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► **To cite this version:**

Hélène Banoun. Why are children and many adults not affected by COVID-19? Role of the host immune response. *Infectious Diseases Research*, 2022, 3 (3), pp.18. 10.53388/IDR20220825018 . hal-03754848

**HAL Id: hal-03754848**

**<https://hal.science/hal-03754848>**

Submitted on 20 Aug 2022

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# Why are children and many adults not affected by COVID-19? Role of the host immune response

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## Competing interests

The author declares no conflicts of interest.

## Acknowledgments

No funding support was obtained.

## Abbreviations

ACE, angiotensin-converting enzyme; TMPRSS2, transmembrane serine protease 2; HCoV, human coronavirus; IFN, interferon; ISGs, IFN-stimulated genes; GM, gut microbiota; MIS-C, multisystem inflammatory syndrome - children; NK, natural killer; TMPRSS2, Type II transmembrane serine protease; URT, upper respiratory tract; SARS-CoV-2, respiratory syndrome coronavirus 2; MERS, middle east respiratory syndrome; RSV, respiratory syncytial virus; ADE, antibody-dependent enhancement; pDCs, plasmaDendriticCells; SLE, systemic lupus erythematosus; PGD2, prostaglandin D2.

## Citation

Banoun H. Why are children and many adults not affected by COVID-19? Role of the host immune response. *Infect Dis Res.* 2022;3(3):18. doi: 10.53388/IDR20220825018.

Executive editor: Na Liu.

Received: 02 August 2022; Accepted: 15 August 2022;

Available online: 19 August 2022.

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## Abstract

Children are less susceptible to COVID-19 than adults: they often have asymptomatic and very rarely severe forms. This protection is valid for all variants of the virus.

The aim here is to compare the immune response of children with that of adults, asymptomatic adults or those with mild disease with those who develop severe Covid.

Several protective factors for children have been mentioned but some of them do not seem to be involved. Indeed, there is no clear difference in the quantity of virus receptors (angiotensin-converting enzyme 2 (ACE2), transmembrane serine protease 2 (TMPRSS2)) present according to age that could explain a lesser entry of the virus into the cells of the nose, oropharynx and lungs of children. In fact, children and adults generally have similar viral loads and respiratory tract excretions.

Most adults, like children, have antibodies (and T cells) that cross-react with human coronavirus (HCoVs) and respiratory syndrome coronavirus 2 (SARS-CoV-2), but this humoral reactivity does not correlate with disease severity in adults; the difference appears to be more qualitative (IgM and anti-S in children and IgG and IgA and anti-N in adults) than quantitative, and mildly affected adults have some of the characteristics of the cross-reactivities of children. At the cellular level, the difference between children and adults lies more in the naivety of the T cells involved. The amount of salivary and mucosal IgA is negatively correlated with age and positively correlated with the absence of Covid infection: these IgAs are different and more effective than serum IgA.

Severe COVID-19 is characterized by hyperinflammation following invasion of the lower respiratory tract when the virus has not been cleared from the upper respiratory tract by innate immunity. Age is associated with an alteration of the immune system, often with a chronic hyperinflammatory state: deficient innate immunity combined with age-related dysregulation of adaptive immunity could cause severe COVID-19.

The innate cellular response in the upper and lower airways is more effective in asymptomatic children and adults: the interferon response is earlier and involves immune rather than epithelial cells, the latter being associated with hyperinflammation. This early response is critical given the ability of SARS-CoV-2 to suppress interferon 1 (IFN-1) responses. Regulatory Treg cells (which prevent the inflammatory response from spiraling out of control) are prevalent in the respiratory tissues of children. The response of myeloid cells (neutrophils and macrophages/monocytes), which are also responsible for hyperinflammation, is also qualitatively different in mildly affected children and adults compared to severe Covid: there is enrichment of classical monocytes and dysfunctional neutrophils in severe cases. It would be useful to explore why the response of children to SARS-CoV-2 is the opposite of that to influenza virus (which causes classical monocyte influx and overproduction of inflammatory cytokines).

Oral dysbiosis is associated with severe COVID-19 and the diversity of the oropharyngeal microbiota is inversely correlated with age.

Mycoplasma co-infections amplify viral replication and are associated with severe Covid; children may have more protective anti-mycoplasma IgG because they are more frequently exposed to community infections.

The role of hyperinflammation in severe COVID-19 justifies the use of immunomodulatory drugs: hydroxychloroquine, ivermectin, anti-histamines, corticosteroids. Probiotics have been used to restore the gut microbiota that interacts with the lung microbiota. Reduction of the permeability of the intestinal barrier has been proposed. Treatment of immune aging with a prostaglandin inhibitor works well in aged mice by restoring dendritic cell migration. Stimulation of innate immunity by a pathogen recognition motif receptor agonist works in mice.

**Keywords:** COVID-19 outcome; children; immune response; inflamm-aging; ACE2; cross-immunity

## Epidemiology of COVID-19

### Morbidity and mortality of COVID-19 in children

Previous close relatives of severe acute SARS-CoV-2, middle east respiratory syndrome (MERS) and SARS-CoV were already less severe in children, such as herpes, varicella, Epstein-Barr, and dengue. In contrast, respiratory syncytial virus (RSV), influenza, rhinoviruses, and metapneumoviruses can all cause severe illness and are among the leading causes of death in children under 5 years of age. Long Covid is less common and severe in children than adults [1].

The review by Zimmerman and Curtis [2] confirm to us that, compared to adults and unlike other respiratory viruses, children infected with SARS-CoV-2 are generally asymptomatic or have mild disease, with significantly lower hospitalization rates and mortality. The sources for the three literature reviews will not be cited again here [1-3].

The data are indeed consistent worldwide. In France, according to Santé Publique France, between March 1, 2020, and April 6, 2021, there were 9 children aged 0-14 years admitted to critical care and 5 deaths, of which only 1 was without comorbidities. (<https://www.santepubliquefrance.fr/maladies-et-traumatismes/maladies-et-infections-respiratoires/infection-a-coronavirus/documents/bulletin-national/covid-19-point-epidemiologique-du-8-avril-2021> SPF Epidemiological Update, April 8, 2021)

According to Geodes, France, ([https://geodes.santepubliquefrance.fr/#c=indicateur&f=09&i=covid\\_hospit\\_clage10.dc&s=2022-06-17&t=a01&view=map1](https://geodes.santepubliquefrance.fr/#c=indicateur&f=09&i=covid_hospit_clage10.dc&s=2022-06-17&t=a01&view=map1)), 41 children aged 0-9 years have died with COVID-19 since March 1, 2020, knowing that since March 31, 2020, a COVID file can be created on strong suspicion of Covid (without biological confirmation).

In the US, according to the CDC, children accounted for 1.3% to 3.1% of total reported hospitalizations, and between 0.1% and 2.0% of all COVID-19 cases in children resulted in hospitalization. Children accounted for 0.00% to 0.19% of all COVID-19 deaths, and 10 states reported no child deaths. In the reporting states, 0.00% to 0.03% of all COVID-19 cases in children resulted in death. (<https://downloads.aap.org/AAP/PDF/AAP%20and%20CHA%20-%20Children%20and%20COVID-19%20State%20Data%20Report%204.1.21%20FINAL.pdf>)

In Europe, of a total of 820,404 symptomatic pediatric cases, hospitalization was reported for 9611 (1.2%) cases, ICU admission for 640 (0.08% of all cases, 6.7% of hospitalized cases) and death for 84 (0.01%). The adjusted odds of hospitalization, ICU admission and death were seven, nine and 27 times higher, respectively, in cases with at least one comorbidity than in those without [4].

In Iceland, a study shows that no children had severe symptoms or were hospitalized. Infants under 6 months of age were not more severely affected than older children [5].

In China, the clinical and epidemiological characteristics of 34 children hospitalized between January and February 2020 in western China show that among children aged 1 month to 12 years, 47% of patients had a coinfection. The current study found that all patients had mild to moderate COVID-19 disease, which is consistent with previous studies. It was also reported that 94% of cases were identified as asymptomatic (4%), mild (51%), or moderate (39%) [6].

In Italy, ECDC confirms that COVID-19 was a mild disease in the general pediatric population in Italy. ([https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.14.2001248#abstract\\_content](https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2021.26.14.2001248#abstract_content))

Also in Australia, most children had a mild illness or were asymptomatic. Two children had post-acute inflammatory conditions COVID-19 temporally associated with SARS-CoV-2. At the most recent review in March 2021, all children had returned to their initial health status and the COVID-19 post-viral symptoms had resolved [7].

According to the Marseille IHU, children appear to be less affected by the disease burden and have less severe symptoms. (<https://www.mediterranee-infection.com/wp-content/uploads/2020/06/302-first-pediatric-patients-COVID-diagnosed-in-Marseille.pdf>)

In COVID-19 in children, rare multisystemic inflammatory

syndromes (multisystemic inflammatory syndromes - Kawasaki like) related to SARS-CoV-2 mimicking a Kawasaki syndrome are found. In the rare cases of Kawasaki-like syndrome, the majority of children recover within a few days, but with possible dilatation of the coronary arteries [8]. There may be an HLA-1 genetic predisposition to multi-inflammatory systemic syndrome (PIMS or MIS-C or Kawasaki-like): HLA class I genes have recently been associated with MIS-C [1].

However, more recently, children appear to be more susceptible to the omicron variant according to a registry-only study: of 462 patients younger than 19 years of age hospitalized for COVID-19 between October 31 and December 11, 2021, during the omicron wave, 7 were ventilated and 4 died, all related to complex underlying comorbidities [9].

But this impression should be interpreted with caution [10]. In children, the fraction of SARS-CoV-2-positive cases admitted to hospital in South Africa doubled during the Omicron wave compared with the Delta wave. However, the in-hospital case fatality rate in children under 5 years of age was 0.5% in the Omicron wave compared with 0.6% in the Delta wave. A similar trend, but with a lower case fatality rate, was observed in older children. The proportion of children under 5 years of age ventilated in the UK was 2.9% in the Omicron wave compared with 5.1% in the other waves. So why are more children admitted with Omicron?

One possibility is that, as with adults, there were more unreported infections in the Omicron wave and this led to more admissions, but the admissions did not result in as many serious outcomes as with the other variants. The length of hospitalization and the type of care provided must be taken into account.

Because Omicron is highly contagious, many hospitalized patients tested will be PCR positive even if the cause of their hospitalization is not COVID-19. The role of co-infections must also be considered: not all germs possibly responsible for symptoms close to those of COVID-19 will be tested when the SARS-CoV-2 PCR is positive.

