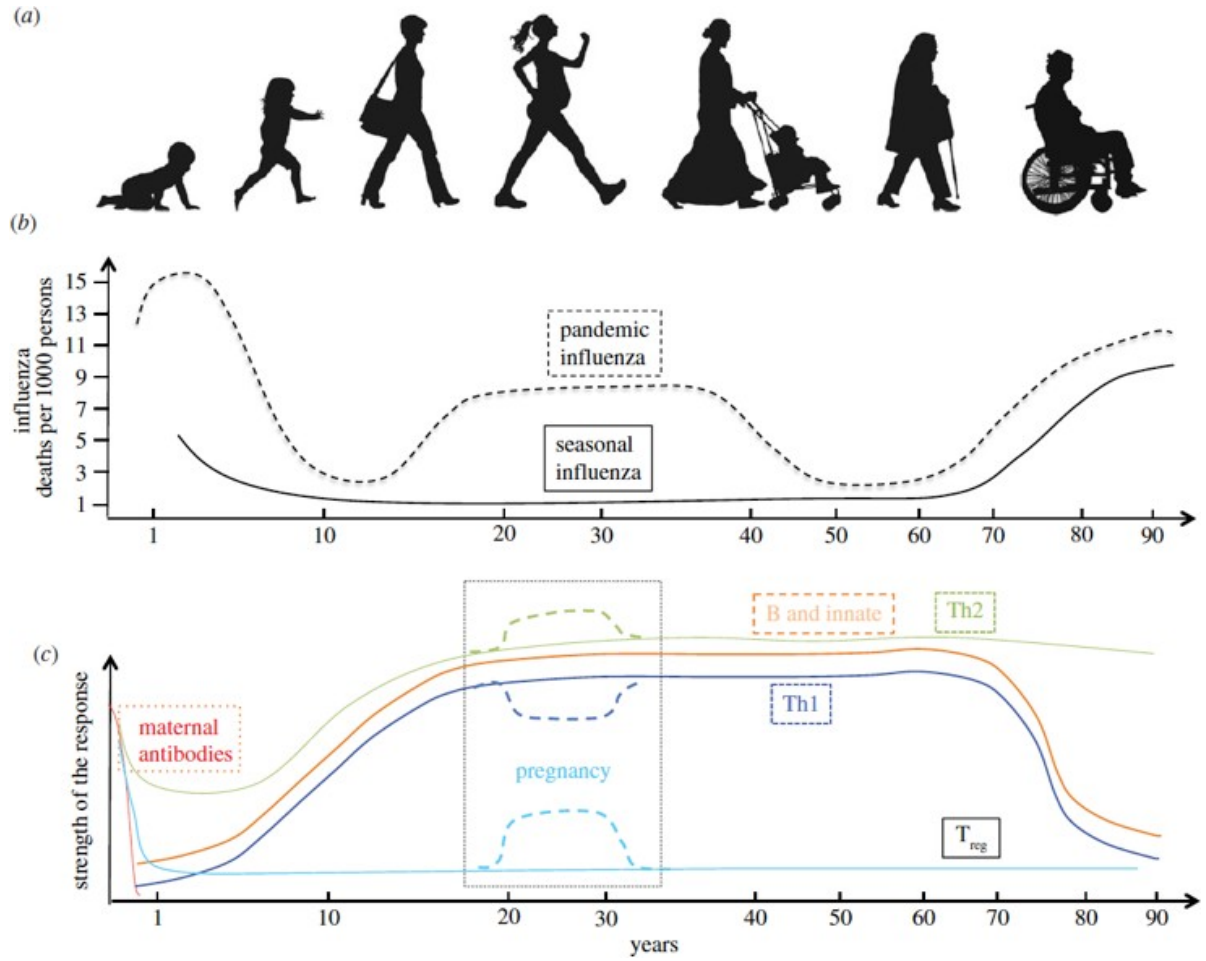
Exp Gerontol. 2019;124:110632. doi:10.1016/j.exger.2019.110632
Immunosenescence: A systems-level overview of immune cell biology and strategies for improving vaccine responses.
Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB.
https://pubmed.ncbi.nlm.nih.gov/31201918/

China Life Sci. 2013;56(5):399-405. doi:10.1007/s11427-013-4478-0
Immunosenescence and age-related viral diseases.
Ma Y, Fang M.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7089158/

[205] https://www.euro.who.int/en/health-topics/communicable-diseases/influenza/vaccination/seasonal-vaccination-policies-and-coverage-in-the-european-region

[206] Jiang N, He J, et al.
Lineage structure of the human antibody repertoire in response to influenza vaccination [published correction appears in Sci Transl Med. 2013 Jul 10;5(193):193er8]. Sci Transl Med. 2013;5(171):171ra19. doi:10.1126/scitranslmed.3004794
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3699344/

Treanor JJ, et al.
Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains.
Clin Infect Dis. 2012;55(7):951-959. doi:10.1093/cid/cis574
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3657521/



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707740/
Immune response to influenza (a) The seven ages of women. **(b)** Schematic graph of excess deaths from seasonal or pandemic influenza over an individual's lifetime represented as the number of deaths per 1000 people. Note that while pregnancy increases the risk of severe influenza, in severe pandemics such as 1918/1919 there were also excess deaths in previously healthy young adults who were not pregnant. **(c)** Schematic graph of the different arms of the immune response to influenza over an individual's lifetime.

Age-associated changes in immune cells and how these changes affect the response to infection and vaccination are summarized schematically: [207]

Innate immunity:

Receptors: TLRs play a key role in the innate immune system as regulators against microbial infections.
Their expression and function in monocytes [(9)], DCs [(10)] and neutrophils [(11)] decrease with advancing age.

Aging also causes **increased gene expression of the NLRP3 inflammasome**, a multiprotein complex activated by DAMPs, (microbial genomes, endotoxins, extracellular ATP, β-amyloid, and intracellular uric acid).[208]

[207] Bibliography reference in superscript in round brackets Oh SJ, Lee JK, Shin OS.
Aging and the Immune System: the Impact of Immunosenescence on Viral Infection, Immunity and Vaccine Immunogenicity.
Immune Netw. 2019;19(6):e37. doi:10.4110/in.2019.19.e37
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6943173/

[208] Latz E, Duewell P.
NLRP3 inflammasome activation in inflammaging.
Semin Immunol. 2018;40:61-73. doi:10.1016/j.smim.2018.09.001
https://pubmed.ncbi.nlm.nih.gov/30268598/

Monocytes: have reduced interferon production and impaired phagocytosis.

Macrophages: very recently, Van Beek et al. [29] proposed that inflammation may lead to the accumulation of alternatively activated macrophages (**M2-like**), which remain pro-inflammatory in tissues.

DCs: Age-associated changes in DC signaling pathways may affect their function and result in dysfunctional secretion of cytokines in response to pathogens or self-DNA and reduced phagocytosis and migration capabilities.

Neutrophils: are important phagocytic cells specialized in early defense against pathogens.

While neutrophil numbers remain unchanged with increasing age, neutrophils in the elderly tend to show dysfunctional phagocytic and chemotactic abilities.

Aging also affects the recruitment of neutrophils, which are the first cells to migrate to the site of infection [40,44]. In addition, neutrophils of the elderly produce fewer neutrophil extracellular traps (NETs)* with a delay in tissue repair and increased susceptibility to antibiotic-resistant infections

** The presence of germs in a wound triggers neutrophil activation and the release of **extracellular traps (NETs)** composed of granular proteins, enzymes, and nuclear material that capture and kill pathogens. After extrusion of the nuclear material, the neutrophils die (**NETosis**)*

Adaptive immunity:

B cells: many older people are known to have limited diversity in their B-cell repertoire, which contributes to increased susceptibility to infectious diseases, poor response to vaccines, and increased likelihood of having self-reactive antibodies.

Aging may cause significant changes in the selection process that leads to the maturation of high-affinity B cells [47], with the formation of an often less diverse B-cell repertoire in old age [8]. It is likely that this loss of diversity is correlated with poor responses to vaccines against many pathogens [6].

T cells: although aging does not change the level of IL-7, a key factor in maintaining T cell homeostasis, it generally decreases the number of naïve T cells and increases the number of senescent T cells [52].

Analysis using the Illumina high-throughput sequencing platform revealed an **age-associated reduction in TCR diversity**, indicating a significant reduction in the number of naïve T cells and TCR-ß diversity by age 40 years [65].

In addition, **thymus involution** results in the formation of defective T cells that induce inflammation, increased susceptibility to infection, and decreased vaccine efficacy.

Since cytokines are key regulatory molecules of the T-cell-mediated immune response, it has been noted that aging-related T-cell defects may originate from **alterations in cytokine production**.

In particular, a shift in the cytokine profile indicates that aged T cells predominantly exhibit a **Th2-type phenotype** [66].

Th17 cells defend the host against extracellular pathogens and are associated with the development of autoimmune diseases and chronic inflammatory diseases in humans [67].

The ratio of Th17 to Treg appears to increase with age, and Schmitt et al. [68] suggest that this variation in the ratio may explain the increased frequency of autoimmune diseases and decreased response to infection in the elderly.

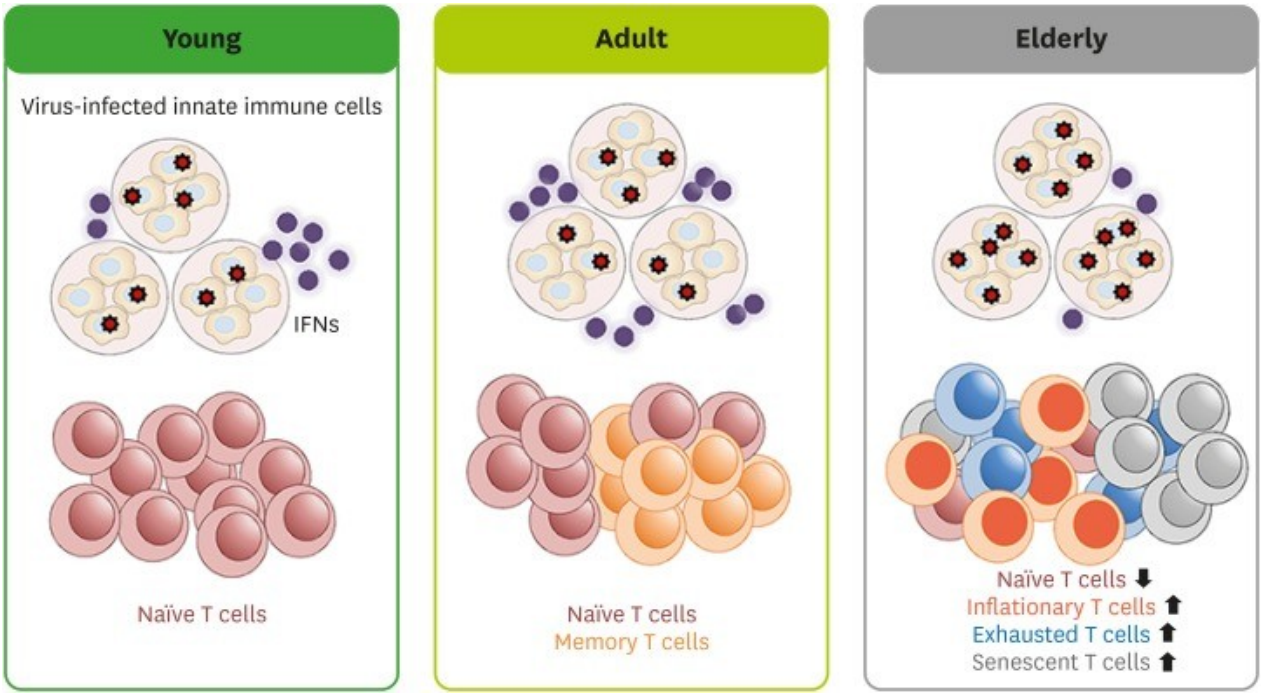
The following figure shows the **impact of immunosenescence on T cells involved in chronic viral infections**.

