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REVIEW

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Children and COVID-19: Microbiological and immunological insights

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Abstract

Since its first description in China, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide being declared a pandemic by the World Health Organization. More than 10.3 million people have been infected and more than 506 000 people died. However, SARS-CoV-2 had a lower impact on the pediatric population. Only about 1% to 2% of infected people are children and few deaths under the age of 14 are described so far. In this article, we discuss microbiological and immunological characteristics of SARS-CoV-2 infection in children highlighting the main differences from adult SARS-CoV-2 infection.

KEYWORDS

children, COVID-19, immunology, novel coronavirus, SARS-CoV-2

1 | INTRODUCTION

Initially described in China, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has now spread all over the world causing more than 10.3 million cases and 506 000 deaths, being declared a pandemic by the World Health Organization. SARS-CoV-2 is having a massive impact on human life, from health to social behaviors and economical assets of every continent, prompting unprecedent efforts from the scientific and medical communities to shed light on this emerging scourge and to rapidly identify effective prophylactic and therapeutic tools. Among the many unknowns, the enigma of SARS-CoV-2 in children is a major one. Children represent about 1% to 2% of the total SARS-CoV-2 burden, critical illness is currently rare and it is concentrated in the youngest infants, while most children develop mild symptoms or are asymptomatic.[1,2] Dong et al[3] described a series of 2143 confirmed or highly suspected Chinese children and reported severe and critical cases in 10.6%, 7.3%, 4.2%, 4.1%, and 3.0% for the age group of less than 1, 1 to 5, 6 to 10, 11 to 15, and more than 15 years, respectively. Understanding the reasons of these differences can help researchers and policymakers to design tailored treatment strategies and prevention measures.

Looking back at the most recent pandemics of the last hundred years, this is not a new scenario. During the 1918 "Spanish flu," people older than 65 years and younger than 15 had little or no change in mortality compared with the previous influenza season, while the other age groups registered higher death rates.[4] Similar differences have been described during the 2009 H1N1 pandemic influenza.[5] Moreover, in contrast to what commonly observed in young children, teenagers, and adults are known to develop severe manifestations of viral infections like rubella, chickenpox, and mononucleosis.[5]

However, we lack a clear understanding of the pathogenesis and clinical mechanisms underlying these age-related differences in the ability to control viral infections in general and SARS-CoV-2 in particular. For this reason, beginning from a summary of current knowledge of the clinical features of coronavirus disease 201 (COVID-19) in children, we review the main epidemiological,

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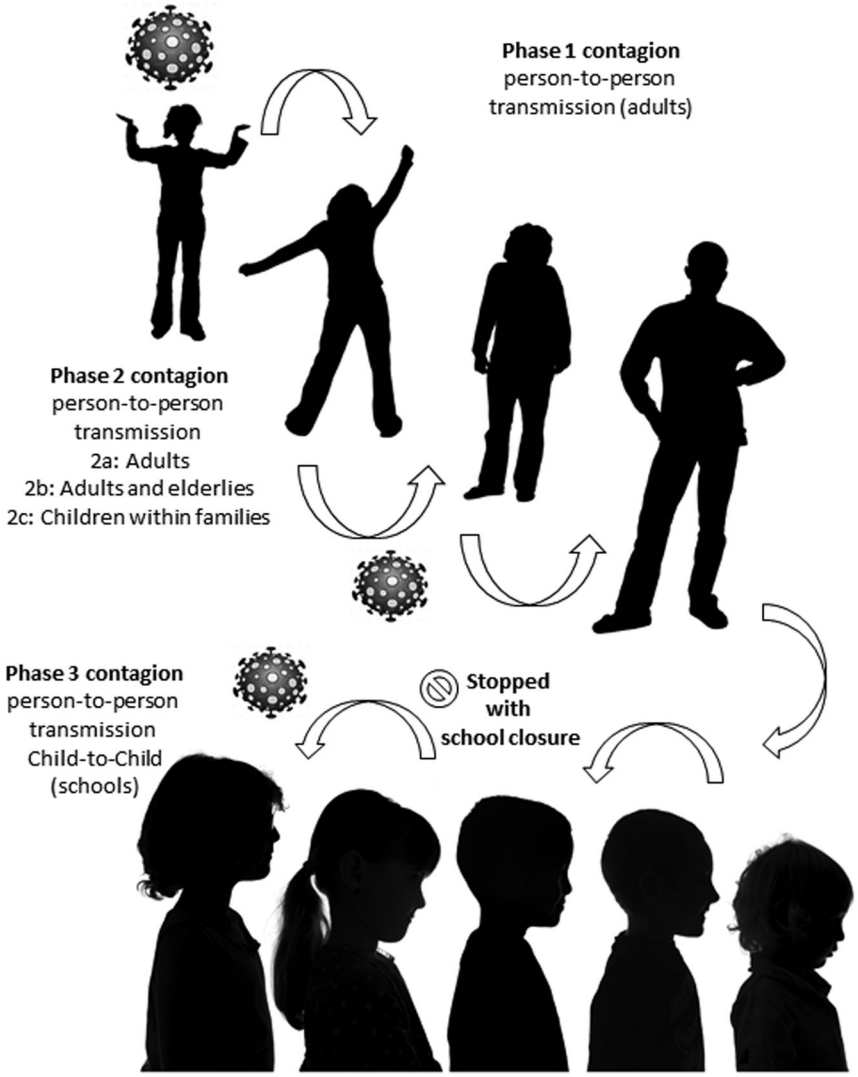