### Immunopathogenesis of COVID-19

According to Bhardwal A et al. [11], viral infection stimulates the host immune system which is initiated by activation of innate immune cells that recognize pathogen-associated molecular patterns. If the innate immune responses fail to eliminate the virus, the adaptive immune system is activated. Induction of innate and adaptive immune cells stimulates the secretion of cytokines or chemokines such as interleukin-6 (IL-6), interferon- $\gamma$  (IFN- $\gamma$ ), interferon- $\gamma$ -inducible protein-10 (IP-10), and monocyte chemoattractant protein-1 (MCP)-1. IFN induces the expression of IFN-stimulated genes (ISGs). Cytokines and chemokines promote the influx of monocytes/macrophages and neutrophils from the blood to the site of infection. These cells will secrete cytotoxic molecules. IFNs promote phagocytosis, activate NKs and initiate the humoral adaptive response. Successful activation of the type 1 IFN response inhibits viral replication and prevents the spread of viral infection. Normally, this innate response is able to eliminate the virus, but sometimes the immune system is dysregulated, resulting in a disruption of immune homeostasis: the intense inflammatory response against the virus leads to excessive production of pro-inflammatory cytokines, which thus promote pulmonary pathogenesis and respiratory failure.

Patients with defects in adaptive immune cells, whether B cells, T cells, or both, usually develop mild or asymptomatic COVID-19 [1]; this supports a primary role for innate immunity.

### SARS-CoV-2 evades the innate immune response by suppressing antiviral type 1 IFN responses.

SARS-CoV-2, like SARS-CoV [12], stimulates lower levels of type 1 and type 3 IFN responses. This leads to limitation of ISG expression (IFN-stimulated gene expression) and induction of high cytokine expression promoting immune cell infiltration. Peripheral blood immune cells from severely ill COVID-19 patients had a reduced type 1 IFN response and higher levels of the inflammatory cytokines IL-6 and TNF- $\alpha$ . Patients with mild symptoms had reduced levels of type 1 and

type 3 IFN responses.

We will see that the temporality of IFN expression must be considered whether it is early or late and that IFN has a double-edged role. We can conclude that SARS-CoV-2 causes dysregulation of innate immunity by modulating type 1 IFN responses.

#### **What protective factors for Covid-19 have been discussed?**

In 2021, Zimmerman and Curtis [2] discussed the reasons for this difference in severity of COVID-19 in children and adults.

Factors that put adults at higher risk include (1) age-related increase in endothelial damage and changes in coagulation function; (2) higher density, increased affinity, and different distribution of angiotensin-converting enzyme 2 ACE2 and transmembrane serine protease 2 TMPRSS2 receptors; (3) preexistence of anti-coronavirus antibodies (including antibody-dependent enhancement (ADE) and T cells); (4) immunosenescence and inflammation; (5) higher prevalence of comorbidities associated with severe COVID-19; and (6) lower vitamin D levels.

Factors that might protect children include (1) differences in innate and adaptive immunity; (2) more frequent recurrent and concurrent infections; (3) preexisting immunity to coronavirus; (4) differences in microbiota; (5) higher levels of melatonin; (6) off-target protective effects of live vaccines; and (7) lower intensity of exposure to SARS-CoV-2.

The authors concluded in 2021 that changes in immune function and endothelial injury and changes in coagulation function could explain the age-related severity gradient.

It should be noted that children are more susceptible to other respiratory viruses than adults, such as RSV or influenza, and they also develop a cytokine storm in response to these viruses, so it will be important to clarify how the difference between the child and adult immune system may contribute to the explanation.

Regarding cross-reactive immunity, Zimmermann and Curtis noted that no age differences are observed in the levels of cross-reactive antibodies to HCoV or the number of reactive T cells. They noted the importance of studying mucosal IgA.

According to Mallapaty's review [13], the reasons why children were resistant to COVID-19 would lie in the innate immune response. Children would mount a less robust adaptive immune response because their innate response is more effective. An overactive adaptive response in adults may be responsible for some of the complications of COVID-19. In contrast to Weisberg SG et al [14], many authors indeed find similar levels of neutralizing antibodies in children compared to adults [15].

Children with disorders affecting their adaptive immune response - humoral or cellular do not have problems when infected with SARS-CoV-2. Among the severe pediatric COVID-19s, there were children whose innate immune response was deficient: the innate system rather than the adaptive system would be able to defeat this virus [14]. Adults patients with adaptive immunity defects have asymptomatic or moderate COVID-19 [16].

For Mallapaty [13], years of exposure to other human coronaviruses could direct the response of the adult immune system to SARS-CoV-2 as it would to other coronaviruses, resulting in a less effective response - a concept known as original antigenic sin (or immune imprinting). In contrast, children might produce a fresh, more refined response to a brand new virus.

In their 2022 review, Zimmerman and Curtis revisit the mechanisms that are likely to contribute to the difference in severity of COVID-19 and emphasize the role of inflammation and innate immunity. Patients with severe COVID-19 have a generalized hyperinflammatory state.

Distinct immune cell subsets associated with asymptomatic infection, disease severity, and viral persistence have been identified in COVID-19 patients. Moreover, regardless of age, the defect in control of viral replication and the tendency to exacerbate lung inflammation and disseminated intravascular coagulation are related to the pathophysiology of COVID-19 [17].

#### **Virus receptors (Age-specific expression of virus receptors: conflicting results)**

**The major receptors involved in SARS-CoV-2 entry.** The angiotensin-converting enzyme 2 (ACE2) receptor, present on multiple cell types throughout the body, is the primary receptor for SARS-CoV-2 entry into human cells. SARS-CoV-2 binds to the ACE2 receptor through the spike protein. Transmembrane serine protease 2 (TMPRSS2) is the major entry-associated protease that cleaves and activates the S protein of SARS-CoV-2, which greatly facilitates viral entry into cells. Furin cleaves the bond between the S1 and S2 subunits, promoting fusion of the virus capsid with the cytoplasmic membrane. ADAM17 is responsible for the excretion of the ACE2 receptor which is detached from the cell membrane and is no longer active [18].

It has been postulated that the different distribution of ACE2 and TMPRSS2 between body sites in children and adults, as well as the lower ACE2 activity for SARS-CoV-2 in children, contribute to age-related differences in the severity of COVID-19. However, the numerous studies on this topic report conflicting results. Some studies show lower expression of ACE2 and TMPRSS2 in the nasal epithelium of children compared with adults, whereas others have found no age-related differences. Therefore, these viral entry factors cannot explain the differences in pathophysiology of SARS-CoV-2 between children and adults.

A recent study of autopsies from subjects aged 24 weeks gestational age to 28 years, all pre-Covid-19, shows that ACE2 expression in the lungs increases with age during the first year of life and stabilizes by 5 years of age. ACE2 in the intestine and stomach decreases with age. In other organs, there is no variation in expression with age (cardiomyocytes, liver, kidney, pancreas, brain) [18].

Results of other studies are contradictory: the confounding factor of ventilation on ACE2 expression must be considered. Children (4-9 years) have lower expression of ACE2 in the nose than older children. But ACE2 expression in the lower airways may play a role in the severity of infection [19].

According to Dioguardi M et al [18], in SARS-CoV-1 infections, the response was already age-dependent and children developed mild disease. Furthermore, downregulation of ACE2 leads to increased inflammation and SARS-CoV-2 downregulates ACE2 once it enters the cell. This is inconsistent with a positive relationship between ACE2 expression and susceptibility to Covid.

Zimmermann and Curtis conclude with studies showing even higher ACE2 expression in children than in adults; ACE2 and TMPRSS2 expression are not age-related and different immune responses determine disease severity. Children can have a high viral load independent of the level of ACE2 expression and it is not the viral load that determines the severity.

Concerning the contradictory aspect of the results of the correlations between ACE2 levels and severity of COVID-19, Bastolla U et al. [20], conclude that the whole renin-angiotensin system must be considered and in particular the ATR2 receptors, which are more expressed in children and which are anti-inflammatory: in children the RAS system is less unbalanced towards the pro-inflammatory side than in adults. We will see below that this balance could indeed explain the protection of children.

#### **Viral load**

According to Zimmerman and Curtis, there is little evidence to support the hypothesis that viral load is responsible for age-related differences in COVID-19 severity. Children and adults generally have similar viral loads and airway excretions. However, one study found significantly higher viral loads in nasopharyngeal specimens from children younger than 5 years of age compared with older children or adults, and some studies that report lower viral loads in children compared with adults. Increased viral load in the blood has also been associated with increased disease severity and increased cytokine storm.

According to Pekayvaz et al, viral loads in the upper airways are equivalent in children, adults with mild and severely affected COVID-19. These equivalent viral loads in the upper airways argue for

additional mechanisms of the mucosal response capable of preventing systemic viral diffusion [21]. The viral load of children hospitalized with COVID-19 was found to be significantly higher than that of adults [22].

According to a preprint from IHU Marseille (<https://www.mediterranee-infection.com/ropen-screening-of-sars-co-v-2-infections-in-the-pediatric-population-in-marseille-southern-france/>), viral loads are roughly equivalent in children and are very slightly significantly lower compared to adults tested in open screening (see figure 3, thus including symptomatic and asymptomatic patients).

According to Rouchka et al. [23], the viral load in the upper respiratory tract varies widely among patients; no viral factor is associated with this variation, which correlates with the host response. Patients who develop mild symptoms have higher viral loads than severe COVID-19 cases (but there are exceptions: severe patients with high viral loads). Mick et al. [24] also found that viral load varies by several orders of magnitude in moderately affected patients and does not differ significantly between children and adults.

### Pre-existing cross-immunity to coronavirus (results between children and adults are contradictory)

Cross-reactive immunity was shown as early as 2020 with common cold coronaviruses, and the possibility of protection against COVID-19 was raised very early as well [25]. It is unfortunate that some recent reviews ignore conflicting studies on this topic: Paul Moss still argues on the basis of a 2020 paper that children and young adults have higher levels of humoral and cellular cross-reactivity between HCoV and SARS-CoV-2 than older people [26]. It highlights the role of cross-reactive CD4<sup>+</sup> T cells in influenza infection to which children are nevertheless more susceptible than adults, in contrast to COVID-19. Recent studies show that children have lower levels of anti-HCoV antibodies than adults [15, 27]. According to Zimmermann and Curtis, commonly circulating human coronaviruses (HCoV-229E, -HKU1, -NL63, and -OC43) are responsible for approximately 6-8% of acute respiratory tract infections, and most people develop immunity to HCoVs during childhood. Therefore, individuals who have not been infected with SARS-CoV-2 may have cross-reactive, neutralizing and non-neutralizing antibodies and T cells against SARS-CoV-2 antigens.

Despite seroconversion at an early age, reinfections with HCoVs later in life are common, and evidence is conflicting as to whether children or adults have higher levels of antibodies and cross-reactive T cells. Children are thought to have less experienced but more polyreactive humoral immunity. Although antibodies to HCoVs may be cross-reactive, they do not necessarily protect against SARS-CoV-2, as they may not be cross-neutralizing.