Mastrocola R, Aragno M, Alloatti G, Collino M, Penna C, Pagliaro P.
Metaflammation: Tissue-Specific Alterations of the NLRP3 Inflammasome Platform in Metabolic Syndrome.
Curr Med Chem. 2018;25(11):1294-1310. doi:10.2174/0929867324666170407123522
https://pubmed.ncbi.nlm.nih.gov/28403789/

For example, aging results in **reduced numbers of naïve $^{CD8+}$ T cells**, **reduced TCR repertoire diversity**, and **high numbers of senescent, exhausted, and inflammatory T cells**

> *In case of cytomegalovirus (CMV) infection, extensive responses are induced by specific $^{CD8+}$ T cells that remain elevated or even increase over time, this phenomenon has been called* ***memory T cell inflation*** [209]

Recently, Tahir et al. [69] defined the **phenotype of senescence-associated T cells (SA-T)** and found that **these cells secrete abundant atypical proinflammatory cytokines**, which accumulate in tissues under metabolic stress and cause persistent inflammation or tumors [70].



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6943173/
The impact of immunosenescence on persistent viral infection and immunity. Aging leads to numerous changes in the major components of the innate and adaptive immune system. In response to viral infection, innate immune cells may trigger activation of IFN pathways to eliminate virus-infected cells. Age-associated defects in innate immune cells can lead to reduced IFN production. Persistent viral infection, such as CMV persistence, can have a profound effect on alterations in adaptive immunity, particularly on T-cell composition and function. In the elderly, there is a reduced number of naïve T lymphocytes, but an increased number of senescent, inflammatory, or exhausted T lymphocytes that are functionally inert or dormant.

Microbiota and senescence [210]

[209] van den Berg SPH, et al.
The hallmarks of CMV-specific CD8 T-cell differentiation.
Med Microbiol Immunol. 2019;208(3-4):365-373. doi:10.1007/s00430-019-00608-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6647465/

[210] Reference for bibliography in superscript in square brackets
Nat Rev Endocrinol. 2018;14(10):576-590. doi:10.1038/s41574-018-0059-4

The average life expectancy for all Italians at birth is estimated at **82.7 years**, the fourth highest in the world and the second highest in Europe [211].

The latest data released by the National Institute of Health (ISS) on June 11, 2020, reported 32,938 patients died positive for COVID-19, with the highest rate (**85.3 percent)** in the over-70 age group and 58.6 percent in the over-80 age group. [212]

The mortality rate is significantly higher among the elderly with pre-existing disease conditions, such as hypertension, cardiovascular disease and diabetes among the most common. [213]
This aspect may explain the high mortality rate in Italy, a country forced to deal with the chronic diseases of old age.

Compared with other European countries, the Italian population has become longer-lived but has chronic diseases that increase the individual's vulnerability to stress and impair the multisystem compensatory effort to restore homeostasis [214]

As already seen, underlying the chronic diseases of old age is the manifestation of inflammaging i.e., a low-grade inflammatory state that occurs in the absence of infection and mainly due to endogenous signals.

The major feature of inflammaging is the activation of the innate immune system, in which macrophages play the major role. [215]

Inflammaging: a new immune-metabolic viewpoint for age-related diseases.
Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A.
https://cris.unibo.it/retrieve/handle/11585/736269/581072/Franceschi_NRE_2018%20Post_Print.pdf

Immunol Invest. 2018;47(8):801-811. doi:10.1080/08820139.2018.1537570
The Impact of the Microbiome on Immunosenescence.
Amsterdam D, Ostrov BE.
https://pubmed.ncbi.nlm.nih.gov/31282802/

[211] Coronavirus Resource Center, Johns Hopkins University. https://coronavirus.jhu.edu/. 2020

[212] https://www.epicentro.iss.it/coronavirus/bollettino/Report-COVID-2019_11_giugno.pdf

[213] Shim E, Tariq A, Choi W, Lee Y, Chowell G.
Transmission potential and severity of COVID-19 in South Korea.
Int J Infect Dis. 2020;93:339-344. doi:10.1016/j.ijid.2020.03.031
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7118661/

[214] Verity R, et al.
Estimates of the severity of coronavirus disease 2019: a model-based analysis [published correction appears in Lancet Infect Dis. 2020 Apr 15] [published correction appears in Lancet Infect Dis. 2020 May 4].
Lancet Infect Dis. 2020;20(6):669-677. doi:10.1016/S1473-3099(20)30243-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7158570/

J Gerontol A Biol Sci Med Sci. 2020;glaa094. doi:10.1093/gerona/glaa094
A geroscience perspective on COVID-19 mortality.
Promislow DEL
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7184466/pdf/glaa094.pdf

Geriatrics (Basel). 2020;5(2):E24. doi:10.3390/geriatrics5020024
COVID-19: A Geriatric Emergency.
Boccardi V, Ruggiero C, Mecocci P.
https://www.mdpi.com/2308-3417/5/2/24/htm

https://www.epicentro.iss.it/coronavirus/bollettino/Infografica_15giugno%20ITA.pdf

[215] NPJ Aging Mech Dis. 2016;2:16018. doi:10.1038/npjamd.2016.18
Macrophages in age-related chronic inflammatory diseases.
Oishi Y, Manabe I.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5515003/

*From an evolutionary point of view, the most difficult events for the survival of an organism are **nutrient deprivation** and **infection by pathogens**; therefore, competition for food and response to infectious diseases are the main factors that determine the ecological dynamics of populations and species [7-9].*

As a result, food sources, metabolism, endocrine responses, innate immunity response and inflammation have evolved together, and the macrophage is a major cell at the interface between metabolism and immunity [10].

Several evidences show that **macrophages and adipocytes have considerable functional overlap**, as both cell types secrete cytokines and can be activated by bacterial products, such as lipopolysaccharides [11], and pre-adipocytes can differentiate into macrophages [12,13].

Another very important consideration is **that nutrition is inevitably linked to immune system activation, as water and food have been heavily contaminated by microbes for much of human evolution**.

In addition to this, the innate immune response is activated when food is ingested: this activation is known as the **postprandial inflammation** and is part of the adaptive response to the meal.

Finally, during an infection a mechanism is activated that leads to a **reduction in food intake so as** to limit the likelihood of ingesting other pathogens and to reduce the likelihood of nutrient epitopes competing with receptors crucial for pathogen detection. [14,16,17].

Chronic infection and inflammation are also linked to **insulin resistance**, which reduces intracellular glucose levels required by most pathogens for replication, and optimizes energy supply to the brain, a crucial organ with a high metabolic cost, so as to protect it from stress due to stimuli such as starvation and infection. [18-20]

During fasting and an infection, fat accumulated in adipose tissue is metabolized to produce energy, while glucose is used as a substrate only for the brain. [21]

Cytomegalovirus (CMV) infection is an example of a common chronic infection associated with immunosenescence and age-related diseases, particularly with type 2 diabetes [27], as it promotes a proinflammatory environment at the level of pancreatic beta cells.

Table 1. Role of macrophage in metabolic control

Metabolic tissue	*Resident macrophage*	*Physiological/pathological role*
Hypothalamus	Microglia	Appetite control
Liver	Kupffer cell	Control of hepatocyte metabolism Liver steatosis and fibrosis Insulin resistance
Pancreatic islet	Resident macrophage	β cell development Islet inflammation and β cell dysfunction
Adipose tissue	Resident macrophage	Lipid handling Adipogenesis Insulin resistance
Skeletal muscle	Resident macrophage	Regeneration Insulin resistance

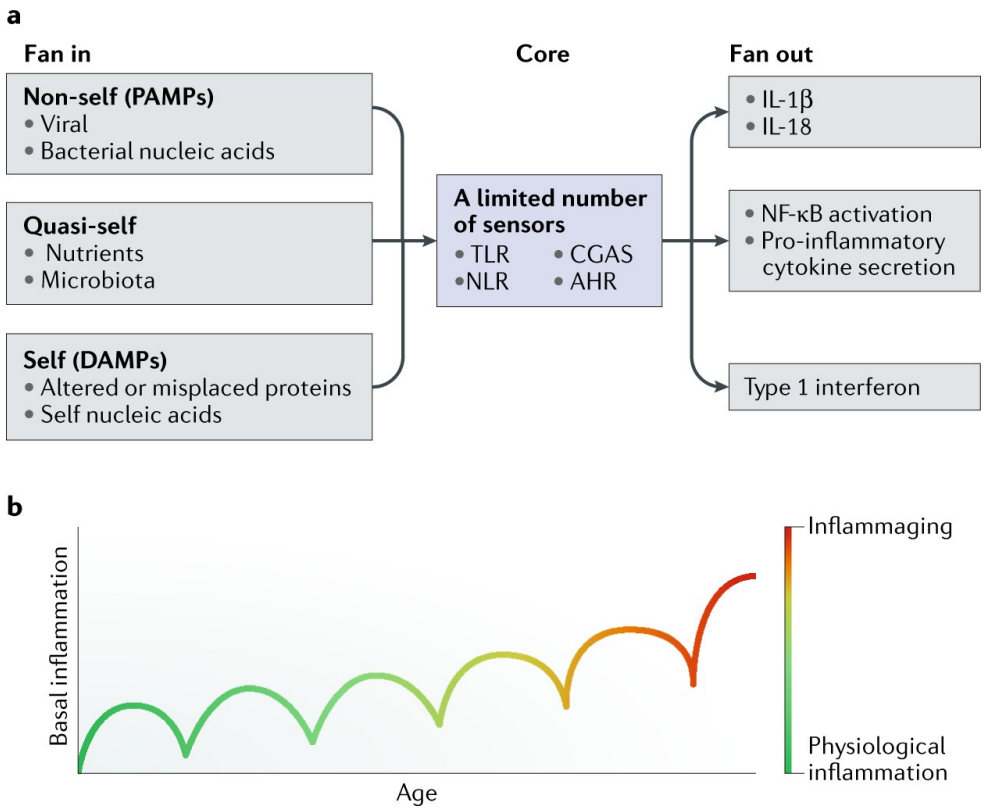
Resident macrophages in metabolic organs are crucial to metabolic control in the steady state. Impairment in the physiological functions of tissue-resident macrophages, which can occur with aging, may contribute to age-related organ dysfunction. Macrophages may also mediate chronic inflammatory processes and tissue dysfunction in obesity.

Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5515003/pdf/npjamd201618.pdf

An important feature of biological complexity is **promiscuity or biological degeneracy**, that is, the ability of structurally different elements to perform the same function, so that if one fails in its function the other elements step in to make up for the deficiency.
There is experimental evidence that the same sensors such as PRRs, including TLRs, NOD-like receptors, cGAS (GMP-AMPc synthase), aryl hydrocarbon receptors (AHRs) and taste receptors have overlapping characteristics.