FIGURE 1 Patterns of SARS-CoV-2 spread within the community. In an initial phase of diffusion (phase 1), the person-to-person spread has been proved, mainly involving young adults. In a second phase, diffusion within work environments and family is described, involving older people. In this phase, sons of infected adults have been infected by the virus. Several studies show that children are the last infected in family clusters. In a third phase, children might contribute with the further spread of the infection, being mainly asymptomatic/paucy-symptomatic and proved to spread the virus by the fecal route for longer periods. The lockdown with school closure has stopped this phase almost in every country. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

microbiological, microbiota, and immunological mechanisms that might help us unravel differences of COVID-19 severity in children and adults.

2 | ASYMPTOMATIC CARRIERS. DO CHILDREN PLAY A ROLE?

Cao et al[6] described the dynamic characteristics of children with SARS-CoV-2 infection. First of all, they noticed a strong similarity with the past outbreaks of severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), when very few pediatric patients were reported.[7,8] Analyzing the current SARS-CoV-2 infection, they found that the first confirmed pediatric case was described in Shenzhen on 20 January 2020.[9] Similarly, in a large series describing 44 672 laboratory-confirmed cases, only 416 (0.9%) were less than 10 years of age and 549 (1.2%) between 10 and 20 years of age.[10] The authors noticed that the number of infections in children increased soon after a significant rise in the number of infected adults. These findings let the authors speculate that during the initial phase of the SARS-CoV-2 outbreak, the infection was disseminated by person-to-person transmission in the community almost exclusively among adults. Only after this stage, dated around mid-January 2020, the virus spread within full family groups from adults to the elderly and, eventually, children. In fact, the first pediatric case was identified in a familial cluster.[9] Other studies described that all children had at least one infected family member and they were always the last infected.[11] On a further phase, when cases raised again, the first infant (3 months old) case was reported from Xiaogan, Hubei province.[12] This was probably the first described case of an infant diagnosed before the onset of the illness in the parents.

The authors hypothesize that in China, if restrictive measures would not have been employed and the transmission further extended, the outbreak could have proceeded into an explosion stage, with children beginning intraschool transmission mixed with a wider community spread.[6] Children at this stage might play a more significant role as the main spreader of SARS-CoV-2 because they are usually asymptomatic or have mild symptoms difficult to distinguish from other viral infections.[6]

The impact of asymptomatic/paucisymptomatic carriers on SARS-CoV-2 spread has been evaluated by Li et al[13] using observations of reported infections in China, mobility data, a networked dynamic metapopulation model, and Bayesian inference. They estimated that 86% of all infections were unreported (95% confidence interval: [82%-90%]) before 23rd January 2020, when travel restrictions began and that undocumented infections were the source of infection for 79% of documented cases. Figure 1 summarizes possible diffusion patterns in the community.

