REVIEW

COVID-19 and the Differences in Physiological Background Between Children and Adults and Their Clinical Consequences

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Summary

The SARS-CoV-2 pandemic has indeed been one of the most significant problems facing the world in the last decade. It has affected (directly or indirectly) the entire population and all age groups. Children have accounted for 1.7 % to 2 % of the diagnosed cases of COVID-19. COVID-19 in children is usually associated with a mild course of the disease and a better survival rate than in adults. In this review, we investigate the different mechanisms which underlie this observation. Generally, we can say that the innate immune response of children is strong because they have a trained immunity, allowing the early control of infection at the site of entry. Suppressed adaptive immunity and a dysfunctional innate immune response is seen in adult patients with severe infections but not in children. This may relate to immunosenescence in the elderly. Another proposed factor is the different receptors for SARS-CoV-2 and their differences in expression between these age groups. In infants and toddlers, effective immune response to viral particles can be modulated by the pre-existing non-specific effect of live attenuated vaccines on innate immunity and vitamin D prophylaxis. However, all the proposed mechanisms require verification in larger cohorts of patients. Our knowledge about SARS-CoV-2 is still developing.

Key words

COVID-19 • Children • Adults • Age-related differences • Disease severity

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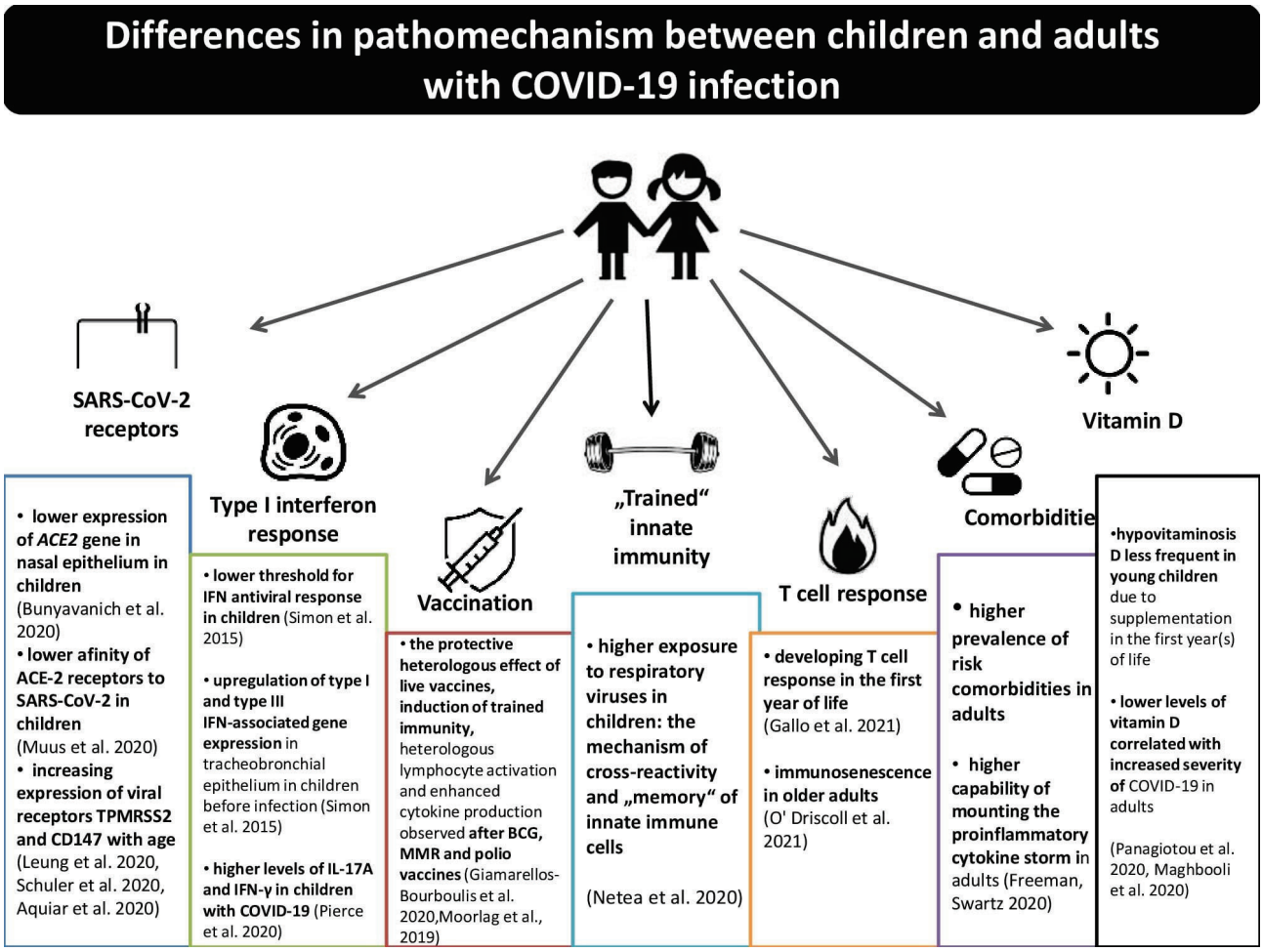

Fig. 1. Main differences in pathomechanism between children and adults with COVID-19 infection.