These receptors are able to recognize altered or degraded self proteins (DAMPs), products of the non-self of viruses and bacteria (PAMPs), and metabolic and nutritional products from the microbiota that can be considered a quasi-self. [6]

During evolution, this set of receptors was optimized to increase inflammation and insulin resistance as a first response to nutrient deprivation and also as a strategy to defend against pathogens. [34,10,35,36]



Taken from
https://www.semanticscholar.org/paper/Inflammaging%3A-a-new-immune%E2%80%93metabolic-viewpoint-for-Franceschi-Garagnani/6247ca2e33d38d072f1f7196e156b43975d00e71

The bowtie of the inflammaging machinery.

a) A heterogeneous and broad spectrum of exogenous and endogenous danger stimuli (fan in) interacts with a limited repertoire of sensors expressed on the cell surface and in the cytoplasm (core) and evokes a limited number of inflammatory responses (fan out). Danger molecules can be non-self (pathogen-associated molecular profiles (PAMPs)), self (damage-associated molecular profiles (DAMPs)), and quasi-self (nutritional and metabolic products of the gut microbiota), and this multitude of stimuli converges on the same evolutionarily selected promiscuous sensors, triggering inflammatory responses. This physiological inflammatory process is critical for survival into middle age.

b) With age, the proinflammatory response usually increases and becomes detrimental in the post-reproductive age. The sum of these responses produces a progressive increase in inflammatory levels that can last for several years or decades, eventually leading to inflammaging. This process is modulated by a variety of factors, including genetics, lifestyle habits, immunobiography, and anatomical variables [61,173].

Excess nutrients and excessive nutrition fit this general scenario, representing particular types of stimuli that fuel inflammation. For simplicity, only some of the inflammatory sensors and compounds have been reported.

AHR, aryl-hydrocarbon receptor; cGAS, GMP-AMP synthase; NF-κB, nuclear factor-κB; NLR, NOD-like receptor; TLR, Toll-like receptor

Taste receptors, particularly G-protein-coupled receptors that detect bitter, salty, and savory tastes, signal the brain to organize eating behavior, that is, to seek out food, select it, and consume it, in response to a wide range of nutritional stimuli.

The same receptors are also exploited for innate and inflammatory immune response to microbial agents. [142]

Interestingly, in the early stage of SARS-Cov-2 infection, ***loss of taste (dysgeusia) and sense of smell (anosmia)*** *occur.* [216]

This could be a signal for the body to direct the innate response toward inflammation and defense against infection rather than toward nutrient assimilation.

Also keep in mind that the virus has colonization in the gut, and therefore food chemosensors (smell and taste) found in the upper respiratory tract could also be present in the gut and act as PRRs to detect and respond to infection.

One example already studied is that of TRPM5, a cation channel essential for signal transduction to the brain for bitter, sweet, and savory flavors (glutamate), which is also expressed in intestinal cells called tuft cells.
TRPM5-dependent signals activate tuft cells involved in the activation of immunity responses following parasitic infection with the production of IL-25, which promotes the rapid expansion of type 2 innate lymphoid cells [145,146].

It follows that taste receptors and chemosensory receptors not only constitute a sensory structure for food, but can also be assumed to behave as PRRs.

On the other hand, several examples are known in which promiscuity or degeneration of the immune receptor response is activated by nutrients.

Specifically, ***during old age a diet rich in saturated fatty acids activates PRRs****, together with material from dead cells due to necroptosis (acting as a DAMPs),* ***activate the inflammatory response in a manner similar to an infection****.*

In healthy subjects, a high-fat diet also increases serum levels of bacterial endotoxin (lipopolysaccharide), which could cause leukocyte activation and inflammation [91].

Bacterial endotoxins *are potent inflammatory compounds circulating at low concentrations in the blood that* ***mimic low-grade bacterial infection****.*

Postprandial elevation of lipopolysaccharide in the circulation contributes to ***metabolic endotoxemia and low-grade inflammation*** [92]*, which appears to play a substantial role in the development and progression of cardiometabolic diseases* [93] *and in promoting aging phenotypes (such as muscle decline and sarcopenia)* [94].

Food intake as an energy source is used by the body for metabolic needs and by gut bacteria for their growth [154].

The gut microbiota shows a **circadian fluctuation** [155], mainly driven by diurnal food intake, leading to a rhythmic abundance of microbial metabolites [1,56,157].

The systemic oscillation of the set of metabolites (metabolome) derived from the gut microbiota regulates host physiology, including metabolic function and drug detoxification [157,158].

Bacterial adhesion to the epithelium shows temporal fluctuations, and disruption of the oscillatory activity of the gut microbiota, following antibiotic treatment or disordered timing of food intake, leads to the

[216] CMAJ. 2020;cmaj.200869. doi:10.1503/cmaj.200869
Anosmia and dysgeusia associated with SARS-CoV-2 infection: an age-matched case-control study
Carignan A, et al.
https://www.cmaj.ca/content/cmaj/early/2020/05/27/cmaj.200869.full.pdf

disorganization of host rhythmicity [158], suggesting that **the gut microbiota acts as a peripheral circadian regulator** [159].

Overall, host-microorganism interaction appears to be essential for maintaining appropriate rhythms by integrating fluctuations in nutritional and environmental signals.

*According to **chronobiomics** [217], this interaction is bidirectional, and the host clock influences microbial community configurations.*

*Moreover, as commensal bacteria compete with invading pathogens, **the oscillation of the gut microbiota contributes to circadian variation in host defense**.*

Circadian disruptions induced by modern lifestyles could lead to dysbiosis and predispose the host to metabolic disorders and inflammation [81,161].

Regular introduction of nutrients into the colon immediately stimulates bacterial growth for 20 minutes. Bacterial molecules and metabolites, the production of which is regulated by bacterial growth stages, control the release of **satiety hormones** in the gut.

Therefore, systemic bacterial molecules are able to act directly on appetite signaling pathways by changing the energy status of both the host and its gut microbiota.

This short-term modulation of intestinal satiety by bacterial growth can be coupled with the long-term control of appetite, which in turn is regulated by neuropeptidergic circuits in the hypothalamus [154].

[217] Bishehsari F, Levi F, Turek FW, Keshavarzian A.
Circadian Rhythms in Gastrointestinal Health and Diseases.
Gastroenterology. 2016;151(3):e1-e5. doi:10.1053/j.gastro.2016.07.036
https://www.gastrojournal.org/article/S0016-5085(16)34832-6/pdf

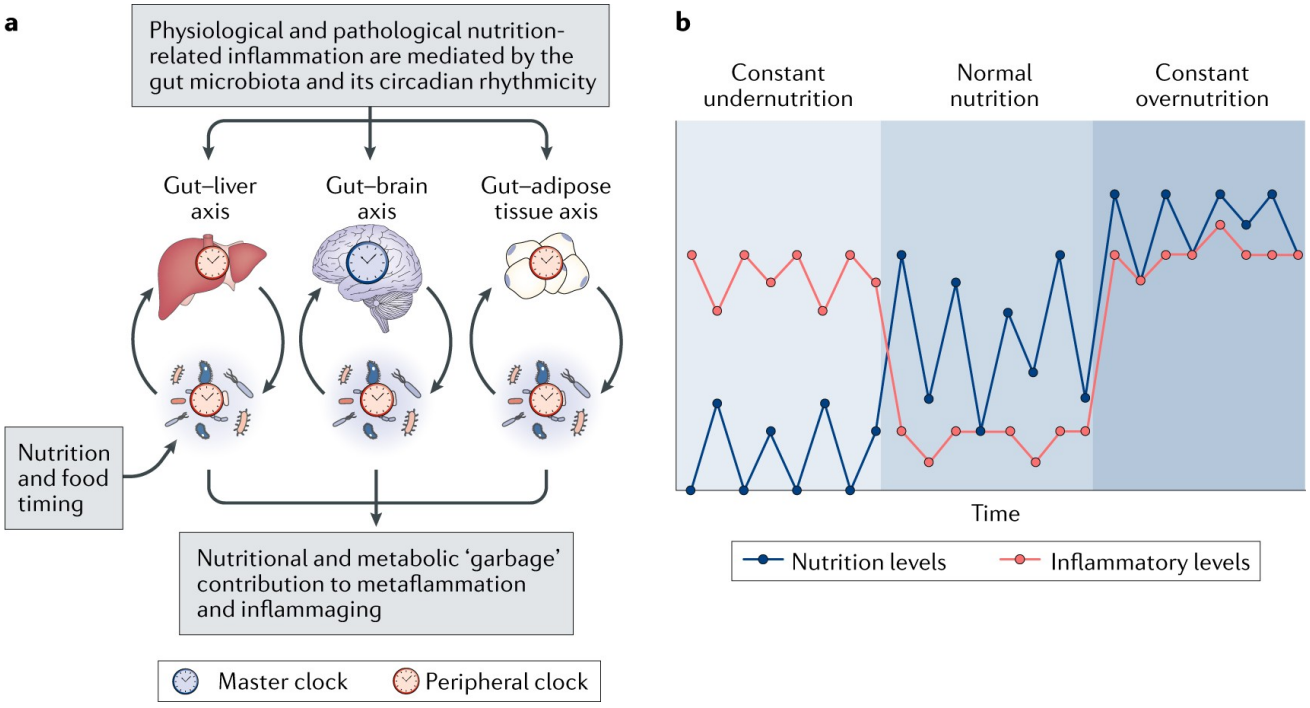
Asher G, Sassone-Corsi P.
Time for food: the intimate interplay between nutrition, metabolism, and the circadian clock.
Cell. 2015;161(1):84-92. doi:10.1016/j.cell.2015.03.015
https://www.sciencedirect.com/science/article/pii/S0092867415003025

Leo V, et al.
Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism.
Cell Host Microbe. 2015;17(5):681-689. doi:10.1016/j.chom.2015.03.006
https://www.sciencedirect.com/science/article/pii/S1931312815001237

Voigt-Zuwala, Robin & Forsyth, Christopher & Green, S.J. & Engen, Phillip & Keshavarzian, A (2016). Circadian Rhythm and the Gut Microbiome.
10.1016/bs.irn.2016.07.002.
https://www.researchgate.net/publication/308007124_Circadian_Rhythm_and_the_Gut_Microbiome

Asher G, Schibler U.
Crosstalk between components of circadian and metabolic cycles in mammals.
Cell Metab. 2011;13(2):125-137. doi:10.1016/j.cmet.2011.01.006
https://www.sciencedirect.com/science/article/pii/S1550413111000076

Mukherji A, Kobiita A, Ye T, Chambon P.
Homeostasis in intestinal epithelium is orchestrated by the circadian clock and microbiota cues transduced by TLRs.
Cell. 2013;153(4):812-827. doi:10.1016/j.cell.2013.04.020
https://www.sciencedirect.com/science/article/pii/S0092867413004625



Taken from
https://www.semanticscholar.org/paper/Inflammaging%3A-a-new-immune%E2%80%93metabolic-viewpoint-for-Franceschi-Garagnani/6247ca2e33d38d072f1f7196e156b43975d00e71
The gut microbiota as a key modulator of nutrition and inflammation. The gut microbiota transforms environmental signals and food molecules into metabolite signals to communicate with different organs and tissues in the host, mediating meal-related inflammation.
a) connections between the gut microbiota and the three metabolically important organs (liver, brain, and adipose tissue) are shown. Gut and liver form a bidirectional link called the gut-liver axis through the portal vein and bile duct. The gut microbiota also establishes a strong bidirectional connection with the central nervous system called the gut-brain axis. The gut microbiota also communicates with the adipose tissue of the host. Communication between the gut microbiota and other organs is also regulated by circadian rhythms, which are driven by a central clock located within the suprachiasmatic nuclei of the hypothalamus, and is mainly stimulated by light signals [174]. In addition, peripheral clocks are found throughout the body in peripheral organs such as the liver, intestines, and adipose tissue [175]. The feeding rhythm and gut microbiota drive the peripheral clock (circadian transcriptional regulation between organs), which in turn can contribute to the regulation of the central clock (transcript expression). Nutritional and metabolic "garbage" contributes to metaflammation and inflammation.
b) Levels of inflammation during periods of constant undernutrition (low caloric intake), normal nutrition (which has periods of feeding and fasting), and overnutrition (high caloric intake) are shown.