According to Monto AS et al., children under 5 years of age are infected more often than older age groups, but thereafter the infection rates are comparable, which cannot explain the protection of children and adolescents against Covid by cross-reactive immunity to HCoVs. Furthermore, the incidence is less than 10% at any age and cannot explain the protection of all children against COVID-19 (figure 4 of [28]).

It may also be useful to include studies that compare cross-reactivity in adults based on the severity of their Covid. Cross-reactions by HCoV type may not give the same protection: sequencing data show that SARS-CoV-2 is more closely related to HCoV-OC43 and HKU1 than to NL63 and 229E [29].

This only partially overlaps with the observation that older adults lack T cells capable of cross-reacting with HCoVs-OC43 and NL63 and SARS-CoV-2 [30].

In a small cohort, there is a correlation between the level of HCoV-OC43 spike cross-reactive antibodies and good prognosis in adult Covid-19 patients (in all severe or deceased patients the antibodies were almost non-existent, variability was greater in mild cases), but only 8% of those tested had humoral immunity to HCoV-OC43 (Figure 5 of Yamaguchi T et al, 2021 [31]). Pre-pandemic sera from most adults have cross-reactivity with SARS-CoV-2 but it does not correlate with protection [32-34].

This pre-existing cross-reactivity appears to exist at all ages but is

qualitatively different.

The antigen with which these cross-reactive antibodies react is also important to consider: protein S (S1 and S2), protein N, and other viral proteins (including nonstructural proteins) are involved. Pre-existing S2-specific antibodies (RBD is located on S1) to HCoV-OC43 are associated with a mild COVID-19. These antibodies are more frequently present in children and adolescents. Regarding antibodies against non-structural proteins (ORF3b, ORF7a and ORF8 - IFN antagonists) the levels of antibodies found in pre-pandemic sera differ markedly from the sera of pediatric COVID-19 patients, they have much less cross-reactive antibodies. This is particularly the case for N and ORF8 (Figure 1-suppl, c, d and e of Hachim et al., 2022 [35]). Unfortunately Hachim et al [35]. do not detail the results as a function of age for prepandemic sera.

Antibody type differs by age in prepandemic sera: Selva KJ et al [36]. find higher IgA and IgG cross-reactive levels in healthy older adults and higher IgM levels in healthy children, suggesting that children have less experienced but more polyreactive humoral immunity [36].

According to Zimmermann and Curtis, there is also a theoretical risk that higher levels of non-neutralizing, cross-reactive anti-HCoVs antibodies will lead to more severe COVID-19 via antibody-dependent enhancement (ADE). In ADE, pre-existing non-neutralizing antibodies can bind to virions, which can then more easily enter and replicate in macrophages and granulocytic cells, leading to higher viral loads.

This more reactive response is also found at the cellular level: T cells that cross-react between commonly circulating HCoVs and SARS-CoV-2 have been identified in a number of studies. Few studies have compared T-cell immunity to HCoVs in different age groups, but one study reported lower levels of T cells against HCoVs in older adults. However, as with cross-reactive antibodies, the role of cross-reactive T cells in relation to SARS-CoV-2 is unclear. It has been suggested that the presence of cross-reactive CD8<sup>+</sup> T cells is associated with milder COVID-19 disease, but also that pre-existing low avidity T cells, which are typically present in greater numbers in the elderly, negatively impact T cell responses to SARS-CoV-2.

At the level of the cross-reaction due to B cells, the difference between children and adults is also mostly qualitative [37]. Dowell et al. point out the different pre-existing cross-reactive T cell response in children, with different cytokine production than in adults [38].

But while cross-reactive CD8<sup>+</sup> T cells likely exist in unexposed individuals, they are not common and thus are unlikely to play a significant role in providing broad pre-existing immunity in the community [39]. However, cross-reactivity to a particular epitope of the N protein exists regardless of age in SARS-CoV-2 uninfected individuals and is consistent with naive T cells capable of broad and strong clonal expansion; the N protein can generate a robust T cell response, is well conserved, and does not recombine frequently. The response to N is rapid after infection and induces a broad spectrum of memory cells that are maintained for 6 months with preserved antiviral activity against different variants [40].

In conclusion, the difference between the cross-reactions between children and adults lies more in the qualitative aspects and the naivety of the T cells involved.

### Cross-protection conferred by off-target live vaccines

One study suggested that live attenuated MMR (measles-mumps-rubella) vaccines may provide cross-protection against COVID-19 [41]. A correlation is found between protection against COVID-19 and the level of anti-mumps antibodies (only in vaccinated and not in naturally infected) and independently of age. This correlation may also reflect a different immune response and not be a causal relationship: Individuals who maintain high mumps antibody levels may be those who have a better immune response to COVID-19. One biological explanation given by the authors is the sequence homology between the SARS-CoV-2 spike and the fusion protein of measles, rubella and mumps viruses: why don't they find a correlation between COVID-19 protection and measles and rubella antibody levels? Why is there no correlation between COVID



protection and mumps antibody levels in elderly people immunized by natural infection either: vaccination would protect better than infection? The other explanation is the non-specific training of innate immunity by live attenuated MMR vaccines; this immunity is also double-edged and this correlation could reverse with age which is strangely not the case here [42].

#### **Immune response to SARS-CoV-2 (Differences between children and adults/ and in adults by severity of infection)**

From a general perspective, age is associated with an altered immune system. Vallet H et al., 2019 [43] describe immunosenescence (aging of the immune system) and inflamm-aging (increased production of proinflammatory cytokines that reflects a basal state of chronic inflammation) and the same team emphasizes the role of stress [44]. Taken together, these changes decrease the ability of the elderly patient to produce an effective immune response. The modifications of the innate immune system are: evolution of monocytes towards a more inflammatory profile, neutrophils towards a suppressive profile, altered capacities of phagocytosis, chemotaxis and production of oxidative stress residue decreased. Concerning lymphocytes, they note a depletion of the T repertoire by restriction of the TCR (T cell receptors): the rate of rearrangements decreases, leading to a loss of diversity of the T compartment and a lesser capacity to recognize a new antigen.

We will find these alterations in the immune cells associated with severe COVID-19. Elderly people do not necessarily have an immunological age corresponding to their real age: it is the basal inflammatory state that determines this immunological age. There are markers of this immunological age [45].

#### **Qualitative differences in infection-induced antibodies, role of IgA (serum and salivary)**

Many authors find similar levels of neutralizing antibodies in children compared to adults [15]: the differences would be more qualitative than quantitative.

**Differences in antibody target.** Infected children develop a more diverse humoral response than adults with antibodies directed against all accessible proteins of the virus. This may be due to differences in virus replication, expression levels, and immune responses in children to the key viral proteins, ORF8 and E (ORF8 appears to play an important role in viral pathogenesis; the extent and rate of virus replication in pediatric cells may be different from adults due to differences in innate pathway activation). Children with COVID-19 have higher levels of antibodies against envelope protein E and against ORF3b, ORF7a, and ORF8 (IFN antagonists) compared with adults and lower levels against spike (S1, S2, and S2'); the pediatric response may abort full virion synthesis by reacting strongly against ORF8 and E. Anti-ORF8 levels were significantly higher in children than in adults, but the observed differences cannot explain the almost complete protection of children: anti-ORF8 was positive in 13.39% of asymptomatic children and 9.21% of asymptomatic adults. Similarly, anti-E levels are significantly higher than in adults but in the same range [35].

Severe adult cases have lower levels of ORF8 antibodies than mild adult cases; therefore, ORF8 antibodies may function as a positive immune correlate anti-ORF8 antibody levels are negatively correlated with IL-6 which independently predicts the severity of COVID. Anti-ORF8 antibodies may have a beneficial role in reducing the cytokine storm since ORF8 plays a role in virus pathogenesis [35].

Weisberg et al [14] found instead anti-spike antibodies in children and no anti-N. However, Hachim et al. [35] found as much anti-N in children as in adults. In contrast to Weisberg SP et al. many authors find an equivalent antibody spectrum in children compared to adults (especially against protein N) [15].

**Differences in antibody type.** Early and transient IgM, IgA, and IgG responses distinguish asymptomatic individuals from patients with mild disease, whose antibody production is slower but more persistent [46].

Severe Covid patients show a delayed anti-RBD IgG and neutralizing antibody response [47]. However, quantitatively, in nasopharyngeal swabs from children and adults who presented to an emergency department, anti-SARS-CoV-2 IgA and IgG were detected at similar levels [28].

Mucosal and serum IgA are poorly studied in COVID-19. Serum IgA may have a dual role as it does for IgG. Instead, Weisberg et al. find IgM in children and IgG and IgA in adults [14]. In adults with severe COVID-19, SARS-CoV-2-specific serum IgA has been reported to activate neutrophils, whereas it was absent in children with mild COVID-19 cited by Brodin P [22].

Serum IgA could contribute to the pathogenic role of antibodies through synergistic effects with IgG by promoting antibody-dependent cellular cytotoxicity (ADCC): Hai-qiong Yu et al. [48] find higher serum IgA values in severe adult patients compared to mild COVID-19 patients; but measurement of serum IgA does not prejudice the level of mucosal IgA, which is also qualitatively different [48]. In another study, serum IgA-dominant responses correlate with the absence of COVID-19 infection but the authors did not investigate nasal IgA levels. COVID-19 infection is associated with the presence of SARS-CoV-2-specific neutralizing serum IgG, T-cell responses, IFN- $\gamma$  response (thus in vitro neutralizing IgG antibodies are not necessarily protective) [49]. For Sheikh-Mohamed et al. [50] asymptomatic pediatric patients have higher IgA levels than symptomatic cases. Patients with IgA deficiency have severe COVID-19. However, the persistence of salivary IgA in adults is associated with the severity of the disease. Dobaño C et al. [51] also find that asymptomatic individuals have more salivary anti-S antibodies than symptomatic patients.

The earliness of the response is critical: mucosal IgA appears as early as the first day of infection with common cold viruses (rhinovirus and coxsackievirus) [52]. Also, for SARS-CoV-2, early responses are dominated by mucosal IgA and neutralizing IgA is detectable in saliva up to 2 months after symptom onset [53].

Tsukinoki et al. [54] suggested that mucosal IgA has a crucial role in protecting mucosal surfaces from SARS-CoV-2 by neutralizing the virus and/or preventing its attachment to epithelial cells in the early stage of viral infection. Salivary IgA (sIgA) is polymeric in contrast to monomeric serum IgA. The sIgA is generated and released in the mucosa, whereas its serum counterpart is, like IgG, derived from a separate source, the bone marrow. IgA is thought to play a more important role than IgG in protecting against SARS-CoV-2 by neutralizing virus infectivity. Serum IgA antibodies to SARS-CoV-2 are half as potent as IgG equivalents. Still, sIgA antibodies in predominantly dimeric mucosal secretions are more than a log more potent than their monomeric serum counterparts. In a Japanese cohort of all ages, from infants to the elderly, 52.8% of individuals tested unexposed to SARS-CoV-2 have virus-reactive sIgA. The amount of sIgA is negatively correlated with age and BMI (obesity index). No correlation is observed with serum IgG levels. A correlation between low levels of sIgA and the incidence of upper respiratory viral infections has been shown [54]. Egwang TG et al. found IgA cross-reactive between HCoVs and SARS-CoV-2 in pre-pandemic milk from Ugandan and American women. The same team had shown the virus neutralizing power of these IgA. These IgAs could participate in the protection of newborns against COVID-19 [55].