Introduction

COVID-19 infection caused by SARS-CoV-2 is one of the main problems in the entire population. It has been more than a year since we have had to modify our lives to fight the virus and stay healthy. Children have accounted for 1.7 % to 2 % of the diagnosed cases of COVID-19. They often have a milder course of the disease than adults, and child deaths have been rare. The documented risk factors for severe disease in children are very young age and underlying comorbidities (Tsabouri *et al.* 2021). Identification of the potential risk factors in both adults and children would be of immense value to all healthcare professionals in predicting the severe course of

the disease (Marin *et al.* 2021). Severe COVID-19 disease is associated with high and persistent viral loads in adults. A strong innate immune response in children is due to trained immunity, which probably allows early control of infection at the site of entry. Suppressed adaptive immunity and a dysfunctional innate immune response is seen in adult patients with severe infections but not in children. This may relate to immunosenescence in the elderly. Insight into the pathophysiological mechanisms of lower severity in children might be important in devising therapeutics for high-risk adults and the elderly (Dhochak *et al.* 2020).

Epidemiology

The novel coronavirus disease is currently the most serious pandemic of the millennium. It has affected over three million of the population in over 185 countries and caused over 220,000 deaths. Around 10 % of hospitalized patients are critical, and mortality in currently known cases is estimated to be 4 to 7 % (Boban *et al.* 2021). Paediatric mortality ranges from 0.1 to 4 % (Taffarel *et al.* 2021). The precise causes of severe disease are not known, but it appears that host factors primarily rather than viral genetic mutations drive the pathogenesis (Gallo *et al.* 2021). **Age** is a major predictor of mortality, and it is therefore considered a key factor in proposed clinical severity risk scores. The infection fatality ratio has been found to be lowest in children aged 5 to 9 years, with a log-linear increase according to age in individuals older than 30 years. Elderly individuals (75 years and over) have a much higher mortality rate (O'Driscoll *et al.* 2021). Males with the disease are more likely than females to have severe symptoms and require hospitalization. The risk of death if infected with SARS-CoV-2 is significantly higher in males than females, particularly in older individuals. Low rates of prepubertal mortality may be due to the different levels of sex hormones (Castagnoli *et al.* 2020, Sharifi 2020). Androgen levels increase in puberty and are higher in males than females and have been hypothesized as having a role in the severity of COVID-19 in patients (Sharifi *et al.* 2020, Wambier *et al.* 2020). Pre-existing **comorbidities**, such as cardiovascular diseases, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, immunosuppression, obesity, and sickle cell disease predispose patients to an adverse clinical course and increased risk of intubation and death. Cancers, particularly hematological and pulmonary malignancies, are also thought to be a major comorbidity associated with poor outcomes in COVID-19 patients. In children, comorbidities include selected primary immunodeficiencies, syndromological diseases, autism, neurological diseases and autoimmune diseases. These conditions and very young age (less than 1 year) are associated with a more severe course of the disease. Trained immunity is also insufficient in the first year of life (Gallo *et al.* 2021).

Clinical picture

The coronavirus disease is spread through human to human transmission, typically through close contact *via* respiratory droplets produced by sneezing, coughing and even breathing and talking. The initial reproductive number of transmissions is estimated to be from 2.2 to 3.9. The incubation period averages between 3 and 5 days, with an interval range of 2 days to 2 weeks (Boban *et al.* 2021). The clinical presentation of COVID-19 infection can involve multiorgan systems, with mild to moderate and severe symptoms (Table 1) (Machhi *et al.* 2020). Symptomatic patients typically manifest sore throat, nasal congestion, fever, malaise, dyspnea and cough from 4 to 7 days. Other clinical symptoms include anosmia, ageusia, muscle weakness, tiredness, headache and diarrhea. Viral pneumonia can develop from day 5 to the second or third week, with hypoxemia depending on the clinical severity (Boban *et al.* 2021). Fever, cough, or shortness of breath were described in 73 % of children and 93 % of adults. Myalgia, sore throat, headache and diarrhea were also less commonly reported by pediatric patients. Children are very often asymptomatic or only have a mild course of the disease. However, children aged less than 1 year accounted for the highest hospitalization rate (Sinaei *et al.* 2021). The occurrence of severe and critical disease in children was 10.6 % in children aged less than 1 year, 7.3 % aged 1 to 5 years, 4.2 % aged 6 to 10 years, 4.1 % aged 11 to 15 years, and 3 % aged 16 to 17 years (Dong *et al.* 2020). Some patients experience a severe course of SARS-CoV-2 infection characterized by hyper-proinflammatory response or a cytokine storm, which leads to **acute respiratory distress syndrome** (**ARDS**). This is more typical in adults, although **multisystem inflammatory syndromes** can also occur in **children** and young **adults** (**MIS-C**, **MIS-A**) (Sinaei *et al.* 2021).