The potential role of children in spreading the infection would be confirmed also by the findings of Xu et al.[14] They evaluated 10 children and followed the pattern of viral excretion from respiratory and gastrointestinal tracts using reverse transcriptase-polymerase chain reaction nasopharyngeal and rectal swab. A total of 8 of 10 patients (including the asymptomatic ones) had persistently positive real-time RT-PCR tests of rectal swabs even after their nasopharyngeal testing had become negative, with rectal viral loads being higher than the nasopharyngeal ones. These findings confirm that in a later phase of the epidemics, children can spread the infection and that school closure is an important preventive strategy. Indeed, the fecal-oral transmission does exist with other respiratory viruses.[15]

To better understand the effectiveness of school closures and other social distancing practices during the COVID-19 pandemic, Viner et al[16] undertook a systematic review that included 16 of 616 identified articles. Data from the SARS outbreak suggested that school closures did not contribute to the reduction of the epidemic burden, while modeling studies of COVID-19 predicted that school closures alone would prevent 2% to 4% of deaths. Although these findings predict that school closure would be less effective than other social distancing interventions, still highlight a potential contribution of children in the contagion chain.

3 | SARS-COV-2/HOST INTERACTION

Growing evidences indicate that angiotensin-converting enzyme II (ACE2) is the host receptor for the SARS-CoV-2.[17,18] Previous studies already showed a positive correlation between ACE2 expression and SARS-CoV infection in vitro.[19,20] The ACE2 was also the cell receptor for SARS-CoV21-23,[21] though the spike (S) protein of SARS-CoV-2 binds ACE2 with approximately 10- to 20-fold higher affinity than the S protein of SARS-CoV, suggesting its direct role for the higher spread of SARS-CoV-2 in human populations compared with previous coronaviruses including SARS-CoV.[21]

As a consequence, since ACE2 expression may influence the virus/host relationship and virus diffusion, it is possible to speculate that a different expression level (or expression pattern) of ACE2 in different tissues might be critical for the susceptibility, symptoms, and outcome of COVID-19 in general,[22] and might also explain why the pediatric population, if they had a lesser expression of ACE2 receptor, presents milder forms of COVID-19.

In support of this hypothesis comes the observation that SARS-CoV or NL63 S protein showed a reduced affinity for some ACE2 variants.[23] However, the genetic basis of ACE2 expression and function in different populations and age groups are still poorly characterized. Cao et al[22] systematically analyzed coding-region variants in ACE2 and the expression of quantitative trait loci variants using the GTEx. They found that the East Asian populations have much higher ACE2 expression in tissues, which may provide a potential explanation for the susceptibility to SARS-CoV-2 of the human population where the virus emerged. We also know from analyses on 430 000 human lung cells (non-SARS-CoV-2 infection) that more than 80% of the ACE2 in the lung was distributed on the surface of type II alveolar epithelial cells (AT2)[24] and this might explain the tropism of the virus for the alveoli and hence for the classical lung disease during COVID-19.

Moreover, the expression of the ACE2 receptor can be influenced by several factors, such as age. For example, in rodents, pulmonary ACE2 expression is developmentally regulated, being highest at an early age and lowest when mice reached adulthood. Interestingly, studies in rat lungs confirmed that ACE2 is predominantly expressed in the alveolar and bronchiolar epithelium, with ACE2 expression dramatically reduced with aging in both genders, with old male rats showing a more pronounced reduction compared with old female rats.[25] It remains to be determined whether ACE2 is differentially regulated in children and adults and the elderly, thus providing a molecular mechanism for the enhanced disease severity in the highest age group.