In mice, only polymeric IgA prevents the spread of influenza infection by blocking it from the upper respiratory tract, suggesting that IgG does not prevent viral infection of the nose. In contrast, IgG, but not pIgA (polymeric IgA), prevents viral pathology in murine lungs. Both types of antibodies are important, with plasma IgG supporting secretory IgA-mediated protection in the nasal compartment, and IgG being the dominant antibody in lung protection [56].

#### **Importance of controlling viral replication in the upper airways by innate immunity**

Coronaviruses have evolved in bats that control infection by their innate immunity enriched in NK (natural killer) and IFN-1 genes and

broad-spectrum nonspecific antibodies. Asymptomatic humans could control the virus with their NK cells and antibodies [46]. As early as late 2020 immunologists from La Jolla (California) proposed that deficient innate immunity associated with age-related dysregulation of adaptive immunity is associated with the immunopathogenesis of severe COVID-19 [57].

Yoshida et al. [58] confirm that the fate of COVID-19 infections is played out in the innate response at the level of upper airway cells. For Kousathanas A et al. [17] also, the innate response is more efficient in children: Epithelial cells, lymphoid cells, alveolar (pulmonary) macrophages, and dendritic cells constitute the first barrier that respiratory viruses (rhinovirus, respiratory syncytial virus, influenza, and coronavirus) encounter from the nasopharynx to the alveolar epithelium.

**The difference is thought to lie in the different cellular composition of the mucosal barrier.** Alveolar macrophages are present in greater numbers in children [18]. Children have pre-existing SARS-CoV-2 reactive B cells in the tonsils.

The number of tonsillar B cells decreases during aging: age-related changes in the cellular composition of the immune system of the upper respiratory mucosa may contribute to the immunopathology of SARS-CoV-2 [59]. According to Mick et al. [24], affected children show a decrease in genes expressed by hair cells but the difference is not significant, in contrast they express many more genes related to basal epithelial cells. Children with COVID-19 express fewer genes related to neutrophil- and mast cell-mediated immunity than children with other non-viral conditions.

Vono M et al. [15] also show a strong reduction of neutrophils in children in the early phase of exposure. Their study involved contacts of infected individuals within household households who were followed up before developing symptoms or not; it should be noted that asymptomatic and PCR-negative contacts did not show changes in cell types or gene expression after contact with the sick: have these individuals developed a protective mechanism not yet identified?

According to Zimmerman and Curtis, 2022, MAIT cells (mucosal-associated invariant T cells, at the crossroads of immunity, inflammation, and the microbial microenvironment) and NK cells are significantly enriched in asymptomatic subjects, whereas NKT (natural killer T) cells increase in symptomatic patients. Classical monocytes are positively correlated with disease severity. In addition, classical monocytes and pDCs (plasma dendritic cells targeting foreign nucleic acids and secreting large amounts of interferon, they are cells of innate immunity) are associated with viral persistence. pDCs contribute to the pathogenesis of SLE (systemic lupus erythematosus) and interferon-related autoimmune diseases. After producing IFN pDCs differentiate and stimulate T cells of the adaptive system [60]. For other authors [61] a reduced frequency of pDCs during the first months of life predisposes to respiratory infections. Vono et al. [15] show that children have more pDCs than adults: pDCs could also have a double-edged role related to interferon production.

Children have more naive lymphocytes. This was pointed out as early as late 2020 by Moderbacher RC et al. [57]: the low frequency of naive T cells is associated with age and disease severity; the lack of coordination of the adaptive response also correlates with age. The decrease in naive CD4<sup>+</sup> lymphocytes is caused by severe COVID-19, whereas the low CD8<sup>+</sup> level in severe patients is observed before the disease and is not an effect of it. The low CD8<sup>+</sup> level associated with age is a factor of disease severity [57]. This may also explain why advanced age is a significant risk factor for the development of severe COVID-19. Older adults are known to have lower numbers of naive CD8<sup>+</sup> T cells, and are therefore less likely to respond effectively to novel viral pathogens [47].

Yoshida et al. [58] confirm that in children there are more naive lymphocytes circulating in the blood than in adults; this results in systemic infection and inflammation in adults, whereas in children the infection remains limited to the upper tract.

Mild and critical COVID-19 differ not only quantitatively in terms of the number of T-cells with good effector function, but also

qualitatively: in critical COVID-19, T-cells are highly dysregulated [62].

For Bhardwal A et al. [11], this different cellular composition of the mucosal barrier would be responsible for an earlier expression of cytokines, in particular IFN-1. Children are observed to have reduced levels of the proinflammatory cytokines TNF- $\alpha$  and IL-6, further reducing neutrophil infiltration and lung injury. Along with the reduced levels of pro-inflammatory cytokines, higher levels of immunomodulatory cytokines such as IL-10 and IL-13 were also observed in children. This may be related to the role of Treg regulatory lymphocytes (which prevent the inflammatory response from spiraling out of control): they are prevalent in the respiratory tissues of children and prevent the exacerbation of the immune response towards exaggerated inflammation.

Studies of IFN-stimulated gene (ISG) expression in the upper respiratory tract show conflicting results. Mick et al. [24] showed in adult hospitalized patients (i.e. in the late stage of infection) that the innate response is attenuated by SARS-CoV-2 compared to other respiratory viruses. The decrease in innate immune responses observed in COVID-19 patients is coupled with attenuated recruitment and activation of pro-inflammatory macrophages in the airway microenvironment. According to Pierce CA et al. [27], children express higher levels of immune cell-associated genes, whereas the expression of epithelial cell-associated genes does not diverge in children compared to adults: this supports the early and vigorous mucosal immune response in children. These are genes associated with IFN signaling and also NLRP3 of the inflammasome and other innate pathways [27].

In children the interferon response is already activated and is weakly increased and involves immune system cells. In adults, the interferon response involves epithelial cells and cells of inflammation: there is proliferation of cytotoxic cells resulting in systemic inflammation. Epithelial cells play a key role in the initiation of the innate response [58]. Vono M et al. [15] show that adults and children have a similar innate response with a more rapid resolution in children (maximum 5 days after exposure with an early IFN response, increased CXCL10 and GM-CSF cytokines, transient lymphopenia and increased monocytes and dendritic cells). Mick et al. [24] find that the differences in ISG expression of children and adults with COVID-19 are subtle and seem unlikely to explain their distinct clinical outcomes. Children have a broader spectrum of immune-related gene expression than adults. The expression of ISGs correlates much better with viral load in children than in adults. Children have more robust innate and especially adaptive immune responses in the upper airways, as well as increased hair cell clearance, which may contribute to their protection. However, Mick et al. [24] studied only hospitalized children and adults, not healthy patients, and they do not have a clear indication of the timing of sampling relative to the onset of symptoms, so their results do not inform about the difference in the earliness of responses between children and adults.

Loske J et al. [63] confirm that epithelial and immune cells in the nose of children are pre-activated and ready to detect the virus. As noted above, SARS-CoV-2 inhibits the interferon response, so cells have an extremely narrow window of opportunity to express IFNs before viral protein production disables the antiviral system. Children clear SARS-CoV-2 much faster than adults: they stop viral replication earlier. This probably also explains the differences between SARS-CoV-2 and other respiratory viruses, including respiratory syncytial virus, influenza A virus, in terms of host-induced response. In contrast, SARS-CoV-2 is very sensitive to IFN treatment before or after infection. Children respond to influenza virus with high airway inflammation correlated with classical monocyte influx and inflammatory cytokine production [64]. It would be interesting to explore the reasons for this opposite response of children to these 2 respiratory viruses.

The same pattern as in children is found in non-pneumonic adult patients infected with SARS-CoV-2: The innate anti-viral response can limit viral spread to the lungs, confining the virus to the upper airways. The genomic approach shows a distinct systemic immune state in

non-pneumonic patients infected with SARS-CoV-2: the interferon-stimulated gene (ISG) is upregulated in circulating immune cells in non-pneumonic patients, NK and T cells have reduced cytotoxic potential, and monocytes show an immunomodulatory phenotype. Conversely, pneumonic patients have a reduced early ISG (interferon-stimulated gene) response in peripheral immune cells (CD4<sup>+</sup> T cells, NK and monocytes) [63, 65], low macrophage/monocyte-directed innate antiviral response and lack of IFN signaling activity [65]; but they show upregulation of some monocytes (IL6R) and increased cytotoxicity of T cells and NK cells [21]. The oral mucosa of severe patients shows evidence of viral activity, cell arrest and viral dissemination to the lower respiratory tract [65]. Channappanavar R et al [12]. had previously shown in mice that SARS-CoV-1 inhibited the early response to interferon and that the late response was associated with the hyperinflammation of severe disease.

In relation to the importance of early detection and reaction to the virus, during infection in vaccinated individuals, the peak of the viral load appears on the first day, whereas the increase in the level of "neutralizing" antibodies appears afterward; this could explain in part the absence of vaccine protection against infections [66].

#### The role of IFN is double-edged

The role of IFN is double-edged: it is protective but may also be associated with severe Covid [58]. Again, it is the earliness of the interferon response that may be the key to limiting viral spread. Effective early IFN responses and rapid return to homeostasis help protect children from COVID-19. Conversely, persistent IFN is associated with deleterious effects on the host [15].

According to Rouchka et al. [23], there is an inverse correlation between interferon expression and disease severity (in symptomatic patients, thus after the prodromal phase). Viral load in URT is correlated with IFN expression. High viral loads would induce a high IFN response in URT. The early IFN response could lead to a protective immune response.

According to Xu G et al. [47], severe patients have a strong transient IFN response before disease deterioration, but then it drops rapidly, whereas mild patients have a weak but stable interferon response throughout the disease. SARS-CoV-2 infection can induce high IFN production that is positively correlated with viral load. In the absence of animal models that can mimic severe COVID-19, it is difficult to determine whether IFNs have a protective or detrimental function.

Brodin P [1] and Chauvineau-Grenier A et al. [67], note that anti-interferon autoantibodies are associated with disease severity: the number of autoantibodies to IFN-I was proportional to increasing age in patients with severe COVID-19 infections.

#### Role of myeloid cells in hyperinflammation

Patients with severe Covid show similarities with other hyperinflammatory conditions (such as hemophagocytic lymphohistiocytosis, Kawasaki) and different respiratory infections (seasonal coronavirus, influenza, bacterial pneumonia). Among the common differentially expressed genes, there is a group of neutrophil-associated genes that reflect a generalized hyperinflammatory state: neutrophil hyperactivation is noted in COVID-19 patients admitted to intensive care units [68].