Gut immune responses during health and disease states are shaped by the gut microbiota, and age-related remodeling could contribute to systemic inflammation, which may directly or indirectly affect its composition. [62] [218]

In particular, changes in the gut microbiota profile in centenarians, in which there is an enrichment of Proteobacteria and a decrease in butyrate-producing bacteria, correlate with a **systemic increase in the levels of the pro-inflammatory cytokines IL-6 and IL-8**. [53].
The Proteobacteria phylum is a group containing many bacteria that have been redefined as **pathobionts**.

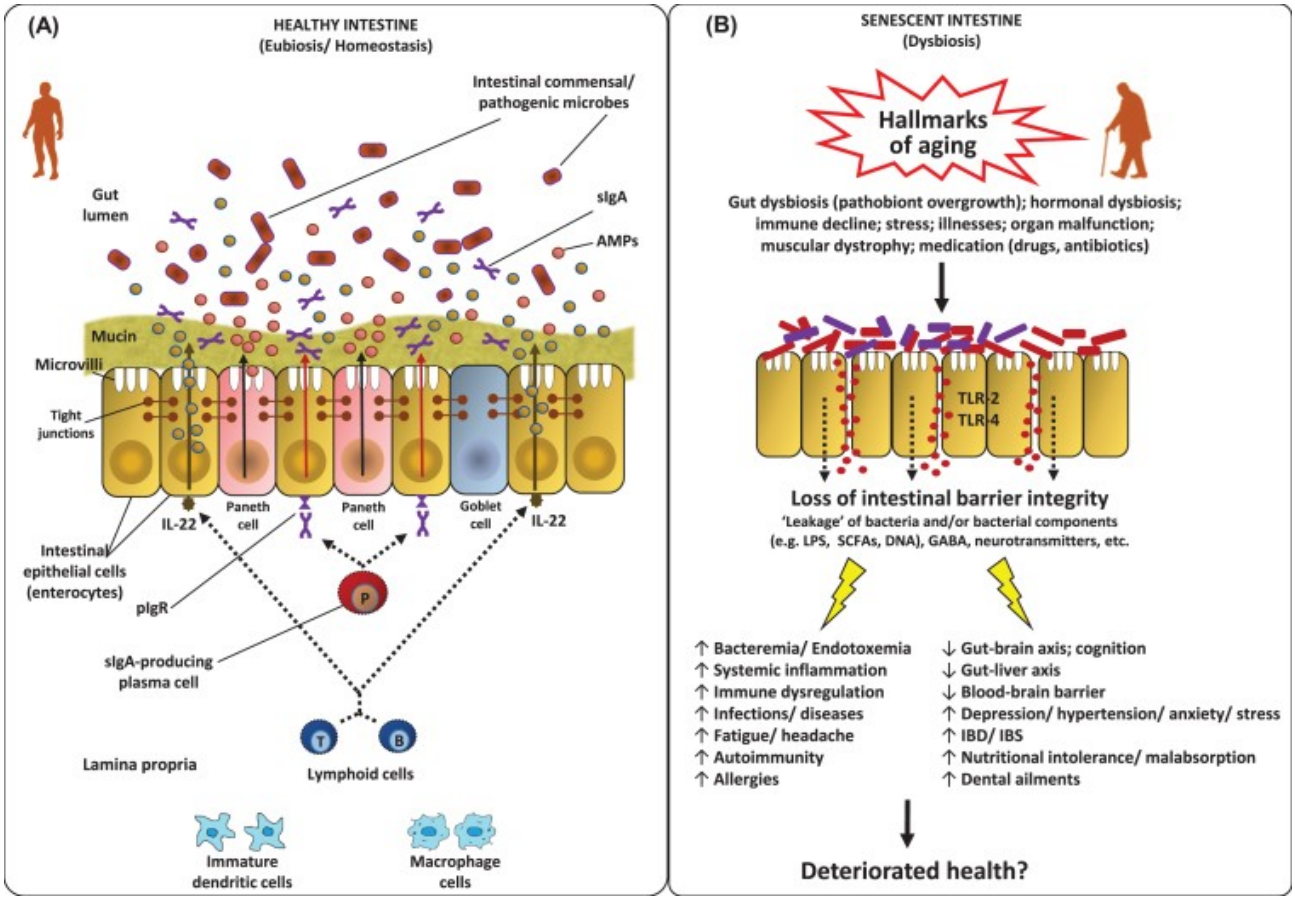
[218] Nagpal R, Mainali R, Ahmadi S, et al.
Gut microbiome and aging: Physiological and mechanistic insights.
Nutr Healthy Aging. 2018;4(4):267-285. Published 2018 Jun 15. doi:10.3233/NHA-170030
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6004897/

Pathobionts are considered minor, opportunistic components of the human gut ecosystem that, under certain circumstances (such as inflammation) can escape surveillance, outcompete mutualistic symbionts, and induce disease. Butyrate is a short-chain fatty acid that is an important source of energy for enterocytes and has been implicated in protection against inflammatory bowel disease [44]

A comprehensive phylogenetic analysis of the human gut **microbiota** of Italians (22-109 years of age) showed that **the main population of the gut microbiota** (which included the dominant symbiotic bacterial taxa of Ruminococcaceae, Lachnospiraceae, and Bacteroidaceae) **shows a decrease in diversity and relative abundance with age** [56].

In extreme longevity (> 105 years), this decline is offset by an increase in probably beneficial and subdominant species of Akkermansia spp., Bifidobacterium spp. and Christensenellaceae.
As a result, an unexpected **increase in diversity** in **gut microbiota composition** compared with younger individuals has been observed in centenarians in Italy and also in China and Japan, despite differences in genetics, lifestyle and diet among people in these countries [60].

Such an extraordinary profile of longevity, is probably a key indicator of health in centenarians and is in contrast to the characteristic decrease in gut microbiota diversity associated with most, if not all, age-related diseases [56,60].



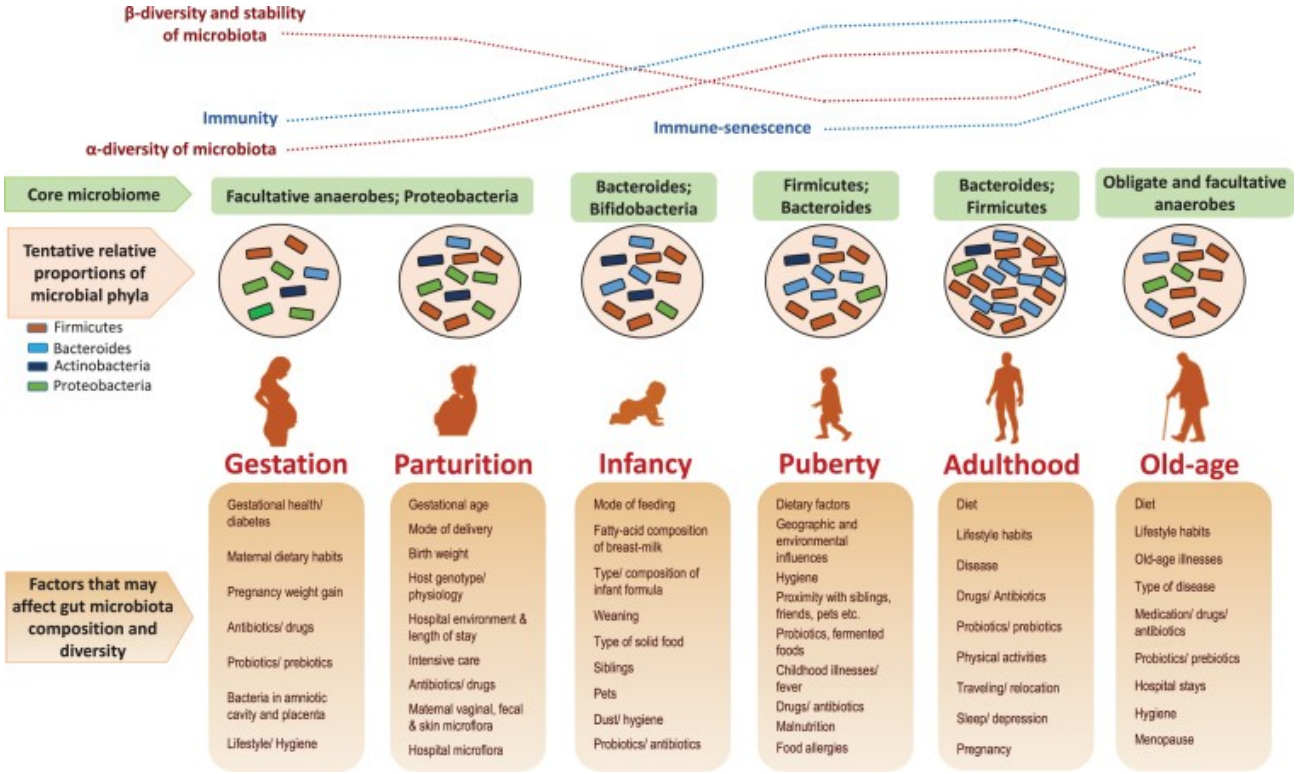
Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6004897/
Characteristics of the homeostatic gut environment (eubiosis) (A) and how a disrupted microbiota (dysbiosis) and gut barrier can cause aging-related diseases (B). Under homeostatic conditions (eubiosis), epithelial cells produce antimicrobial peptides (AMPs) in response to interleukins (e.g., IL-22) and also express receptors for molecular profiling recognition (e.g., Toll-like receptors; TLRs). Gut microbes regulate mucus secretion and AMP production and regulate/enhance intestinal barrier integrity through the production of short-chain fatty acids (SCFAs). Calyciform cells produce mucus to limit pathobiont invasion. Lymphoid cells (e.g., TH17 cells) play a role in host defense by producing IL-22. Dendritic cells (DCs) induce activation and differentiation of naïve B cells to produce plasma cells that release commensal-specific IgA into the lamina propria. IgA is transported into the intestinal lumen as secreted IgA (sIgA) through receptors (polymeric immunoglobulin receptor pIgR), where after sIgA binds to microbes

commensals and to soluble antigens, thereby limiting their adherence to the host epithelium and leakage across the intestinal barrier. However, in cases of dysbiosis and/or senescent environment, altered microbiota composition and weakened intestinal permeability
/ disrupted can lead to increased adherence and leakage of various microbes and microbial by-products across the intestinal barrier, thereby provoking hyperinflammatory responses that ultimately increase host susceptibility to various intestinal and

*The mechanism that links nutrient excess and inflammation is the so-called **meta-inflammation process (metaflammation - metabolic inflammation)**, which is a chronic, sterile low-grade inflammation sustained by high nutrient intake that alters the inflammatory and metabolic response of cells, tissues, and organs.* [219]

Metainflammation could precede and contribute to inflammation and vice versa, and age-related metabolic diseases in turn could be considered manifestations of the acceleration of aging (which in turn accelerates aging itself). [220]

*This unifying hypothesis indicates that an individual's metabolic history likely influences an individual's **immunobiography** and **inflammatory phenotype,** thus determining the risk of developing age-related chronic metabolic diseases.*