Other mechanisms may be responsible for the higher rates of COVID-19 pneumonia in adults compared with children. The mucociliary clearance (MCC) is one important mechanism of defense in preventing that viral and bacterial infections reach the lower airways.[26-28] ACE2 is expressed in the ciliated cells of the respiratory epithelium where it can serve as a target for coronavirus attachment and internalization.[29,30] Intriguingly, old mice show a significantly reduced MCC function in the upper and lower airways compared to young mice, due to reduced secretion of Cl^- and Muc5b, the major secreted mucin, and most importantly reduced ciliary beat frequency.[28] Hence, the damage on the upper respiratory epithelium in the elderly may further impair cilia function, hampering viral clearance, and increasing the possibility for the virus to reach the lung alveoli and promote the pathological process that leads to pneumonia. Recent data showing that cystic fibrosis is less likely to develop severe COVID-19 does not lend support to this hypothesis, though factors other than cilia function, may be responsible for this outcome (eg, use of long term azithromycin or other inhalers; presence of thick mucus).[31]

These data suggest that differences exist in the host/pathogen interaction in both the upper and lower respiratory tract in different age groups, highlighting potential clues in understanding why only a minority of children are infected with SARS-CoV-2.

4 | MICROBIOTA

Several studies in the last decade documenting the impact of the microbiota on the innate and adaptive immunity. The interplay between microbes and host is dynamic, beginning from birth and evolving over time.[32] Evidences show that microbiota abnormalities (or its absence in

experimental conditions) have consequences on the development of lymphoid tissues, production of secretory immunoglobulin A, abnormalities in intestinal T cell development, and absorption of dietary elements contributing the to the immunity.[33-37] Also, the microbiota is able to influence the host/virus relationships.[38-40]

The influence of the intestinal microbiota composition on vaccine responses in adults and children has been recently reviewed, highlighting the interplay between host immune responses and viral infections.[41] In infants, a prevalence of the phylum Actinobacteria is associated with both higher humoral and cellular vaccine responses to oral, and parenteral vaccines,[42] while a prevalence of the phylum Proteobacteria is associated with lower humoral and cellular responses to the same vaccines.[42] Also, in infants and adults, a higher presence of the phylum Firmicutes is associated with higher humoral and cellular responses to oral vaccines,[43,44] while the phylum Bacteroidetes is associated with lower humoral responses to oral vaccines in infants.[43] These differences in microbiota compositional translate in relevant functional differences.[45] Children have an overrepresentation of the glycan degradation pathways, riboflavin, pyridoxine, and folate biosynthesis pathways and of catabolic pathways (valine, leucine, and isoleucine degradation), as compared to biosynthetic pathways in adults (valine, leucine, and isoleucine biosynthesis).[45] Hence, microbiota composition can influence vaccine responses, especially early in life and, since differences in microbiota compositions in age groups have been clearly documented,[32] we can expect that it might influence immune responses to viral infections as well. It remains to be determined the impact of microbiome composition on SARS-CoV-2 pathogenesis and COVID-19 severity and whether different age-related microbiota compositions may help explain the better outcomes observed in children.

5 | IMMUNE PATHOGENESIS OF COVID-19

Autopsy studies of COVID-19 pneumonia show that inflammatory response plays a primary role: edema and inflammatory cell infiltration, severe exfoliation of alveolar epithelial cells, alveolar septal widening, damage to alveolar septa, necrosis, infiltration, and hyperplasia.[46] Interferons (IFNs), defensins, dendritic cells, T cells, and humoral responses play primary roles in the host/virus interplay.

When a virus invades the host, pattern recognition receptors recognize the viral nucleic acid and a cascade of reactions begins, eventually promoting the synthesis of type I IFNs. They, in turn, activate the JAK-STAT signal pathway.[47,48] IFNs represent the main antiviral agents, limiting virus spread, and play an immunomodulatory role to promote macrophage phagocytosis. Thus, blocking the production of IFNs has a direct effect on the survival of the virus in the host.[49,50] In fact, although coronaviruses are sensitive to IFNs, it has been shown that the N-protein can act as an antagonist of immune escape protein and host IFN response. Indeed, it acts as a Papain-like Protease (PLpro) that hampers IRF3 phosphorylation that, in turn, inhibits type I IFN induction.[51-54]