Comparison of cell profiles between mild and severe patients shows that the exaggerated inflammatory response to COVID-19 would be of myeloid origin. The differences are mainly qualitative. According to the study of innate immune cells sought in bronchoalveolar washings of COVID-19 patients (according to the severity of the disease), the number of lymphocytes was globally reduced, while inflammatory myeloid cells, mainly classical monocytes, were increased. The peripheral adaptive immune response to SARS-CoV-2 is characterized by an enrichment of classical monocytes and dysfunctional neutrophils in critical cases of COVID-19 (where, monocytes were much more frequent and characterized by a chronic hyperinflammatory phenotype). In mild COVID-19, macrophages characterized by anti-inflammatory, pro-phagocytic and antigen

presentation facilitating functions were enriched [62].

Late IFN induction in severe patients explains the attraction of monocytes to the site of infection and the lack of NK recruitment. The monocyte/NK ratio is less than 1 in asymptomatic individuals and increases in mildly affected patients; in severe patients the absence of NK is associated with monocyte expansion [46]. The proportion of abnormal monocytes appears early and continues to increase throughout severe disease [47].

There may be a kind of innate immune memory in COVID-19: Long-term adaptive changes have been observed in monocytes after COVID-19 infection; monocyte-mediated training of innate immunity may prevent reinfections. But it could also lead to harmful hyperactivation as in multisystemic inflammatory syndromes (You M et al.) [69]. The double-edged role of innate immune memory was highlighted by Netea MG et al [42].

Patients with defects in neutrophil function have moderate Covid [16]. However for Neeland et al [70], the infection in children is characterized by an increased activation of neutrophils and a low circulation of monocytes, dendritic cells and NK, in contrast, in adults only a reduction of the non-classical fraction of monocytes is observed. But Vono et al. [15] show a strong reduction of neutrophils in children in the early phase of exposure: this discrepancy would be due to the difference in the observation period in the 2 studies. In exposed but uninfected individuals (children and adults), the immune response is characterized by a low density of immature neutrophils. So this may also be a qualitative rather than quantitative difference?

#### Role of coinfections and microbiota

##### Oral and nasopharyngeal microbiota

The gut microbiota (GM) is closely related to respiratory infections : it can distally drive host response to pneumonia. The possible mechanisms include the regulation of extraintestinal T cell populations, development of oral immune tolerance through regulatory T cells (Tregs), production of short-chain fatty acids (SCFAs), and regulation of systemic inflammation [71]. SARS-CoV-2 infection begins in the oral cavity and is influenced by its microbiota. COVID-19 patients have a significantly altered microbiota compared to influenza patients or healthy individuals. Oral dysbiosis is associated with severe COVID-19: the oral microbiota plays a role in the activation of innate and humoral immunity against SARS-CoV-2. The same is true for the nasal microbiota. The relative amount of many bacterial genera correlates with the severity of COVID-19 (these are *Bifidobacterium*, *Lactobacillus*, *Solobacterium*, *Proteus*, *Cupriavidus*, and *Paracoccus*) [72].

Altered salivary microbiota in severe patients is associated with diarrhea in severe patients. The diversity of salivary and nasopharyngeal microbiota is inversely correlated with age and may predispose older individuals to severe disease [72]. In addition, Natarajan A et al. [73] suggested that long COVIDs are associated with persistence of viral RNA in the gut and signs of digestive dysfunction. Moreover, Suskun et al. [74] show that the GM of children with MIS-C (multisystemic inflammatory syndrome or Kawasaki like) after COVID-19 is altered compared to healthy children or those with acute uncomplicated COVID-19. A mechanism has been proposed to explain how the GM can protect against COVID-19 infection both locally and systemically: the production of SCFAs (short-chain fatty acids) by commensal bacteria capable of fermenting fibers downregulates ACE2, decreases viral load, increases immune responses, and may influence the propensity for infection-induced hypercoagulation [71]. Moreover the commensal GM can induce pre-existing cross-reactive antibodies against the spike protein [75, 76].

##### Co-infections

The late presentation of MIS-C (systemic multi-inflammatory syndromes of childhood) could be explained by the need for a co-infecting pathogen. This could be due to prolonged viral shedding in the intestines of children and disruption of the integrity of the intestinal barrier in patients with MIS-C. Since most children with



MIS-C have intestinal symptoms and often inflammation of the terminal ileum, SARS-CoV-2 virus could then persist in the gut of children more than in adults and, in genetically susceptible individuals, resulting in local T-cell activation and inflammation, loss of gut barrier integrity, and release of superantigens into the bloodstream. This could then potentially explain the systemic hyperinflammatory responses observed in children and young people with MIS-C [1].

In 1990, Professor Luc Montagnier highlighted the role of mycoplasmas in AIDS [77]. Mycoplasma and chlamydia are capable of amplifying the intracellular multiplication of viruses (see the patent <https://patents.google.com/patent/WO1994002630A1/fr> Method of amplification of infectious agents in 'in vitro' diagnosis on cell cultures and Chowdury IH et al. [78] and Milligan WH, Fletcher RD [79]). Other studies have shown the potentiation of HIV infection by mycoplasmas [80]. A correspondence was found between the presence of one of the potential "co-infectants" and co-amplifiers of viral replication (Mycoplasma pneumoniae) and the proliferation peaks of respiratory viruses (RSV and Influenza) [81].

Bacteria present in the dysbiosis of the surrounding microbiomes (oral, pulmonary or digestive) are associated with severe COVID-19: A Chinese study illustrates that co-infected (and not super-infected) persons entering the hospital die 20 times more than those without co-infections [82]. Varying rates of co-infections and/or superinfections are found in patients with COVID-19 infection. When they occur, these infections appear to be associated with both the severity of COVID-19 infection and poorer outcomes [83].

Therapeutic evidence of this involvement may be provided by the positive responses of some COVID-19 patients to antibiotic and antimalarial therapy: these responses could be the result of suppression of Mycoplasma species and other bacterial co-infections in subsets of patients [84, 85]. Huang et al. [85] report a COVID-19 patient co-infected with *Mycoplasma pneumoniae* who recovered well after combination therapy.

Returning to children who are highly affected by community-acquired *Mycoplasma pneumoniae* infections and have anti-mycoplasma antibodies. Bobin et al. [86] show that anti-mycoplasma IgG positivity is a potential protective factor against SARS-CoV-2 infection. And indeed, nearly one-third of children with COVID-19 had a co-infection, most commonly caused by *Mycoplasma pneumoniae*. In children, co-infection did not result in significant exacerbation of clinical manifestations [87].

#### How do we translate all this complex immunological data into treatment?

Inflammation-aging has been described along with avenues to address it (obesity control, exercise, restoration of balanced gut microbiota) [88]. It is plausible to suggest that altered immune response during aging increases the severity of COVID-19 in the elderly [11].

#### Immunomodulators and corticosteroids

Inhibition of the inflammatory response caused by a dysregulated immune response may be an effective therapy to prevent COVID-19. Immunomodulatory drugs therefore have a place; hydroxychloroquine (Million et al.) [89] and ivermectin (Kory et al.) [90] used in the treatment of COVID-19 are immunomodulators [91], ivermectin [92].

In a randomized clinical trial (2,100 participants) in the UK, the synthetic glucocorticoid dexamethasone was found to suppress deaths by one-third in intubated patients with COVID-19. Dexamethasone had no impact on non-severe patients, but was highly effective in severe intubated patients and reduced their risk of death by 20%. Another study reported that short-term treatment with dexamethasone significantly reduced C-reactive protein levels and length of hospital stay in COVID-19 patients [93].

#### Antihistamines

Antihistamines have shown anti-viral action against influenza, RSV and Zika viruses and their ability to inhibit cellular entry of Ebola and

hepatitis C viruses; they are also able to inhibit histamine-mediated production of inflammatory cytokines; (Some antihistamines have anti-viral activity in vitro possibly through interaction with virus receptors (ACE2 and sigma-1) [94]. Dr S Arminjon had identified as early as February 2020 that the immune system could cause a deleterious reaction to the virus and that histamine could be involved in this hyperinflammation (<https://www.francesoir.fr/opinions-tribunes/hypothese-covid-19-anti-histaminiques-pertinence-actualisee-selon-evolution-des>), this hypothesis has been confirmed by clinical studies (<https://www.researchgate.net/profile/Annwyne-Houldsworth-2/post/Are-Angiotensin-Receptor-Blocker-Trials-as-Therapy-Inhibiting-COVID-19-Infection-Successful/attachment/5eb02081c005cf0001882c60/AS%3A887470908530693%401588600961846/download/Antihistamines+as+a+therapeutic+care+plan+of+of+Covid-19+About+26+cases+%281%29.pdf>) and also, Reznikov LR et al. [94]; Palma G et al. [95]; Ciprandi et al. [96]; Freedberg DE et al. [97]; Hogan Ii RB et al. [98]; Morán Blanco JI et al. [99]).

#### Gut microbiota (GM) and COVID-19

We have seen that since alteration of the gut microbiota causes several diseases, it is also possible that the gut microbiota is altered during SARS-CoV-2 infection. The lung also has its own microbiota, called the pulmonary microbiota. It has been revealed that the GM regulates lung health and that lung inflammation also has an effect on the GM [100]. Thus, GM and lung microbiota are interconnected with each other and affect their respective development. A clinical trial (NCT04355741) is underway to test the role of GM in COVID-19.

Yonker et al. [101] tested an inhibitor of zonulin (a protein that increases intestinal permeability) with positive results in children with MIS-C (multi-inflammatory syndrome): MIS-C is thought to be associated with increased permeability of the intestinal barrier allowing passage of intestinal virions into the general circulation.

#### Probiotics for the treatment and management of COVID-19

Probiotics modulate GM, regulate the immune system and maintain the integrity of the gut. The National Administration of Traditional Chinese Medicine of China and the National Health Commission of China has also proposed the use of probiotics for the treatment of SARS-CoV-2 infection (Mak JWY et al., 2020 [102], for a review). Administration of probiotics-*Enterococcus faecalis*, *Lactobacillus rhamnosus* GG, and *Bacillus subtilis* to severely ill patients with pneumonia (on ventilator support) significantly reduced the need for intubation. Probiotics can also be used to prevent dysbiosis in COVID-19. Another mechanism by which probiotics may prevent SARS-CoV-2 infection is through modulation of the immune system. Probiotics have shown anti-inflammatory properties. The probiotic combination of *L. rhamnosus*, *B. lactis*, and *B. longum* decreased the production of IL- $\beta$  and IL-6 and downregulated the expression of IL-10.