Table 1. Clinical presentation of COVID-19 infection.

Clinical presentation of COVID-19 infection				
Respiratory system	**Nervous system**	**Gastrointestinal system**	**Cardiovascular system**	**Uropoetic system**
Mild disease				
Cough Sore throat Rhinorrhea Sneezing Dry cough	Hyposmia-anosmia Hypogeusia-ageusia Visual disturbance Fatigue, somnolence Depressed mood Anxiety, Insomnia Anger, Fear	Nausea Vomiting Diarrhea	Chest pain Arrythmia Sinus tachycardia Blood coagulation	Proteinuria Hematuria
Moderate disease				
Pneumonia Dyspnoea Moderate hypoxemia	Headaches Dizzines, Myalgia Ataxia, Encephalopathy Depression	Loss of apetite Abdominal pain Bloating	Cardiac inflammation Thromboembolism Cytokine storm	Acute renal injury
Severe disease				
Severe hypoxemia Acute respiratory distress syndrome Respiratory failure and death	Cerebrovascular disease Meningoencephalitis Seizures Gullain-Barré syndrome Coma	Gastrointestinal bleeding Gastrointestinal viral dissemination	Cardiomyopathy Acute heart failure Pulmonary embolism Disseminated intravascular coagulation	Renal failure

MIS-C can develop 4 to 6 weeks after primary COVID-19 infection, which is usually mild or asymptomatic. The main symptoms are persistent fever (more than 3 to 5 days), gastrointestinal symptoms (abdominal pain, vomiting, diarrhea), evidence of mucocutaneous inflammation (rash, conjunctivitis, oromucosal changes), lymphopenia, and high levels of circulating acute phase reactants (CRP, IL-6, ferritin and procoagulant factors). Severe MIS-C includes hypotension or shock and evidence of cardiac involvement with myocarditis, myocardial dysfunction and coronary artery changes. Fatal cases are rare (2 %) (Vogel *et al.* 2021). The syndrome has clinical similarities with Kawasaki disease (KD) but also some distinct features. MIS-C affects older children and adolescents, which is in marked contrast to the epidemiology of KD, which occurs predominantly in children 5 years of age or less and with a peak incidence at 9 to 11 months of age. MIS-C manifests with a higher incidence of myocardial dysfunction and gastrointestinal symptoms than KD. Thrombocytopenia is typical of MIS-C and usually not observed in KD. Kawasaki disease shock syndrome (KDSS), a rare form of KD, has many similarities to MIS-C and is often associated with myocarditis and prolonged myocardial dysfunction (Yasuhara *et al.* 2021).

A subset of adult patients experiences a severe hyperinflammatory response known as **MIS-A** during primary SARS-CoV-2 infection. MIS-A is a severe illness in a person aged over 21 years, with laboratory evidence of current or previous (within 12 weeks) SARS-CoV-2 infection, severe extrapulmonary organ dysfunction (including thrombosis), laboratory evidence of severe inflammation, and the absence of severe respiratory disease. The severity of cardiac dysfunction, incidence of thrombosis and mortality of MIS-A may be

higher than MIS-C (Vogel *et al.* 2021).

Other hyperinflammatory syndromes are primary and secondary **haemophagocytic lymphohistiocytosis** (**HLH**) characterized by fever, elevated liver enzymes, hyperferritinaemia, hypertrygliceridaemia, hypofibrinogenaemia, thrombocytepenia and splenomegaly. SARS-CoV-2, like other respiratory viruses, may be considered a potential etiological trigger of HLH. Mehta *et al.* (2020) have suggested a close association and recommended that all patients with severe COVID-19 should be screened for HLH. Loscocco (2020) argue that most of these patients develop ARDS, which shares some HLH features, rather than present systemic macrophage activation (macrophage activation syndrome, MAS), which is the hallmark of HLH. The frequency of HLH in adult patients with severe systemic COVID-19 was described as less than 5 %. In children with MIS-C, the estimated frequency of MAS is higher than in adults (Retamozo *et al.* 2021, Mehta *et al.* 2020, Loscocco 2020).