A cytokine profile resembling Hemophagocytic lymphohistiocytosis syndrome is associated with COVID-19 disease severity, characterized by increased secretion of interleukin (IL)-2, IL-7, granulocyte-colony stimulating factor, IFN-γ inducible protein 10 (IP-10), monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumor necrosis factor (TNF)-α.[55] However, among these cytokines IFN-α, TNF-α, IL-6, and IL-1 are of particular importance.[56] Binding of SARS-CoV-19 to toll-like receptors triggers the pathway leading to the expression and secretion of IL-1, followed by inflammasome activation. High levels of ATP are correlated with activation of the P2X7 receptor, which belongs to the P2 (purinerg 2) receptor family, having cellular toxicity and mediating autoinflammation. This receptor causes the activation of the inflammasome with the production of mature ILs. IL-1β is then secreted outside the macrophage mediating lung inflammation, fever and fibrosis, and provoking severe respiratory problems. Immune cells are attracted to the place of infection by IL-8, a chemokine that is generated at the inflammatory site.[56,57] IL-1 generated during inflammation by immune cells, fibroblasts, and endothelial cells are a response to the pathogenic virus and play an important role in the pathogenesis of both acute and chronic obstructive respiratory disease and in the progression of pulmonary fibrosis.[57]

T cells, CD4+ T cells, and CD8+ T cells play a significant antiviral role. CD4+ T cells promote the production of virus-specific antibodies by activating T dependent B cells.[46] CD8+ T cells are cytotoxic and can kill virus-infected cells. Studies showed that about 80% of total infiltrative inflammatory cells in the pulmonary interstitium in coronaviruses infected patients are CD8+ cells, highlighting both their primary role in virus clearing and inducing immune injury.[58] Additionally, T helper cells produce proinflammatory cytokines via the NF-kB signaling pathway.[59] However, MERS-CoV induces T cell apoptosis, somehow prolonging the infection and promoting viral survival.[60]

Reports show that humoral immunity is essential to control the persistent phase of Coronaviruses infection.[61-63] The complement also plays a vital role in the host immune response to coronaviruses. C3a and C5a have potent proinflammatory properties and can trigger inflammatory cell recruitment and neutrophil activation. SARS-CoV infection activates the complement pathway and complement signaling contributes itself to disease.[64]

5.1 | Immune system differences between adults and children

Except for viral pathogenicity, the human inflammatory response plays a crucial role in SARS-CoV-2 induced lung injury cases. Therefore, it is important to control cytokine production and inflammatory responses, given that they are responsible for the accumulation of cells and fluids. Do adults and children have differences in immune responses?

The impact of respiratory virus infections on the health of children and adults can be very significant. In early life when the adaptive functions are still underdeveloped, the innate immune system is predominant, while the adaptive immunity plays a fundamental role in the adults.[65] Moreover, physiological ageing is accompanied by decline in immune system function and immune alteration during ageing increases susceptibility to infections.