#### Treatment of immune aging

In mice, as in humans, the severity of SARS, MERS and COVID-19 infections increases with age. Researchers have tested an antagonist of a prostaglandin (PGD2 or ecosanoids) that increases in the lungs with age and prevents the proper migration of dendritic cells (cells of innate immunity that collaborate with B cells of adaptive immunity). In aged mice, high levels of ecosanoids (prostaglandin D2) are correlated with poor clinical outcomes. Aged mice that do not express PGD2 (prostaglandin D2) are protected against severe Covid. PGD2 is responsible for impaired migration of dendritic cells to lymph nodes resulting in a suboptimal T response. PGD2 increases with inflammation-aging and oxidative stress. Asapiprant, a specific PGD2/PTGDR (PTGD2 receptor) antagonist accelerates viral clearance and decreases pathological changes in the lungs. Asapiprant increases respiratory dendritic cell migration from the lungs to the lymph nodes [102]. Asapiprant has already been tested in clinical trials for allergic rhinitis and a clinical trial is underway for Covid (<https://clinicaltrials.gov/ct2/show/NCT04705597>).

### Stimulation of innate immunity

A team from Yale University [103] has identified an antiviral drug that activates the innate immune system and appears to have strong prophylactic and therapeutic potential in mice, meaning it could both prevent and treat COVID-19 in humans.

Innate immunity relies on the identification of pathogen-associated molecular patterns, or PAMPs, by pattern recognition receptors. Among the most important pattern recognition receptors are Toll-like receptors and retinoic acid-inducible gene I (RIG-I) receptors. Detection of PAMPs by these receptors activates signaling pathways that induce the production of interferons, interferon-stimulated genes, and inflammatory cytokines, which in turn trigger the adaptive immune response.

The drug tested by Mao et al. [104] in mouse models is a RIG-I agonist called Stem-loop RNA 14 (SLR14). Previous research had already established that SLR14 generates a robust innate immune response in mice and that it could produce an equally potent response in humans. Mao et al. found that administration of SLR14 to mice four hours after infection with wild-type SARS-CoV-2 significantly reduced symptoms and improved survival. In contrast, mice that did not receive the drug succumbed to infection by day eight. The drug also works in immunodeficient mice that lack functional B cells and T cells (so the adaptive response is not required for recovery). SLR14 stimulates rapid interferon proliferation in the airways and is much less expensive than interferon-based drugs. Mao et al. also suggest that other modulators of innate immunity, such as poly (I:C) and diABZI, a small molecule and STING agonist would be a useful addition to an anti-SARS-CoV-2 drug strategy. If this is true for SARS-CoV-2, it is likely to be true for many other RNA viruses that mimic the RIG-I immune signaling pathway.

### Conclusion

Morbidity and mortality data show that overall, children are little affected by COVID-19 (often asymptomatic, with severe cases occurring in children with serious comorbidities). In adults, 40% of cases are asymptomatic according to Ma Q et al [105].

A review of the reasons for the natural protection of children (and some adults) against COVID-19 emphasizes the baseline inflammatory state of infected individuals and the role of co-infections and the respiratory and digestive tract microbiota. Indeed, other factors invoked seem to play a minor role (quantity of virus receptors, viral load, pre-existing cross immunity with common cold coronaviruses).

Age is associated with an altered immune system, often with a chronic hyperinflammatory state (inflamm-aging); deficient innate immunity combined with age-related dysregulation of adaptive immunity could cause severe COVID-19. The response of children and adults with little disease is early and depends on good innate immunity in the mucous membranes; this would be related to the role of mucosal IgA, early cellular innate response mediated by controlled interferon and cytokine production. Adaptive immunity seems to play a minor, if any, role, at worst deleterious. The microbiota of the respiratory tract and especially the intestinal tract participates in the protection against COVID-19 and dysbiosis is correlated with severe COVID-19 as well as the presence of co-infections.

From a preventive point of view, it is therefore necessary to promote the maintenance of a low inflammatory basal state by fighting against inflamm-aging which is favored by obesity, lack of physical exercise and dysregulated microbiota.

From a therapeutic point of view, this justifies the use of immunomodulatory and antihistamines drugs, the restoration of a balanced microbiota, the treatment of immune aging and the stimulation of innate immunity.

### References

1. Brodin P. SARS-CoV-2 infections in children: understanding diverse outcomes, *Immun* 2022;55:201-209.

2. Zimmermann P, Curtis N. Why Does the Severity of COVID-19 Differ With Age? *Pediatr Infect Dis J* 2021;41:e36–e45. <http://dx.doi.org/10.1097/INF.0000000000003413>
3. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. *Arch Dis Child* 2020;106:429–439. <http://dx.doi.org/10.1136/archdischild-2020-320338>
4. Bundle N, Dave N, Pharris A, et al. COVID-19 trends and severity among symptomatic children aged 0–17 years in 10 European Union countries, 3 August 2020 to 3 October 2021. *Eurosurveillance*, 2021;26(50):2101098. <https://doi.org/10.2807/1560-7917.ES.2021.26.50.2101098>
5. Thors V, Bjornsdottir KL, Love T, Haraldsson A. SARS-CoV-2 Infections in Icelandic Children: Close Follow-Up of All Confirmed Cases in a Nationwide Study. *Pediatr Infect Dis J*. 2022. <http://dx.doi.org/10.1097/INF.0000000000003626>
6. Zhang C, Gu J, Chen Q, et al. Clinical and epidemiological characteristics of pediatric SARS-CoV-2 infections in China: A multicenter case series. *PLoS Med* 2020;17(6):e1003130. <http://dx.doi.org/10.1371/journal.pmed.1003130>
7. Say D, Crawford N, McNab S, et al. Post-acute COVID-19 outcomes in children with mild and asymptomatic disease. *The Lancet Child & Adolesc Health* 2021;5:e22–e23. [http://dx.doi.org/10.1016/S2352-4642\(21\)00124-3](http://dx.doi.org/10.1016/S2352-4642(21)00124-3)
8. Mercier JC, Maroni A, Levy M, Melki I, Meinzer U, Gaschnigard J, Beyler C, Santos A. COVID-19 chez l'enfant : syndrome inflammatoire multi-systémique lié à SARS-CoV-2 mimant un syndrome de Kawasaki [COVID-19 in children: SARS-CoV-2-related inflammatory multisystem syndrome mimicking Kawasaki disease]. *Bull Acad Natl Med*. 2021;205(6):579-586. <http://dx.doi.org/10.1016/j.banm.2020.11.018>
9. Cloete J, Kruger A, Masha M, et al. Paediatric hospitalisations due to COVID-19 during the first SARS-CoV-2 omicron (B.1.1.529) variant wave in South Africa: a multicentre observational study. *Lancet Child Adolesc Health*. 2022;6(5):294-302. [http://dx.doi.org/10.1016/S2352-4642\(22\)00027-X](http://dx.doi.org/10.1016/S2352-4642(22)00027-X)
10. Sigal A, Milo R, Jassat W. Estimating disease severity of Omicron and Delta SARS-CoV-2 infections. *Nat Rev Immunol* 2022;22:267–269. <http://dx.doi.org/10.1038/s41577-022-00720-5>
11. Bhardwaj A, Sapra L, Saini C, et al. COVID-19: Immunology, Immunopathogenesis and Potential Therapies. *Int Rev Immunol* 2021;41:171–206. <http://dx.doi.org/10.1080/08830185.2021.1883600>
12. Channappanavar R, Fehr AR, Vijay R, et al. Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-2-Infected Mice. *Cell Host Microbe* 2016;19(2):181–193. <http://dx.doi.org/10.1016/j.chom.2016.01.007>
13. Mallapaty S. Kids and COVID: why young immune systems are still on top. *Nature* 2021;597(7875):166–168. <http://dx.doi.org/10.1038/d41586-021-02423-8>
14. Weisberg SP, Connors TJ, Zhu Y, et al. Distinct antibody responses to SARS-CoV-2 in children and adults across the COVID-19 clinical spectrum. *Nat Immunol*. 2021;22(1):25-31. <http://dx.doi.org/10.1038/s41590-020-00826-9>
15. Vono M, Huttner A, Lemeille S, et al. Robust innate responses to SARS-CoV-2 in children resolve faster than in adults without compromising adaptive immunity. *Cell Reports* 2021; 37: 109773. <http://dx.doi.org/10.1016/j.celrep.2021.109773>
16. Meyts I, Buccioli G, Quinti I, et al. Coronavirus disease 2019 in patients with inborn errors of immunity: An international study. *J Allergy Clin Immunol* 2021;147(2):520–531.