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6004897/
Characteristics of the homeostatic gut environment (eubiosis) (A) and how a disrupted microbiota (dysbiosis) and gut barrier can cause aging-related diseases (B). Under homeostatic conditions (eubiosis), epithelial cells produce antimicrobial peptides (AMPs) in response to interleukins (e.g., IL-22) and also express receptors for molecular profiling recognition (e.g., Toll-like receptors; TLRs). Gut microbes regulate mucus secretion and AMP production and

[219] Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A.
Inflammaging: a new immune-metabolic viewpoint for age-related diseases.
Nat Rev Endocrinol. 2018;14(10):576-590. doi:10.1038/s41574-018-0059-4
https://pubmed.ncbi.nlm.nih.gov/30046148/

[220] Prattichizzo F, et al.
Inflammageing and metaflammation: The yin and yang of type 2 diabetes.
Ageing Res Rev. 2018;41:1-17. doi:10.1016/j.arr.2017.10.003
https://pubmed.ncbi.nlm.nih.gov/29081381/

They regulate/improve the integrity of the intestinal barrier through the production of short-chain fatty acids (SCFA). Calyciform cells produce mucus to limit pathobiont invasion. Lymphoid cells (e.g., TH17 cells) play a role in host defense by producing IL-22. Dendritic cells (DCs) induce activation and differentiation of naïve B cells to produce plasma cells that release commensal-specific IgA into the lamina propria. IgA is transported into the intestinal lumen as secreted IgA (sIgA) through receptors (polymeric immunoglobulin receptor pIgR), where after sIgA binds to commensal microbes and soluble antigens, thereby limiting their adherence to the host epithelium and leakage across the intestinal barrier. However, in cases of dysbiosis and/or senescent environment, altered microbiota composition and weakened intestinal permeability
/ disrupted can lead to increased adherence and leakage of various microbes and microbial by-products across the intestinal barrier, thereby provoking hyperinflammatory responses that eventually increase host susceptibility to various intestinal and systemic disorders through perturbations of the brain-gut axis, liver-gut axis, etc.

Microbiota and COVID-19 [221]

As discussed above, signals derived from the gut microbiota direct the pro- and anti-inflammatory responses of immune cells, thus influencing susceptibility to various diseases [222].
The homeostasis of the gut immune system is regulated of the balance between the pro-inflammatory responses of Th17 and those of regulatory T cells (Tregs), which in turn is controlled by commensal microorganisms [223].

To overcome an infection such as coronavirus infection, ***it is critical to maintain a healthy gut microbiome so*** *that the immune system can respond optimally to prevent a series of potentially damaging immune overreactions to the lungs and other vital organs as occurs in severe complications from Covid-19.*

The older segment of the population is more susceptible to complications from COVID-19 also due to a ***higher frequency of gut dysbiosis*** *due to aging and/or continued use of microbiota-modifying drugs and improper diet,[224] particularly in the community-dwelling long-term care elderly. [225]*

Study of the fecal microbiome of patients hospitalized for COVID-19 showed that significant alterations were present compared with controls, characterized by **enrichment of opportunistic pathogens and depletion of beneficial commensals**, at the time of hospitalization and throughout its duration.

---

[221] Dhar D, Mohanty A.
Gut microbiota and Covid-19- possible link and implications.
Virus Res. 2020;285:198018. doi:10.1016/j.virusres.2020.198018
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7217790/

[222] Negi S, Das DK, Pahari S, Nadeem S, Agrewala JN.
Potential Role of Gut Microbiota in Induction and Regulation of Innate Immune Memory.
Front Immunol. 2019;10:2441. doi:10.3389/fimmu.2019.02441
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6842962/

[223] Round JL, O'Connell RM, Mazmanian SK.
Coordination of tolerogenic immune responses by the commensal microbiota.
J Autoimmun. 2010;34(3):J220-J225. doi:10.1016/j.jaut.2009.11.007
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3155383/

[224] Kim S, Jazwinski SM.
The Gut Microbiota and Healthy Aging: A Mini-Review.
Gerontology. 2018;64(6):513-520. doi:10.1159/000490615
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6191326/

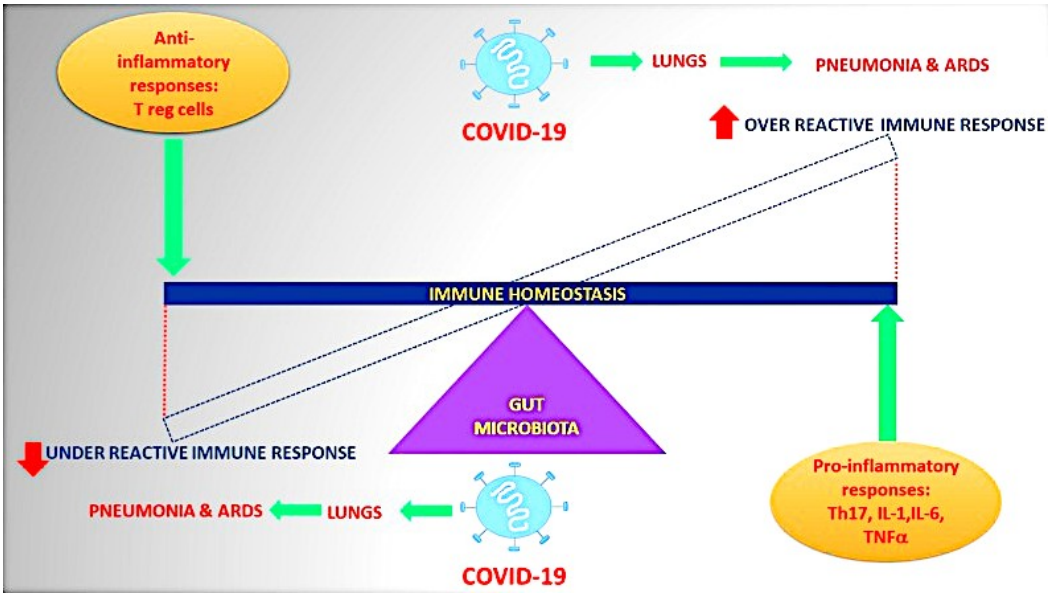
Nagpal R, et al.
Gut microbiome and aging: Physiological and mechanistic insights.
Nutr Healthy Aging. 2018;4(4):267-285. doi:10.3233/NHA-170030
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6004897/

[225] Araos R, Andreatos N, Ugalde J, Mitchell S, Mylonakis E, D'Agata EMC.
Fecal Microbiome Among Nursing Home Residents with Advanced Dementia and Clostridium difficile.
Dig Dis Sci. 2018;63(6):1525-1531. doi:10.1007/s10620-018-5030-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6434537/

Symbiont depletion and gut dysbiosis persisted even a f t e r SARS-CoV-2 elimination (determined by pharyngeal swabs) and resolution of respiratory symptoms.

The abundance of Coprobacillus, Clostridium ramosum, and Clostridium hathewayi was correlated with the severity of COVID-19, while there was an inverse correlation between the abundance of Faecalibacterium prausnitzii (an anti-inflammatory bacterium) and disease severity.

Over the course of hospitalization, Bacteroides dorei, Bacteroides thetaiotaomicron, Bacteroides massiliensis, and Bacteroides ovatus, which downregulate ACE2 expression in the murine intestine, were inversely correlated with SARS- CoV-2 viral load in fecal samples from patients. [226]



Taken from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7217790/
Possible role of gut microbiota in modulating the immune response in Covid-19.
The gut microbiota may act on the immune response thereby influencing disease progression. Both overactive and hypoactive immune response, probably mediated by the gut microbiota, can lead to serious clinical adverse events.

[226] Zuo T, Zhang F, et al.
Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization
Gastroenterology. 2020;S0016-5085(20)34701-6. doi:10.1053/j.gastro.2020.05.048
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7237927/

Gender difference

Data to date show that the entire population with SARS-CoV2 consists **of 58% male subjects**.[227] The difference in the number of reported cases by gender increases progressively in favor of male subjects up to the ≥60-69 (66.6%) and ≥70-79 (66.1%) age group, with the exception of the 20-29 years and the 30-39 years group where the number of female subjects is slightly higher.

Higher lethality is in favor of male subjects in all age groups. Deaths among 30-39 year olds are 82.4% male; 40-49 year olds are 73.1% male; 50-59 year olds are 78.5% male; 60-69 year olds are 79.7% male; 70-79 year olds are 79.6% male; and 80-89 year olds are 66.9% male. [228]

*A **higher male risk predisposition** occurs when weak immune responses underlie significant tissue damage by viral infection, while a **higher female risk predisposition** occurs when strong immune responses promote tissue damage.*

It has been seen that in the case of coronavirus infections, **estrogens have a protective effect**, as they increase the initial production of type I interferons with antiviral activity necessary to block virus replication, and stimulate type 2 response-mediated repair activity, while **androgens have immunosuppressive effects**.

It follows that in men the poor immune response results in greater severe tissue damage.

Immune regulatory genes encoded by the X chromosome in the female gender cause lower viral load levels and less inflammation than in men, while $^{CD4+}$ T cells are higher with a better immune response. In addition, women generally produce higher levels of antibodies that remain longer in the circulation.

Levels of immune cell activation are higher in women than in men, and this is related to TLR7 stimulation* and IFN production.

** TLR7 is expressed in innate immune cells that recognize single-stranded RNA viruses and promote the production of antibodies against the virus and the generation of pro-inflammatory cytokines including members of IL-6 and IL-1.*

---

[227] Zhou F., Yu T., Du R.
Clinical course and risk factors for mortality of adults in patients with COVID-19 in Wuhan, China: a retrospective cohort study.
Lancet. 2020 doi: 10.1016/S0140-6736(20)30566-3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7270627/

Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention.
Zhonghua Liu Xing Bing Xue Za Zhi. 2020;41(2):145-151. doi:10.3760/cma.j.issn.0254-6450.2020.02.003
https://pubmed.ncbi.nlm.nih.gov/32064853/

Guan W., Liang W., Zhao Y.
Comorbidity and its impact on 1590 patients with Covid-19 in China: a nationwide analysis.
Eur Respir J. 2020 doi: 10.1183/13993003.00547-2020.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7098485/

[228] Walter L.A., McGregor A.J..
Sex- and gender-specific observations and implications for COVID-19.
West J Emerg Med. 2020 doi: 10.5811/westjem.2020.4.47536.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7234726/

Pozzilli P, Lenzi A.
Commentary: Testosterone, a key hormone in the context of COVID-19 pandemic
Metabolism. 2020;108:154252. doi:10.1016/j.metabol.2020.154252
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7185012/

In women, inflammatory **IL-6** production after viral infection is lower than in men and is often correlated with better longevity.
TLR7 is higher in women than in men, and its biallelic (i.e., due to both X chromosomes) expression leads to higher immune responses and increases resistance to viral infections.

In addition, there are loci on the X chromosome that code for genes involved in immune cell regulation such as FOXP3 and transcription factors for Treg involved in virus pathogenesis.
The **X chromosome** influences the immune system by acting on many other proteins, including TLR8, CD40L, and CXCR3, which can be overexpressed in women and affect the response to viral infections and vaccinations.