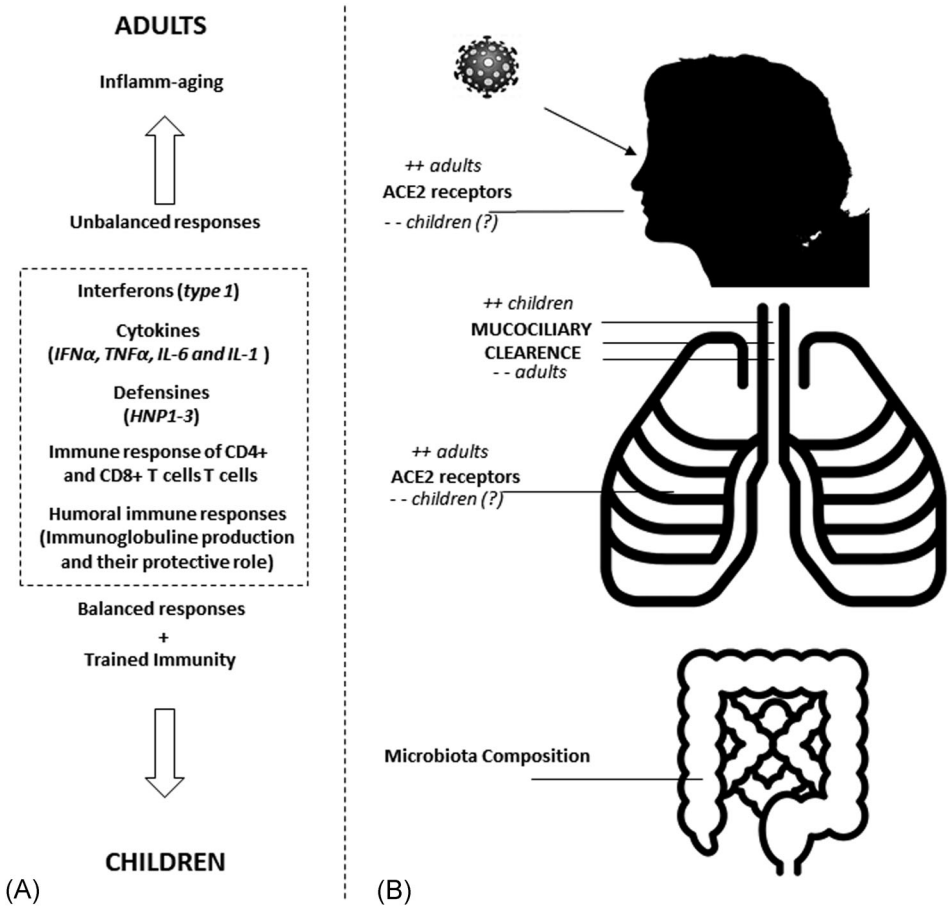

FIGURE 2 COVID-19 is the result of a complex interaction between the virus and the host. A, The immune system plays a significant role in the pathogenesis of SARS-CoV-2 clinical consequences. A proinflammatory background and response to infection may contribute to more severe clinical manifestations of COVID-19 in adults. B, This panel summarizes potential differences in the host-virus interaction in adults and children. ACE2 receptors expression in the upper and lower respiratory tract may be different in adults and children. An impaired mucociliary clearance is described in adults and elders, potentially contributing to more severe lung involvement in adults. Microbiota has proven impact on immune responses to infection. ACE2, angiotensin-converting enzyme II; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Comparisons among age groups from infants through adults reveals progressive declines in the percentage of total lymphocytes and absolute numbers of T and B cells.[66]

The percentages of T cells increase with age from infant to adulthood and then decline. The CD8 T cell percentages increased from childhood to adult in analysis of Valiathan et al[66] and this is supported by previous reports[67] which showed that the numbers of both CD4 and CD8 T cells increase with age. Interestingly, the CD8 T cell percentages decreased significantly in elderly people compared to adults. The B cells showed a continuous decrease from childhood to elderly which is consistent with previous reports.[67] Changes in T cell homeostasis with ageing are associated with a decline in immunity and an increase in inflammation. Increased accumulation of regulatory T cells contributes to impaired CD8 and NK cell activities.[66] Although these differences can play a role in the different COVID-19 manifestations in children and adults, probably the different patterns of cytokine responses in different age groups are more significant in this regard.

Numerous evidences show that cytokine dysregulation plays a key role in the natural remodeling of the immune system in the elderly, with evidence pointing to an inability to fine-control systemic inflammation. This reshaping of cytokine expression pattern with a progressive tendency toward an unbalanced proinflammatory phenotype has been called "inflamm-aging."[68-70] Inflamm-aging is characterized by a subclinical, low-grade, chronic systemic proinflammatory state which contributes to a greater predisposition to illness and worsening of chronic diseases.[71-73] Thus, inflamm-aging seems to be associated with increased morbidity and mortality in the elderly[71] in general, and this can contribute to more significant manifestations of viral diseases, and COVID-19, in adults and elderlies.