- <http://dx.doi.org/10.1016/j.jaci.2020.09.010>
17. Kousathanas A, Pairo-Castineira E, Rawlik K, et al. Whole-genome sequencing reveals host factors underlying critical COVID-19. *Nature* 2022;607(7917):97–103. <http://dx.doi.org/10.1038/s41586-022-04576-6>
  18. Dioguardi M, Cazzolla AP, Arena C, et al. Innate Immunity in Children and the Role of ACE2 Expression in SARS-CoV-2 Infection. *Pediatr Rep* 2021;13(3):363–382. <http://dx.doi.org/10.3390/pediatric13030045>
  19. Patel AB, Verma A. Nasal ACE2 Levels and COVID-19 in Children. *JAMA* 2020;323(23):2386–2387. <http://dx.doi.org/10.1001/jama.2020.8946>
  20. Bastolla U, Chambers P, Abia D, et al. Is Covid-19 Severity Associated With ACE2 Degradation? *Front Drug Discov* 2022;11:789710. <http://dx.doi.org/10.3389/fddsv.2021.789710>
  21. Pekayvaz K, Leunig A, Kaiser R, et al. Protective immune trajectories in early viral containment of non-pneumonic SARS-CoV-2 infection. *Nat Commun* 2022;13(1):1018. <http://dx.doi.org/10.1038/s41467-022-28508-0>
  22. Yonker LM, Neilan AM, Bartsch Y, et al. Pediatric Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): Clinical Presentation, Infectivity, and Immune Responses. *J Pediatr* 2020;227:45–52.e5. <http://dx.doi.org/10.1016/j.jpeds.2020.08.037>
  23. Rouchka EC, Chariker JH, Alejandro B, et al. Induction of interferon response by high viral loads at early stage infection may protect against severe outcomes in COVID-19 patients. *Sci Rep* 2021;11(1):15715. <http://dx.doi.org/10.1038/s41598-021-95197-y>
  24. Mick E, Kamm J, Pisco AO, et al. Upper airway gene expression reveals suppressed immune responses to SARS-CoV-2 compared with other respiratory viruses. *Nat Commun* 2020;11(1):5854. <http://dx.doi.org/10.1038/s41467-020-19587-y>
  25. Banoun H. Covid19: cross-immunity with other coronaviruses, immunopathological phenomena-update-august20. Unpublished 2020. <http://rgdoi.net/10.13140/RG.2.2.35117.92648>
  26. Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol* 2022;23:186–193. <http://dx.doi.org/10.1038/s41590-021-01122-w>
  27. Pierce CA, Sy S, Galen B, et al. Natural mucosal barriers and COVID-19 in children. *JCI Insight* 2021;6(9):e148694. <http://dx.doi.org/10.1172/jci.insight.148694>
  28. Monto AS, DeJonge PM, Callear AP, et al. Coronavirus Occurrence and Transmission Over 8 Years in the HIVE Cohort of Households in Michigan. *J Infect Dis* 2020;222(1):9–16. <http://dx.doi.org/10.1093/infdis/jiaa161>
  29. Gussow AB, Auslander N, Faure G, et al. Genomic determinants of pathogenicity in SARS-CoV-2 and other human coronaviruses. *Proc Natl Acad Sci USA* 2020;117:15193–15199. <http://dx.doi.org/10.1073/pnas.2008176117>
  30. Saletti G, Gerlach T, Jansen JM, et al. Older adults lack SARS CoV-2 cross-reactive T lymphocytes directed to human coronaviruses OC43 and NL63. *Sci Rep* 2020;10(1):21447. <http://dx.doi.org/10.1038/s41598-020-78506-9>
  31. Yamaguchi T, Shinagawa T, Kobata H, et al. Immunity against seasonal human coronavirus OC43 mitigates fatal deterioration of COVID-19. *Int J Infect Dis* 2021;109:261–268. <http://dx.doi.org/10.1016/j.ijid.2021.07.015>
  32. Anderson EM, Goodwin EC, Verma A, et al. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. *Cell* 2021;184(7):1858–1864.e10. <http://dx.doi.org/10.1016/j.cell.2021.02.010>
  33. Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science* 2020;370:1339–1343. <http://dx.doi.org/10.1126/science.abe1107>
  34. Majdoubi A, Michalski C, O'Connell SE, et al. A majority of uninfected adults show preexisting antibody reactivity against SARS-CoV-2. *JCI Insight* 2021;6(8):e146316. <http://dx.doi.org/10.1172/jci.insight.146316>
  35. Hachim A, Gu H, Kavian O, et al. SARS-CoV-2 accessory proteins reveal distinct serological signatures in children. *Nat Commun* 2022;13(1):2951. <http://dx.doi.org/10.1038/s41467-022-30699-5>
  36. Selva KJ, van de Sandt CE, Lemke MM, et al. Systems serology detects functionally distinct coronavirus antibody features in children and elderly. *Nat Commun* 2021;12(1):2037. <http://dx.doi.org/10.1038/s41467-021-22236-7>
  37. Yang F, Nielsen SCA, Hoh RA, et al. Shared B cell memory to coronaviruses and other pathogens varies in human age groups and tissues. *Science* 2021;372:738–741. <http://dx.doi.org/10.1126/science.abc6648>
  38. Dowell AC, Butler MS, Jinks E, et al. Children develop robust and sustained cross-reactive spike-specific immune responses to SARS-CoV-2 infection. *Nat Immunol* 2022;23(1):40–49. <https://doi.org/10.1038/s41590-021-01089-8>
  39. Swaminathan S, Lineburg KE, Ambalathingal GR, et al. Limited Recognition of Highly Conserved Regions of SARS-CoV-2. *Microbiol Spectr* 2022;10(1):e0278021. <http://dx.doi.org/10.1128/spectrum.02780-21>
  40. Peng Y, Felce SL, Dong D, et al. An immunodominant NP105–113-B\*07:02 cytotoxic T cell response controls viral replication and is associated with less severe COVID-19 disease. *Nat Immunol* 2021;23:50–61. <http://dx.doi.org/10.1038/s41590-021-01084-z>
  41. Gold JE, Baumgartl WH, Okyay RA, et al. Analysis of Measles-Mumps-Rubella (MMR) Titers of Recovered COVID-19 Patients. *mBio* 2020;11(6):e02628-20. <http://dx.doi.org/10.1128/mBio.02628-20>
  42. Netea MG, Domínguez-Andrés J, Barreiro LB, et al. Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 2020;20:375–388. <http://dx.doi.org/10.1038/s41577-020-0285-6>
  43. Vallet H, Fali T, Sauce D. Le vieillissement du système immunitaire : du fondamental à la clinique. [Aging of the immune system: From fundamental to clinical data]. *Rev Med Interne* 2019;40(2):105–111. <http://dx.doi.org/10.1016/j.revmed.2018.07.005>
  44. Fali T, Vallet H, Sauce D. Impact of stress on aged immune system compartments: Overview from fundamental to clinical data. *Exp Gerontol* 2018;105:19–26. <http://dx.doi.org/10.1016/j.exger.2018.02.007>
  45. Sayed N, Huang Y, Nguyen K, et al. An inflammatory aging clock (iAge) based on deep learning tracks multimorbidity, immunosenescence, frailty and cardiovascular aging. *Nat Aging* 2021;1:598–615. <http://dx.doi.org/10.1038/s43587-021-00082-y>
  46. Carsetti R, Zaffina S, Piano Mortari E, et al. Different Innate and Adaptive Immune Responses to SARS-CoV-2 Infection of Asymptomatic, Mild, and Severe Cases. *Front Immunol* 2020;11:610300. <http://dx.doi.org/10.3389/fimmu.2020.610300>
  47. Xu G, Qi F, Wang H, et al. The Transient IFN Response and the Delay of Adaptive Immunity Feature the Severity of COVID-19. Xu G, Qi F, Wang H, et al. The Transient IFN Response and the Delay of Adaptive Immunity Feature the Severity of COVID-19. *Front Immunol* 2022;12:5846. <http://dx.doi.org/10.3389/fimmu.2021.816745>
  48. Yu HQ, Sun BQ, Fang ZF, et al. Distinct features of SARS-CoV-2-specific IgA response in COVID-19 patients. *Eur Respir J*. 2020;56(2):2001526. <http://dx.doi.org/10.1183/13993003.01526-2020>
  49. Hennings V, Thörn K, Albinsson S, et al. The presence of serum anti-SARS-CoV-2 IgA appears to protect primary health care workers from COVID-19. *Eur J Immunol* 2022;52(5):800–809.

- <https://doi.org/10.1002/eji.202149655>
50. Sheikh-Mohamed S, Sanders EC, Gommerman JL, Tal MC. Guardians of the oral and nasopharyngeal galaxy: IgA and protection against SARS-CoV-2 infection. *Immunol Rev* 2022;309(1):75-85. <http://dx.doi.org/10.1111/imr.13118>
  51. Dobaño C, Alonso S, Fernández de Sevilla M, et al. Antibody conversion rates to SARS-CoV-2 in saliva from children attending summer schools in Barcelona, Spain. *BMC Med* 2021;19(1):309. <http://dx.doi.org/10.1186/s12916-021-02184-1>
  52. Butler WT, Waldmann TA, Rossen RD, Douglas RG Jr, Couch RB. Changes in IgA and IgG concentrations in nasal secretions prior to the appearance of antibody during viral respiratory infection in man. *J Immunol* 1970;105(3):584-591. <https://pubmed.ncbi.nlm.nih.gov/4318484/>
  53. Sterlin D, Mathian A, Miyara M, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci Transl Med* 2021;13(577):eabd2223. <http://dx.doi.org/10.1126/scitranslmed.abd2223>
  54. Tsukinoki K, Yamamoto T, Saito J, et al. Prevalence of Salivary IgA Reacting with SARS-CoV-2 among Japanese People Unexposed to the Virus. *medRxiv* 2022. <https://doi.org/10.1101/2022.01.09.22268986>
  55. Egwang TG, Owalla TJ, Okurut E, et al. Differential pre-pandemic breast milk IgA reactivity against SARS-CoV-2 and circulating human coronaviruses in Ugandan and American mothers. *Int J Infect Dis* 2021;112:165-172. <http://dx.doi.org/10.1016/j.ijid.2021.09.039>
  56. Renegar KB, Small PA Jr, Boykins LG, Wright PF. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. *J Immunol* 2004;173(3):1978-1986. <http://dx.doi.org/10.4049/jimmunol.173.3.1978>
  57. Rydzynski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* 2020;183(4):996-1012.e19. <http://dx.doi.org/10.1016/j.cell.2020.09.038>
  58. Yoshida M, Worlock KB, Huang N, et al. Local and systemic responses to SARS-CoV-2 infection in children and adults. *Nature* 2021;602(7896):321-327. <http://dx.doi.org/10.1038/s41586-021-04345-x>
  59. Liu Y, Budyłowski P, Dong S, et al. SARS-CoV-2-Reactive Mucosal B Cells in the Upper Respiratory Tract of Uninfected Individuals. *J Immunol* 2021;207(10):2581-2588. <http://dx.doi.org/10.4049/jimmunol.2100606>
  60. Li S, Wu J, Zhu S, et al. Disease-Associated Plasmacytoid Dendritic Cells. *Front Immunol* 2017;8:1268. <http://dx.doi.org/10.3389/fimmu.2017.01268>
  61. Upham JW, Zhang G, Rate A, et al. Plasmacytoid dendritic cells during infancy are inversely associated with childhood respiratory tract infections and wheezing. *J Allergy Clin Immunol* 2009;124(4):707-713.e2. <http://dx.doi.org/10.1016/j.jaci.2009.07.009>
  62. Wauters E, Van Mol P, et al. Discriminating mild from critical COVID-19 by innate and adaptive immune single-cell profiling of bronchoalveolar lavages. *Cell Res* 2021;31:272-290. <http://dx.doi.org/10.1038/s41422-020-00455-9>
  63. Loske J et al., 2022 Loske J, Röhm J, Lukassen S, et al. Pre-activated antiviral innate immunity in the upper airways controls early SARS-CoV-2 infection in children. *Nat Biotechnol* 2021;40:319-324. <http://dx.doi.org/10.1038/s41587-021-01037-9>
  64. Oshansky CM, Gartland AJ, Wong SS, et al. Mucosal Immune Responses Predict Clinical Outcomes during Influenza Infection Independently of Age and Viral Load. *Am J Respir Crit Care Med* 2014;189:449-462. <http://dx.doi.org/10.1164/rccm.201309-1616OC>
  65. Gómez-Carballa A, Rivero-Calle I, Pardo-Seco J, et al. A multi-tissue study of immune gene expression profiling highlights the key role of the nasal epithelium in COVID-19 severity. *Environ Res* 2022;210:112890. <http://dx.doi.org/10.1016/j.envres.2022.112890>
  66. Koutsakos M, Lee WS, Reynaldi A, et al. The magnitude and timing of recalled immunity after breakthrough infection is shaped by SARS-CoV-2 variants. *Immunity* 2022;55:1316-1326.e4. <http://dx.doi.org/10.1016/j.immuni.2022.05.018>
  67. Chauvineau-Grenier A, Bastard P, Servajean A, et al. Autoantibodies Neutralizing Type I Interferons in 20% of COVID-19 Deaths in a French Hospital. *J Clin Immunol* 2022;42:459-470. <http://dx.doi.org/10.1007/s10875-021-01203-3>
  68. Schimke LF, Marques AHC, Baiocchi GC, et al. Severe COVID-19 Shares a Common Neutrophil Activation Signature with Other Acute Inflammatory States. *Cells* 2022;11(5):847. <http://dx.doi.org/10.3390/cells11050847>
  69. You M, Chen L, Zhang D, et al. Single-cell epigenomic landscape of peripheral immune cells reveals establishment of trained immunity in individuals convalescing from COVID-19. *Nat Cell Biol* 2021;23(6):620-630. <http://dx.doi.org/10.1038/s41556-021-00690-1>
  70. Neeland MR, Bannister S, Clifford V, et al. Innate cell profiles during the acute and convalescent phase of SARS-CoV-2 infection in children. *Nat Commun* 2021;12(1):1-5. <http://dx.doi.org/10.1038/s41467-021-21414-x>
  71. Chunxi L, Haiyue L, Yanxia L, et al. The Gut Microbiota and Respiratory Diseases: New Evidence. *J Immunol Res* 2020;2020:2340670. <http://dx.doi.org/10.1155/2020/2340670>
  72. Kim JG, Zhang A, Rauseo AM, et al. The salivary and nasopharyngeal microbiomes are associated with SARS-CoV-2 infection and disease severity. *bioRxiv* 2022. <https://doi.org/10.1101/2022.05.31.494162>
  73. Natarajan A, Zlitni S, Brooks EF, et al. Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection. *Med* 2022;3:371-387.e9. <http://dx.doi.org/10.1016/j.medj.2022.04.001>
  74. Suskun C, Kilic O, Yilmaz Ciftdogan D, et al. Intestinal microbiota composition of children with infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and multisystem inflammatory syndrome (MIS-C). *Eur J Pediatr* 2022;181(8):3175-3191. <https://doi.org/10.1007/s00431-022-04494-9>
  75. Jia L, Weng S, Wu J, et al. Pre-existing antibodies targeting a linear epitope on SARS-CoV-2 S2 cross-reacted with commensal gut bacteria and shaped vaccine induced immunity. *medRxiv* 2021. <https://doi.org/10.1101/2021.07.13.21260404>
  76. Ninnemann J, Budzinski L, Bondareva M, et al. Induction of cross-reactive antibody responses against the RBD domain of the spike protein of SARS-CoV-2 by commensal microbiota. *bioRxiv* 2021. <https://doi.org/10.1101/2021.08.08.455272>
  77. Balter M. Montagnier Pursues the Mycoplasma-AIDS Link: In the face of stubborn skepticism from the AIDS research community, Luc Montagnier presents new results. *Sci* 1991;251(4991):271-271. <https://doi.org/10.1126/science.1670968>
  78. Chowdhury IH, Munakata T, Koyanagi Y, Kobayashi S, Arai S, Yamamoto N. Mycoplasma can enhance HIV replication in vitro: a possible cofactor responsible for the progression of AIDS. *Biochem Biophys Res Commun* 1990;170(3):1365-1370. [http://dx.doi.org/10.1016/0006-291x\(90\)90545-x](http://dx.doi.org/10.1016/0006-291x(90)90545-x)
  79. Milligan WH, Fletcher RD. Effect of Mycoplasma pneumoniae on poliovirus replication. *Infect Immun* 1975;11(3):607-608. <http://dx.doi.org/10.1128/iai.11.3.607-608.1975>
  80. Rapelanoro Rabenja F, Lepère P, Escarguel C, Pelissier C, Lamarque P, Malvy D. Prévalence de l'infection par mycoplasmes urogénitaux chez la femme atteinte par le VIH à