However, biallelic expression of X-linked genes can also promote harmful autoimmune and inflammatory responses.[229]

Sexual difference or sexual dimorphism in immunity (particularly autoimmunity) is influenced by the gut microbiota.
Sex differences in the gut microbiome are partially driven by sex hormones, which in turn contribute to sex differences in immunity and susceptibility to a multitude of infections and chronic diseases. [230]

*The **microgenderome** defines the interaction between the microbiota, sex hormones, and the immune system and involves bidirectional interactions between the microbiota, hormones, immunity, and susceptibility to disease.* [231] *The growing number of microbiome studies are revealing bidirectional activity between the microbiota and the endocrine system in which bacteria are able to produce hormones (e.g., serotonin, dopamine, and somatostatin), respond to host hormones (e.g., estrogen), and regulate the homeostasis of host hormones by inhibiting their gene transcription (e.g., prolactin) or converting them (e.g., glucocorticoids to androgens)* [232].

Specifically**, bacteria metabolize sex steroids through hydroxysteroid dehydrogenase** (HSD), which affects the balance between active and inactive steroids. Genes for HSD are encoded in the genomes of Actinobacteria, Proteobacteria and Firmicutes, which colonize the human gastrointestinal [tract233].

---

[229] J Biol Regul Homeost Agents. 2020 Apr 7;34(2). doi: 10.23812/Editorial-Conti-3.
Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection.
Conti P1, Younes A2.
https://pubmed.ncbi.nlm.nih.gov/32253888/

Fish EN.
The X-files in immunity: sex-based differences predispose immune responses.
Nat Rev Immunol. 2008;8(9):737-744. doi:10.1038/nri2394
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7097214/

[230] Galligan C.L., Fish E.N. (2015) Sex Differences in the Immune Response. In: Klein S., Roberts C. (eds)
Sex and Gender Differences in Infection and Treatments for Infectious Diseases. Springer, Cham

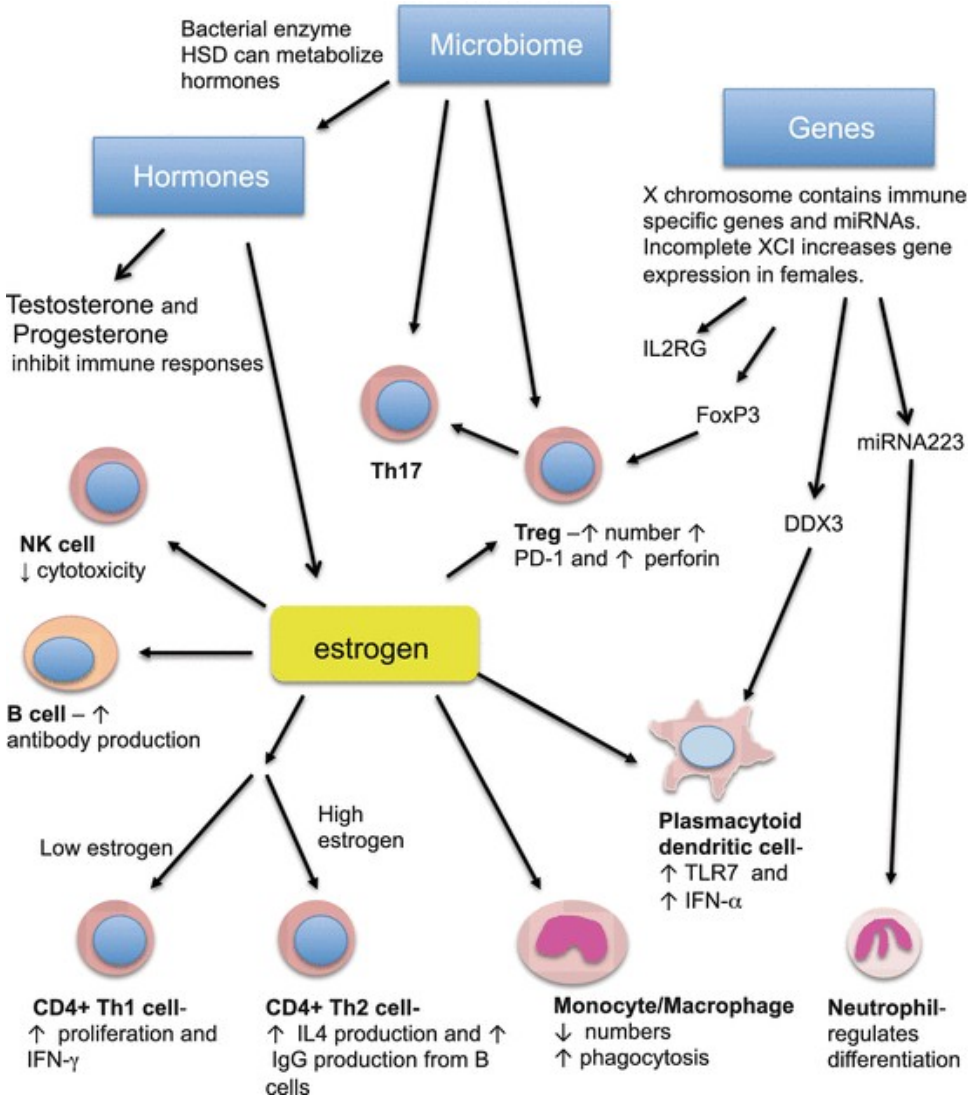
[231] Elderman M, de Vos P, Faas M.
Role of Microbiota in Sexually Dimorphic Immunity.
Front Immunol. 2018;9:1018. doi:10.3389/fimmu.2018.01018
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5992421/

[232] Neuman H, Debelius JW, Knight R, Koren O.
Microbial endocrinology: the interplay between the microbiota and the endocrine system.
FEMS Microbiol Rev. 2015;39(4):509-521. doi:10.1093/femsre/fuu010
https://academic.oup.com/femsre/article/39/4/509/2467625

[233] Vemuri R, Sylvia KE, Klein SL, et al.
The microgenderome revealed: sex differences in bidirectional interactions between the microbiota, hormones, immunity and disease susceptibility.
Semin Immunopathol. 2019;41(2):265-275. doi:10.1007/s00281-018-0716-7
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6500089/

Ma ZS, Li W.
How and Why Men and Women Differ in Their Microbiomes: Medical Ecology and Network Analyses of the Microgenderome. A

It should be mentioned that in vivo studies in mice have shown that the composition of the microbiota before and after puberty was not different in females, while the composition deviated after puberty in males, suggesting that **male sex hormones may play an important role in sexual differences in the gut microbiota**. [234]



Tratto da https://link.springer.com/chapter/10.1007/978-3-319-16438-0_1
Specific sex differences influence the immune response. Hormones, genes, and the microbiome all influence the immune response. Estrogen has direct effects on immune cells. Bacterial hydroxysteroid dehydrogenase (HSD), microRNA (miRNA)

dv Sci (Weinh). 2019;6(23):1902054. doi:10.1002/advs.201902054
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6891928/

Rizzetto L, Fava F, Tuohy KM, Selmi C.
Connecting the immune system, systemic chronic inflammation and the gut microbiome: The role of sex.
J Autoimmun. 2018;92:12-34. doi:10.1016/j.jaut.2018.05.008
https://pubmed.ncbi.nlm.nih.gov/29861127/

Kisiela M, Skarka A, Ebert B, Maser E.
Hydroxysteroid dehydrogenases (HSDs) in bacteria: a bioinformatic perspective.
J Steroid Biochem Mol Biol. 2012;129(1-2):31-46. doi:10.1016/j.jsbmb.2011.08.002
https://pubmed.ncbi.nlm.nih.gov/21884790/

[234] Kim YS, Unno T, Kim BY, Park MS.
Sex Differences in Gut Microbiota.
World J Mens Health. 2020;38(1):48-60. doi:10.5534/wjmh.190009
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6920072/

It is important to remember that the ACE2 (angiotensin-converting enzyme 2) TMPRSS2 (transmembrane serine protease 2) receptors to which SARS-Cov-2 binds in order to enter cells are both predominantly distributed in intestinal tissues [235] *and* ***TMPRSS2 has a particular tropism for prostate tissue****, a feature that could explain the* ***higher incidence of severe forms of COVID-19 for the male gender, with the regulation by sex hormones of the cytokine storm*** [236].

[235] EXPRESSION OF SARS-COV-2 RECEPTOR
https://nebion.com/resources/covid-19/

https://www.proteinatlas.org/ENSG00000130234-ACE2/tissue
https://www.proteinatlas.org/ENSG00000184012-TMPRSS2/tissue

Int J Oral Sci. 2020 Feb 24;12(1):8. doi: 10.1038/s41368-020-0074-x.
High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa.
Xu H1, Zhong L1, Deng J1, Peng J1, Dan H1, Zeng X1, Li T2, Chen Q1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7039956/

Cancer Discov. 2020 Apr 10. pii: CD-20-0451. doi: 10.1158/2159-8290.CD-20-0451.
TMPRSS2 and COVID-19: Serendipity or opportunity for intervention?
Stopsack KH1, Mucci LA2, Antonarakis ES3, Nelson PS4, Kantoff PW
https://cancerdiscovery.aacrjournals.org/content/10/6/779.full-text.pdf

[236] Gender differences in COVID-19: possible mechanisms
https://www.epicentro.iss.it/coronavirus/sars-cov-2-differenze-genere

Ital J Gender-Specific Med 2020; 6(2): 49-50 DOI 10.1723/3351.33219
Gender differences in COVID-19: some open questions
Anna Ruggieri, Maria Cristina Gagliardi
https://www.gendermedjournal.it/articoli.php?archivio=yes&vol_id=3351&id=33219

Scientific World Journal. 2014;2014:159150. doi:10.1155/2014/159150
Sex hormones and immune dimorphism.
Bhatia A, Sekhon HK, Kaur G.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4251360/

Autoimmun Rev. 2020;102571. doi:10.1016/j.autrev.2020.102571
Transcriptional landscape of SARS-CoV-2 infection dismantles pathogenic pathways activated by the virus, proposes unique sex-specific differences and predicts tailored therapeutic strategies
Fagone P, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7252184/

Front Immunol. 2018 Jul 20;9:1653. doi: 10.3389/fimmu.2018.01653.
Sex Hormones Regulate Innate Immune Cells and Promote Sex Differences in Respiratory Virus Infection.
Kadel S1,2, Kovats S1,2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6062604/

PLoS Pathog. 2016 Feb 18;12(2): e1005374. doi: 10.1371/journal.ppat.1005374.
SeXX Matters in Infectious Disease Pathogenesis.
vom Steeg LG1, Klein SL1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4759457/

J Allergy Clin Immunol. 2013 Dec;132(6):1263-76; quiz 1277. doi: 10.1016/j.jaci.2013.06.006. epub 2013 Aug 1.
Viral infection of the lung: host response and sequelae.
Yoo JK1, Kim TS, Hufford MM, Braciale TJ.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3844062/.

Semin Immunopathol. 2016 Jul;38(4):471-82. doi: 10.1007/s00281-016-0558-0.
The host immune response in respiratory virus infection: balancing virus clearance and immunopathology.
Newton AH1,2, Cardani A1,3, Braciale TJ
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4896975/

Androgenic hyperstimulation due to activation of the TMPRSS2 receptor has been noted in men, leading to to thinning hair, increased body hair and acne. [237]
Viral infection in male reproductive organ tissues due to the presence of the ACE2 receptor has been preliminarily associated with **hypogonadism due to increased luteinizing hormone**. [238]

Androgen sensitivity may be an important factor in disease severity, and would also explain severe cases in female patients who have metabolic syndrome or use birth control methods with progestin hormones that bind to the androgen receptor.

Several studies have shown that androgen sensitivity is associated with the CAG triplet repeat in the first exon of the androgen receptor (AR) gene.
A shorter CAG triplet repeat predisposes men to develop androgenetic alopecia, acne, and oily skin, and this could be associated with increased severity and mortality of COVID-19 disease.