This low-grade, chronic systemic proinflammatory state consists of elevated levels of proinflammatory cytokines, such as IL-1, IL-6, and TNF-α, interestingly the same involved in COVID-19. IL-6 is considered the cytokine of gerontologists. Combined with TNF-α, it induces CRP production, which is useful as an inflammatory marker in the aging process and most commonly used in clinical practice.

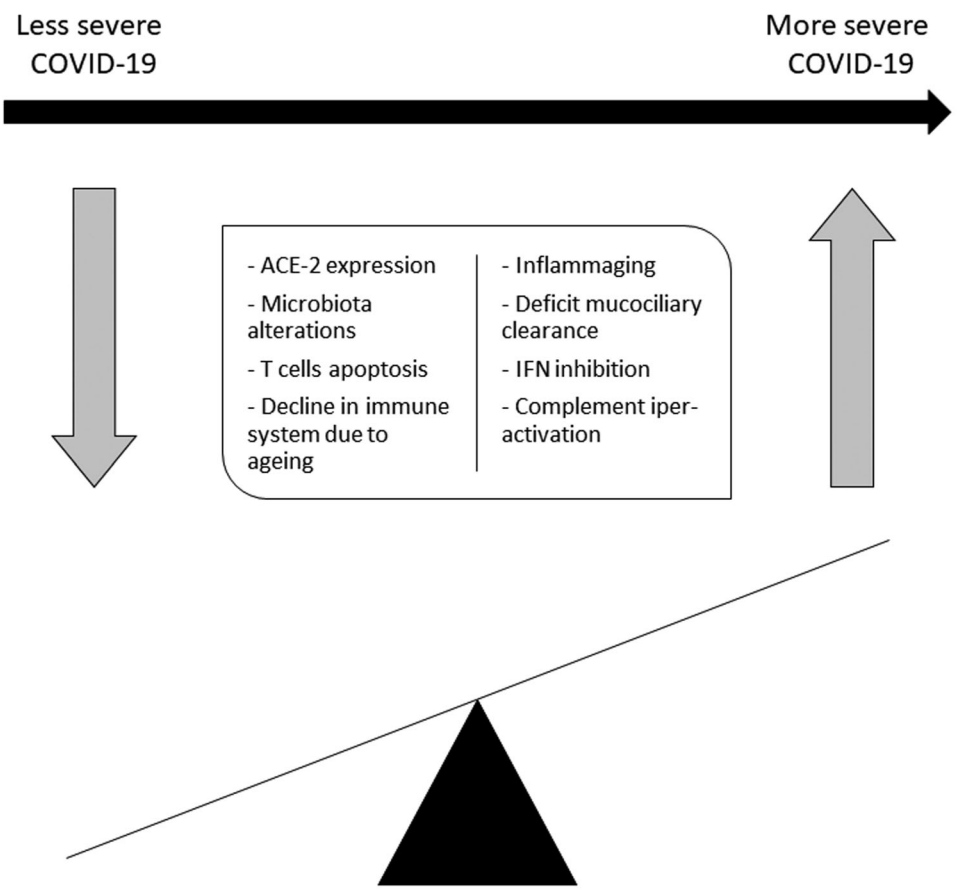

FIGURE 3 Summary of the different immune mechanisms of COVID-19. COVID-19, coronavirus disease 2019

Milan-Mattos et al[71] evaluated the influence of age and gender on levels of C-reactive protein, IL-6, and TNF-α, clearly showing a positive correlation between high-sensitivity C-reactive protein and IL-6 as a function of age. The analysis of the TNF-α level did not demonstrate differences among the five groups, but it was possible to identify a trend toward increased levels of this inflammatory marker in the older.

Importantly, obesity is associated with increased inflammatory markers, particularly IL-6, since adipocytes are sources of IL-6.[74] Several authors also showed that IL-6 is increased in postmenopausal women.[66,71] These two factors also might contribute to the differences between adults and children.