- Bangui (République Centrafricaine) [Prevalence of urogenital mycoplasma infection in women infected with HIV in Bangui (Central African Republic)]. *Sante* 1998;8(3):189-192. French. <https://pubmed.ncbi.nlm.nih.gov/9690318/>
81. Layani-Milon MP, Gras I, Valette M, et al. Incidence of upper respiratory tract *Mycoplasma pneumoniae* infections among outpatients in Rhône-Alpes, France, during five successive winter periods. *J Clin Microbiol* 1999;37(6):1721-1726. <http://dx.doi.org/10.1128/JCM.37.6.1721-1726.1999>
  82. He S, Liu W, Jiang M, et al. Clinical characteristics of COVID-19 patients with clinically diagnosed bacterial co-infection: A multi-center study. *PLoS One* 2021;16(4):e0249668. <http://dx.doi.org/10.1371/journal.pone.0249668>
  83. Feldman C, Anderson R. The role of co-infections and secondary infections in patients with COVID-19. *Pneumonia(Nathan)* 2021;13(1):5. <http://dx.doi.org/10.1186/s41479-021-00083-w>
  84. Nicolson GL, de Mattos GF. COVID-19 coronavirus: Is infection along with *Mycoplasma* or other bacteria linked to progression to a lethal outcome? *Int J Clin Med* 2020;11(05):282. <https://doi.org/10.4236/ijcm.2020.115029>
  85. Huang AC, Huang CG, Yang CT, Hu HC. Concomitant infection with COVID-19 and *Mycoplasma pneumoniae*. *Biomed J* 2020;43(5):458-461. <http://dx.doi.org/10.1016/j.bj.2020.07.002>
  86. Mi B, Chen L, Panayi AC, et al. Serum *Mycoplasma pneumoniae* IgG in COVID-19: A protective factor. medRxiv 2020. <https://doi.org/10.1101/2020.04.12.20060079>
  87. Li Y, Wang H, Wang F, et al. Co-infections of SARS-CoV-2 with multiple common respiratory pathogens in infected children: A retrospective study. *Med(Baltimore)* 2021;100(11):e24315. <http://dx.doi.org/10.1097/MD.00000000000024315>
  88. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci* 2014;69:S4-S9. <http://dx.doi.org/10.1093/gerona/glu057>
  89. Million M, Lagier JC, Tissot-Dupont H, et al. Early combination therapy with hydroxychloroquine and azithromycin reduces mortality in 10,429 COVID-19 outpatients. *Rev Cardiovasc Med* 2021;22(3):1063-1072. <http://dx.doi.org/10.31083/j.rcm2203116>
  90. Kory P, Meduri GU, Varon J, Iglesias J, Marik PE. Review of the Emerging Evidence Demonstrating the Efficacy of Ivermectin in the Prophylaxis and Treatment of COVID-19. *Am J Ther* 2021;28(3):e299-e318. <http://dx.doi.org/10.1097/MJT.0000000000001377>
  91. Chandler LC, Yusuf IH, McClements ME, Barnard AR, MacLaren RE, Xue K. Immunomodulatory Effects of Hydroxychloroquine and Chloroquine in Viral Infections and Their Potential Application in Retinal Gene Therapy. *Int J Mol Sci* 2020;21(14):4972. <http://dx.doi.org/10.3390/ijms21144972>
  92. Sajid MS, Iqbal Z, Muhammad G, Iqbal MU. Immunomodulatory effect of various anti-parasitics: a review. *Parasitology* 2006;132(Pt3):301-313. <http://dx.doi.org/10.1017/S0031182005009108>
  93. Ledford H. Coronavirus breakthrough: dexamethasone is first drug shown to save lives. *Nature* 2020;582(7813):469. <http://dx.doi.org/10.1038/d41586-020-01824-5>
  94. Reznikov LR, Norris MH, Vashisht R, et al. Identification of antiviral antihistamines for COVID-19 repurposing. *Biochem Biophys Res Commun* 2021;538:173-179. <http://dx.doi.org/10.1016/j.bbrc.2020.11.095>
  95. Palma G, Pasqua T, Silvestri G, et al. PI3K $\delta$  Inhibition as a Potential Therapeutic Target in COVID-19. *Front Immunol* 2020;11:2094. <http://dx.doi.org/10.3389/fimmu.2020.02094>
  96. Ciprandi G, Licari A, Filippelli G, Tosca MA, Marseglia GL. Children and adolescents with allergy and/or asthma seem to be protected from coronavirus disease 2019. *Ann Allergy Asthma Immunol* 2020;125(3):361-362. <http://dx.doi.org/10.1016/j.anai.2020.06.001>
  97. Freedberg DE, Conigliaro J, Wang TC, et al. Famotidine Use Is Associated With Improved Clinical Outcomes in Hospitalized COVID-19 Patients: A Propensity Score Matched Retrospective Cohort Study. *Gastroenterology* 2020;159(3):1129-1131.e3. <https://doi.org/10.1053/j.gastro.2020.05.053>
  98. Hogan Ii RB, Hogan Iii RB, Cannon T, et al. Dual-histamine receptor blockade with cetirizine - famotidine reduces pulmonary symptoms in COVID-19 patients. *Pulm Pharmacol Ther* 2020;63:101942. <https://doi.org/10.1016/j.pupt.2020.101942>
  99. Morán Blanco JI, Alvarenga Bonilla JA, Homma S, Suzuki K, Fremont-Smith P, Villar Gómez de Las Heras K. Antihistamines and azithromycin as a treatment for COVID-19 on primary health care - A retrospective observational study in elderly patients. *Pulm Pharmacol Ther* 2021;67:101989. <https://doi.org/10.1016/j.pupt.2021.101989>
  100. Zhang D, Li S, Wang N, Tan HY, Zhang Z, Feng Y. The Cross-Talk Between Gut Microbiota and Lungs in Common Lung Diseases. *Front Microbiol* 2020;11:301. <http://dx.doi.org/10.3389/fmicb.2020.00301>
  101. Yonker LM, Neilan AM, Bartsch YY. Pediatric SARS-CoV-2: clinical presentation, infectivity and immune response. *J Pediatr* 2020;227:45-52.e5. <https://doi.org/10.1016/j.jpeds.2020.08.037>
  102. Mak JWY, Chan FKL, Ng SC. Probiotics and COVID-19: one size does not fit all. *Lancet Gastroenterol Hepatol* 2020;5(7):644-645. [http://dx.doi.org/10.1016/S2468-1253\(20\)30122-9](http://dx.doi.org/10.1016/S2468-1253(20)30122-9)
  103. Wong LR, Zheng J, Wilhelmsen K, et al. Eicosanoid signalling blockade protects middle-aged mice from severe COVID-19. *Nature* 2022;605(7908):146-151. <http://dx.doi.org/10.1038/s41586-022-04630-3>
  104. Mao T, Israelow B, Lucas C, et al. A stem-loop RNA RIG-I agonist protects against acute and chronic SARS-CoV-2 infection in mice. *J Exp Med* 2021;219(1):e20211818. <http://dx.doi.org/10.1084/jem.20211818>
  105. Ma Q, Liu J, Liu Q, et al. Global Percentage of Asymptomatic SARS-CoV-2 Infections Among the Tested Population and Individuals With Confirmed COVID-19 Diagnosis:A Systematic Review and Meta-analysis. *JAMA Netw Open* 2021;4(12):e2137257. <http://dx.doi.org/10.1001/jamanetworkopen.2021.37257>