An interesting observation supporting this hypothesis is the **particularly high mortality rate observed in African American COVID-19 patients, who** as an ethnic group tend to carry a shorter version of the CAG repeat in the androgen receptor gene. [239]

This theory also supports the low incidence of the disease in males up to puberty, where testosterone levels are very low. [240]
However, it should be remembered that COVID-19 is a multifactorial syndrome and mortality is related to numerous individual, environmental and social factors.

J Immunol. 2017 May 15;198(10):4046-4053. doi: 10.4049/jimmunol.1601896.
Sex-Based Differences in Susceptibility to Severe Acute Respiratory Syndrome Coronavirus Infection.
Channappanavar R1, Fett C1, Mack M2, Ten Eyck PP3, Meyerholz DK4, Perlman S5.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5450662/

[237] J Am Acad Dermatol. 2020 Apr 10. pii: S0190-9622(20)30608-3.
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is likely to be androgen mediated
Wambier CG1, Goren A2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7151476/

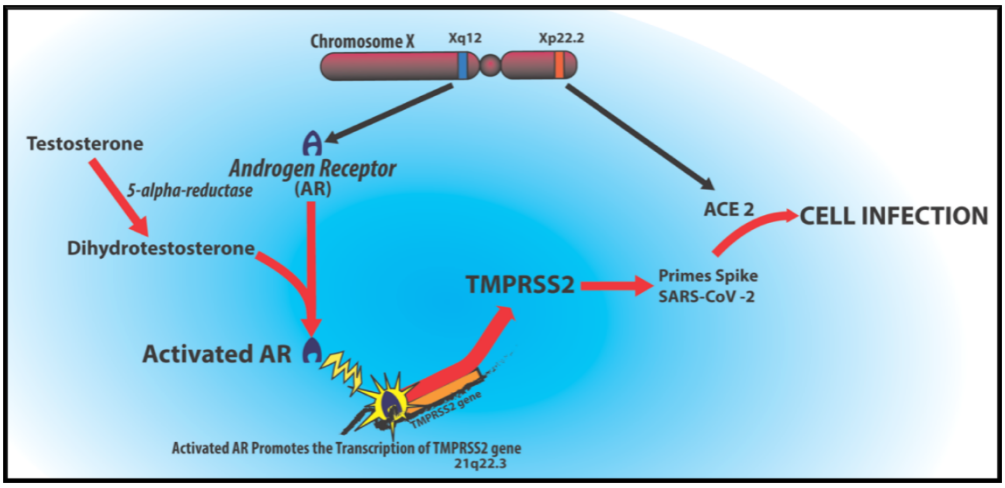
J Cosmet Dermatol. 2020 Apr 16. doi: 10.1111/jocd.13443.
A preliminary observation: male pattern hair loss among hospitalized COVID-19 patients in Spain - A potential clue to the role of androgens in COVID-19 severity.
Goren A et al
https://onlinelibrary.wiley.com/doi/epdf/10.1111/jocd.13443

[238] medRxiv 2020.03.21.20037267 doi.org/10.1101/2020.03.21.20037267
Effect of SARS-CoV-2 infection upon male gonadal function: A single center-based study
Ling Ma, Wen Xie, Danyang Li, Lei Shi, Yanhong Mao, Yao Xiong, Yuanzhen Zhang, Ming Zhang
https://www.medrxiv.org/content/10.1101/2020.03.21.20037267v1.full.pdf
[239] Ann Epidemiol. 2020;10.1016/j.annepidem.2020.05.003. doi:10.1016/j.annepidem.2020.05.003
Assessing Differential Impacts of COVID-19 on Black Communities
Millett GA, Jones AT, Benkeser D, et al.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7224670/

JAMA. 2020;323(19):1891–1892. doi:10.1001/jama.2020.6548
COVID-19 and African Americans.
Yancy CW.
https://jamanetwork.com/journals/jama/fullarticle/2764789

Bennett CL, Price DK, Kim S, et al.
Racial variation in CAG repeat lengths within the androgen receptor gene among prostate cancer patients of lower socioeconomic status.
J Clin Oncol. 2002;20(17):3599-3604. doi:10.1200/JCO.2002.11.085
https://pubmed.ncbi.nlm.nih.gov/12202660/

[240] Wambier, Carlos & Goren, Andy & Ossimetha, Angelina & Nau, Gerard & Qureshi, Abrar.
(2020). Androgen-driven COVID-19 pandemic theory. 10.13140/RG.2.2.21254.11848.
https://www.researchgate.net/publication/340548509_Androgen-driven_COVID-19_pandemic_theory



Taken from https://www.researchgate.net/publication/340548509_Androgen-driven_COVID-19_pandemic_theory
Theoretical limiting role of androgens in CoVID-19 infection. Red arrows show the pathway of SARS- CoV-2 infection mediated by androgen activity. Dihydrotestosterone (DHT) is the most potent androgenic hormone and requires intracellular 5- alpha-reductase activity. Testosterone is considered the main androgenic hormone, which activates the androgen receptor with less affinity than DHT in cells not expressing 5-alpha-reductase.

THE AUTOIMMUNE/INFLAMMATORY SYNDROME

An immune system can be considered effective only when it can identify and destroy pathogen-infected cells, while distinguishing these cells from healthy ones. If immune tolerance is broken, the immune system cannot discriminate between self-antigens and foreign antigens, and the manifestation of **autoimmune disease**, i.e., **unwanted destruction of healthy cells, occurs**.

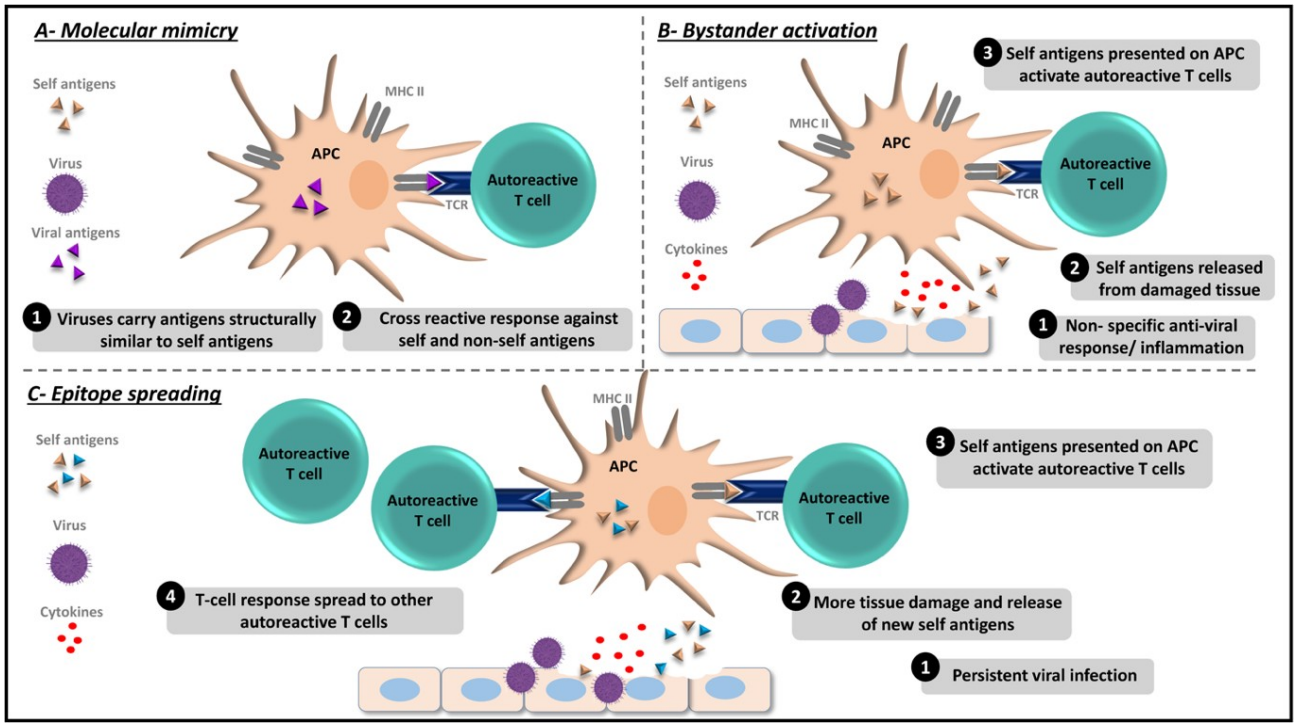
Under normal conditions, foreign epitopes are presented on antigen-presenting cells (APCs) to T cells, and this results in proliferation of T cells and induction of their effector function.

While this mechanism is responsible for proper clearance of infection, ***cross-reactivity between epitopes associated with foreign and self antigens can lead to a T-cell response against healthy host cells****.*

Although ***genetic predisposition*** *is known* ***to*** *play a very significant role, it is believed that some additional* ***environmental triggers*** *are necessary for the onset of autoimmunity, and these are usually represented by* ***infections****.* [241]

[241] Committee to Review Adverse Effects of Vaccines; Institute of Medicine; Stratton K, Ford A, Rusch E, et al, editors. Adverse Effects of Vaccines: Evidence and Causality. Washington (DC): National Academies Press (US); 2011 Aug 25. 3, Evaluating Biological Mechanisms of Adverse Events. Available from: https://www.ncbi.nlm.nih.gov/books/NBK190017/

Smatti MK, Cyprian FS, Nasrallah GK, Al Thani AA, Almishal RO, Yassine HM.
Viruses and Autoimmunity: A Review on the Potential Interaction and Molecular Mechanisms.
Viruses. 2019;11(8):762. doi:10.3390/v11080762
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6723519/



Taken from https://www.mdpi.com/1999-4915/11/8/762/htm
Mechanisms of virus-induced autoimmunity.
(A) Molecular mimicry model: (1) Viruses carry epitopes that are structurally similar to self-epitopes. (2) Presentation of viral epitopes by antigen-presenting cells (APCs) activates autoreactive T cells that bind to both self and non-self antigens and induce tissue damage.
(B) "Bystander" activation model: (1) Nonspecific, hyper-reactive antiviral immune responses lead to release of self-antigens and release of inflammatory cytokines from damaged tissue. (2) Self antigen is captured and presented by APCs. (3) Self-reactive T cells activated by APCs, lead to tissue destruction.
(C) Epitope spreading pattern: (1) Persistent viral infection. (2) Continuous tissue damage and release of new antigens. (3) Autoantigens are detected and presented by APCs. (4) Nonspecific activation of more autoreactive T cells leading to autoimmunity.