Excess oxidative stress and DNA damage trigger the inflammasome, stimulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and the IL-1β mediated inflammatory cascade. Autophagy, the cell machinery process that removes damaged proteins and large aggregates, is also stronger at an older age and in age-related disease, causing damaged material to accumulate and reduce cellular efficiency. Senescent cells increase with age and in age-related diseases, and the associated secretome produces a self-perpetuating intracellular signaling loop and inflammatory cascade involving the NF-κB, IL-1α, TGF-β, and IL-6 pathway that participates in the proinflammatory milieu. The molecular processes that damp down inflammation include the resolvin family of bioactive molecules, which have been much less evaluated in aging or age-related disease,[68] again potentially contributing in differences of COVID-19 manifestations in adults and children.

Other evidences that aging influences the immune response to viral infections come from Chason et al,[75] which studied how aging affects the nasal epithelium, the main target of influenza infection. Studying the human nasal epithelial cell cultures from older adults, they found increased levels of nasal cytokines and reduction of clearance pathways and antiviral molecules during influenza infection compared with younger patients. Taken together, these results indicate that aging is associated with important changes in the nasal epithelium, contributing to increased severity of disease in older adults through impaired clearance of infected cells.

Also, Moliva et al[76] showed that age-associated changes in the alveolar lining fluid (ALF) may increase susceptibility to *M. tuberculosis* infection and disease by altering soluble mediators of innate immunity. They found the amplification of pro-oxidative and proinflammatory pathways in elderly-ALF and decreased binding capability of surfactant-associated SP-A and SP-D to *M. tuberculosis*. Also, human macrophages infected with elderly-ALF exposed to M. tuberculosis had reduced control and fewer phagosome-lysosome fusion events. In the in vivo animal model, exposure to elderly-ALF exacerbated *M. tuberculosis* infection in young mice. Considering that the innate immunity plays a

primary role in the host response to SARS-CoV-2 and that trained innate immunity in children may be a critical component in preventing the emergence of COVID-19, it can be hypothesized that differences in ALF may contribute to the differential outcomes observed in children and elderly following SARS-CoV-2 infection.

Another potential difference between adults and children resides in the so called "trained immunity." It is well known that young children are exposed to higher frequency than adults and elderly to bacterial and viral infections and are immunized with live attenuated vaccines. These events are known to stimulate the innate immune system, inducing a memory like response termed "trained immunity," which enhances nonspecifically antimicrobial responses.[77] In fact, some authors,[78] speculated that the low incidence of COVID-19 in children living in middle and low-income countries and China may be explained with the routinely use of bacillus calmette–guérin (BCG). Indeed, BCG is a strong inducer of innate and "trained" immunity, inducing "protection" against several infectious agents[79] and in boosting immune responses induced by other vaccines including influenza.[80] The potential beneficial effects of BCG against SARS-CoV-2 infection are being evaluated in a trial aimed at assessing protection in Healthcare Workers Against (ClinicalTrials.gov Identifier: NCT04327206). Altogether, the presented data show that the immune system presents age-related differences that need further investigation to understand how they impact disease severity in adults and children. Figure 2 summarizes immune responses and SARS-CoV-2/host interactions in adults and children.

6 | CONCLUSIONS

In conclusion, growing evidences on larger studies and number of patients are confirming that adults and children have, in general, significantly different clinical/laboratory COVID-19 manifestations. Although the specific reasons of these differences are still unknown, available data suggest that differences in ACE2 expression and basal and active inflammatory/cytokine production (inflamm-aging) may all play a role, while the impact of microbiota and airway clearance mechanisms need to be clarified. Many of the theories presented are speculative, but they may be important research questions (Figure 3). Further studies aimed to study all these aspects are needed to better understand these differences. In particular, it would be important to characterize ACE2 expression in the upper and lower respiratory tract in different age groups; assess in innate and adaptive immune responses including cytokines production following SARS-CoV-2 infections; define the impact of microbiota compositions and how it influences innate/adaptive immune responses to viruses. Understanding these aspects might contribute to a better knowledge of the pathophysiology of COVID-19 and, therefore, speed up the development of therapeutic strategies.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTION

All authors have read and approved the manuscript for submission; have made a substantial contribution to the conception, design, gathering of data and a contribution to the writing and intellectual content of the article.

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