The **presence of autoantibodies** in patients who developed Covid-19 caused by molecular mimicry between autoantigens and SARS-CoV-2 proteins may suggest that an **autoimmune/inflammatory mechanism** may be an additional event determining the severity of the disease. [242]

[242] Autoimmun Rev. 2020 Mar 24:102524. doi: 10.1016/j.autrev.2020.102524.
Could Sars-coronavirus-2 trigger autoimmune and/or autoinflammatory mechanisms in genetically predisposed subjects?
Case F1, Costa L2, Ruscitti P3, Navarini L4, Del Puente A2, Giacomelli R3, Scarpa R2.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7271072/

Shoenfeld Y.
Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning.
Autoimmun Rev. 2020;19(6):102538. doi:10.1016/j.autrev.2020.102538
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7131471/

Clin Exp Immunol. 2005 Sep;141(3):500-8.
Antibody to severe acute respiratory syndrome (SARS)-associated coronavirus spike protein domain 2 cross-reacts with lung epithelial cells and causes cytotoxicity.
Lin YS et al
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1809466/

Yonggang Zhou, et al
Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients,
National Science Review, , nwaa041, https://doi.org/10.1093/nsr/nwaa041
https://academic.oup.com/nsr/advance-article/doi/10.1093/nsr/nwaa041/5804736

J Transl Autoimmun. 2020; 3: 100051.10.1016/j.jtauto.2020.100051
Pathogenic priming likely contributes to serious and critical illness and mortality in COVID-19 via autoimmunity
James Lyons-Weiler
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7142689/

In a retrospective study, the clinical, autoimmune, and laboratory characteristics of 21 patients who had had laboratory-confirmed severe and critical cases of coronavirus disease 2019 (COVID-19) from the intensive care unit of Huangshi Central Hospital, Hubei Province, China, were analyzed. [243]

The prevalence of anti - 52 kDa SSA/Ro antibody, anti - 60 kDa SSA/Ro antibody, and antinuclear antibody was 20%, 25%, and 50%, respectively, which confirms that **autoimmune phenomena exist in COVID - 19 subjects** and that the present findings call for a strategy of prevention of immune dysfunction and optimal immunosuppressive therapy.

Other researchers also tested the serum of ten COVID-19 patients for rheumatoid factor (RF), anti-cyclic peptide containing anti-citrulline antibody (anti-CCP antibody) and anti-neutrophil cytoplasmic antibody (ANCA), which are indices of immune rheumatic disease, in the serum of ten COVID-19 patients.
Results showed that anti-CCP antibody was increased in two patients, while RF and ANCA were negative in all subjects. [244]

Of note, the research group of Caso F. et al. showed that **the radiological aspects of lung involvement in COVID-19, resemble the characterization of pneumonia from autoimmune diseases**, such as rheumatoid arthritis (RA), systemic sclerosis, and eosinophilic granulomatosis with polyangiitis [245].

The similarity of the clinical manifestations of CODIV-19 with autoimmune syndromes noted by rheumatologists supports this mechanism, as shown in the following table: [246]

Table 1 Manifestations associated with coronavirus disease 19 (COVID-19) mimicking rheumatic syndromes

1. Arthralgias and Myalgias
2. Cytopenias: leucopenia (predominantly lymphopenia); thrombocytopenia
3. Acute interstitial pneumonia-like presentation
4. Myocarditis
5. Secondary hemophagocytic lymphohistiocytosis and cytokine storm
6. Possible greater risk of venous thromboembolism

Tratta da https://link.springer.com/content/pdf/10.1007/s10067-020-05073-9.pdf

J Biomed Biotechnol. 2008;2008:326464.
Peptide mimicrying between SARS coronavirus spike protein and human proteins reacts with SARS patient serum.
Hwa KY1, Lin WM, Hou YI, Yeh TM.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2254527/

[243] Zhou Y, et al.
Clinical and Autoimmune Characteristics of Severe and Critical Cases of COVID-19
Clin Transl Sci. 2020;10.1111/cts.12805. doi:10.1111/cts.12805
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7264560/

[244] Gao ZW, Wang X, Lin F, Dong K.
The correlation between SARS-CoV-2 infection and rheumatic disease.
Autoimmun Rev. 2020;19(7):102557. doi:10.1016/j.autrev.2020.102557
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7194066/

[245] F. Case, et al.
Could Sars-coronavirus-2 trigger autoimmune and/or autoinflammatory mechanisms in genetically predisposed subjects?
Autoimmun. Rev., 19 (5) (2020), p. 102524, 10.1016/j.autrev.2020.102524
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7271072/

[246] Misra DP, Agarwal V, Gasparyan AY, Zimba O.
Rheumatologists' perspective on coronavirus disease 19 (COVID-19) and potential therapeutic targets
Clin Rheumatol. 2020;1-8. doi:10.1007/s10067-020-05073-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7145936/

The existence of autoantibodies suggests ***an increased risk of autoimmune diseases*** *in some patients with COVID-19, and* ***further studies*** *in larger groups of patients and long-term* ***studies are certainly recommended*** *to clarify these observations.*

It has also been suggested that **molecular mimicry between human coronavirus (HCV) 229E and the myelin basic protein of** the central nervous system could contribute to the **pathogenesis of multiple sclerosis**, as self-reactive T cells specific for myelin components capable of cross-reacting with HCV 229E have been isolated and coronavirus particles have been found in patients with multiple sclerosis.

Because SARS-Cov-2 virus (and the vaccine) also exhibits such mimicry, due to its high sequence homology with HCV 229E, it is important to assess its consequences both following infection and vaccination as a potential adverse reaction. [247]

In a recent study, Prof. Kanduc and Prof. Shoenfeld sought to answer, through a bioinformatics proteomics study, the question of why SARS-CoV-2 so aggressively attacks the respiratory system.

Their results report **extensive peptide sharing between the SARS-CoV-2 spike glycoprotein and lung surfactant-related proteins** (13 out of 24 peptides).

Analyses using the Immune Epitope DataBase (IEDB) resource show that many shared peptides have immunological potential, suggesting **that immune responses following SARS-CoV-2 infection could**

---

[247] Lancet. 2003 Jun 14;361(9374):2081.
Severe acute respiratory syndrome coronavirus and viral mimicry.
Chew FT, Ong SY, Hew CL.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7135708/

Ann Neurol. 1996 Feb;39(2):233-40.
Myelin basic protein and human coronavirus 229E cross-reactive T cells in multiple sclerosis.
Talbot PJ1, Paquette JS, Ciurli C, Antel JP, Ouellet F.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159680/

Science. 1980 Aug 22;209(4459):933-4.
Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients.
Burks JS, DeVald BL, Jankovsky LD, Gerdes JC.
https://pubmed.ncbi.nlm.nih.gov/7403860/

Viruses. 2019 Dec 20;12(1). pii: E14. doi: 10.3390/v12010014.
Human Coronaviruses and Other Respiratory Viruses: Underestimated Opportunistic Pathogens of the Central Nervous System?
Desforges M1, Le Coupanec A1, Dubeau P1, Bourgouin A1, Lajoie L2, Dubé M1,3, Talbot PJ1.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7020001/

J Virol. 2018 Aug 16;92(17). pii: e00404-18. doi: 10.1128/JVI.00404-18. Print 2018 Sep 1.
Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43.
Dubé M#1, Le Coupanec A#1, Wong AHM2,3, Rini JM2,3, Desforges M4, Talbot PJ4.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6096804/

Adv Exp Med Biol. 2014;807:75-96. doi: 10.1007/978-81-322-1777-0_6.
Neuroinvasive and neurotropic human respiratory coronaviruses: potential neurovirulent agents in humans.
Desforges M1, Le Coupanec A, Brison E, Meessen-Pinard M, Talbot PJ.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7121612/

Virus Res. 2014 Dec 19;194:145-58. doi: 10.1016/j.virusres.2014.09.011.
Human coronaviruses: viral and cellular factors involved in neuroinvasiveness and neuropathogenesis.
Desforges M1, Le Coupanec A2, Stodola JK2, Meessen-Pinard M2, Talbot PJ3.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7114389/

Acta Neurol Taiwan. 2005 Sep;14(3):113-9.
Neurological manifestations in severe acute respiratory syndrome.
Tsai LK1, Hsieh ST, Chang YC.
https://pubmed.ncbi.nlm.nih.gov/16252612/

lead to cross-reactions with lung surfactant and related proteins, contributing to SARS-CoV-2-associated lung diseases.

> *Based on these results, the authors point out the **risk of using vaccines containing the whole SARS-Cov-2 virus antigen** and suggest the selection of unique, noncross-reactive peptides for immunotherapy with IVIG.* [248]

Equally, Dr. JL Wyler, studied the sequence homology between SARS-Cov-2 proteins and human proteins and confirmed the **high sequence homology and potential risk of autoimmunity**.

He also hypothesizes that exposure to these specific peptides, either through infection or vaccination, could cause **enhanced pathogenicity as a result of future exposure due to new pandemics, or outbreaks of infection, or through mass vaccination programs on a global scale**, and because this phenomenon is plausible, he considers it essential to evaluate it before proceeding with the use of vaccines on humans against SARS-Cov2. [249]

Finally, another research group [250] tested 5 different blood samples positive for SARS- CoV-2 [251] IgG and IgM antibodies. Measurements included anti-nuclear antibody (ANA), anti-extractable nuclear antigen (ENA), double-stranded DNA (dsDNA), actin antibody, mitochondrial antibody, rheumatoid factor (RF), and C1q immune complexes.

3 of the 5 samples had significant increases in ANA, ENA, actin, and mitochondrial antibodies, but not against dsDNA or RF.
This prompted researchers to study the cross-reactivity between SARS-CoV-2 and tissue autoimmune target proteins.

This study found that 21 out of 50 tissue antigens had moderate to strong reactions with antibodies against SARS-CoV-2. This finding is a sufficiently robust indication of the **cross-reaction between SARS-CoV-2 proteins and a variety of tissue antigens, in** addition to lung tissue, which could lead to autoimmunity against connective tissue, cardiovascular, gastrointestinal, and nervous systems, even in the medium and long term, after resolution of acute illness. [252]

---

[248] Clin Immunol. 2020;215:108426. doi:10.1016/j.clim.2020.108426
On the molecular determinants of the SARS-CoV-2 attack
Kanduc D, Shoenfeld Y.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7165084/

[249] J Transl Autoimmun. 2020;3:100051. doi:10.1016/j.jtauto.2020.100051
Pathogenic Priming Likely Contributes to Serious and Critical Illness and Mortality in COVID-19 via Autoimmunity
Lyons-Weiler J.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7142689/

[250] Vojdani A, Kharrazianb D.
Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases [published online ahead of print, 2020 May 24]. Clin Immunol. 2020;108480. doi:10.1016/j.clim.2020.108480
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7246018/

[251] Whitman J.D., Hiatt J., Mowery C.T., Shy B.R., Yu R., Yamamoto T.N.
Test performance evaluation of SARS-CoV-2 serological assay.
MedRxiv. 2020 doi: 10.1101/2020.04.25.20074856. (preprint)

[252] Salehi S, Reddy S, Gholamrezanezhad A.
Long-term Pulmonary Consequences of Coronavirus Disease 2019 (COVID-19): What We Know and What to Expect
J Thorac Imaging. 2020;10.1097/RTI.0000000000000534. doi:10.1097/RTI.0000000000000534
https://journals.lww.com/thoracicimaging/Citation/9000/Long_term_Pulmonary_Consequences_of_Coronavirus.99412.aspx

https://www.vox.com/2020/5/8/21251899/coronavirus-long-term-effects-symptoms

https://elemental.medium.com/the-long-term-health-impacts-of-being-infected-with-the-coronavirus-d3a03f3cb6e8

The authors conclude with an important reflection:

"In light of the information discussed above on the cross-reactivity of SARS-CoV-2 proteins with human tissues and the possibility of inducing autoimmunity, either exacerbating existing conditions of poor health or leading to unforeseen consequences, it would be very prudent to do more in-depth research on the ability of SARS-CoV-2 antigens to induce autoimmune reactions.

The promotion and implementation of such an aggressive ***"immune passport"*** *program worldwide in the absence of thorough and meticulous safety studies may come at a monumental cost to humanity in the form of another epidemic, this time of a rising tide of autoimmune diseases and the increased years of suffering that will accompany them."*

DR. LORETTA BOLGAN

Doctor of Pharmaceutical Chemistry and
Technology Ph.D. in Pharmaceutical Sciences
Scientific Advisor *loretta.bolgan@gmail.com*
Dolo (VE)