Physiological background of COVID-19 infection

To explore the differences between children and adults with COVID-19 infection, we should start with the physiological background of COVID-19 infection. The main differences between children and adults are in the **expression of viral receptors**, which are required for viral entry, **innate immune response and cytokine production**, and **mechanisms of adaptive immune response**. Children and adults have many factors which contribute to a different course of COVID-19. These factors include comorbidities, trained immunity by viral exposure to other respiratory viruses, serum levels of vitamin D, etc. This section of the article examines the pathophysiology of COVID-19 and its differences between children and adults.

SARS-CoV-2 characteristics

The novel coronavirus named SARS-CoV-2 belongs to the *Coronaviridae* family of viruses. Coronaviruses are large enveloped viruses (80 to 120 nm) which contain single-stranded ribonucleic acid (RNA). Their genome is one of the largest RNA viruses, containing 27,000 to 32,000 nucleotides. They are divided into four genera according to specific genomes: alpha, beta, gamma, and delta. The alpha and beta coronaviruses infect only mammals. The most dangerous of the coronaviruses, SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus), MERS-CoV (Middle East Respiratory Syndrome Coronavirus) and SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) are betacoronaviruses (Rabi *et al.* 2020). The genome of SARS-CoV-2 has an 82 % nucleotide identity with SARS-CoV and contains 14 open reading frames (ORFs) (Chan *et al.* 2020). It encodes 16 non-structural proteins (Nsp1-16), 4 structural proteins and 9 accessory proteins (ORF 3a, 3b, 6, 7a, 7b, 8, 9b, 9c, and 10) (Chan *et al.* 2020). The structural proteins are spike (S), membrane (M), nucleocapsid (N) and viral envelope (E). The most important of them is the spike protein, which is a binding protein that interacts with cell surfaces. Mutations in the spike protein structure determine the diversity of SARS-CoV-2 coronavirus variants, affect their antigenicity and the binding of neutralizing antibodies (Harwey *et al.* 2021). To understand the differences between the immune response in children and adults and the possible modifying factors, it is necessary to mention the life cycle of SARS-CoV-2.

SARS-CoV-2 receptors

The most common manner of virus transmission is droplet infection. SARS-CoV-2 interacts with the airway epithelium through the S protein. Investigations of the SARS-CoV infection have found that the virus interacts with **angiotensin converting enzyme 2 (ACE2) receptors**, an observation confirmed by other SARS-CoV-2 studies (Chen *et al.* 2020, Walls *et al.* 2020). ACE2 receptors are expressed predominantly in the upper airway epithelium, lungs (type II pneumocytes), kidney and bladder, myocardium, ileum, and colon (Salamanna *et al.* 2020). SARS-CoV-2 bound to ACE2 receptors in the lungs forms a complex. This complex is subsequently proteolytically cleaved by type 2 transmembrane protease (TMPRSS2), leading to S protein activation and facilitating SARS-CoV-2 entry into cells (Hoffmann *et al.* 2020). The level of ACE2 expression in these organs is relatively low, and according to current knowledge, other receptors are involved in the pathogenesis of SARS-CoV-2 penetration into cells (Fig. 2).

The first is **CD147** (**basigin/EMMPRIN/ extracellular matrix metalloproteinase inducer**) and the second is **GRP78/ BiP** (**Glucose Regulating Protein 78/Binding immunoglobulin protein**). CD147 is a transmembrane glycoprotein, a member of the immunoglobulin superfamily. CD147 is a pleiotropic

molecule critical during fetal development and retinal function and has been shown to play a role in thymic T cell development and many neurological processes (Muramatsu *et al.* 2003, Zhou *et al.* 2005). Wang *et al.* (2020) reported a direct interaction between CD147 and the SARS-CoV-2 spike protein. The loss or blockage of CD147 inhibits SARS-CoV-2 replication; by contrast, CD147 overexpression encourages virus infection. SARS-CoV-2 virions enter host cells through the CD147-spike protein route by endocytosis. The presence of CD147 on peripheral lymphocytes can explain the lymphopenia observed in patients with severe COVID-19 infection (Wang *et al.* 2020). GRP78 is a chaperone usually located in the endoplasmic reticulum of cells. Upon cell stress, overexpression of GRP78 initiates and the proteins translocate to cell membranes, where they can mediate virus entry (Ibrahim *et al.* 2019). This has been observed in studies of SARS-CoV-2 (Ibrahim *et al.* 2020).

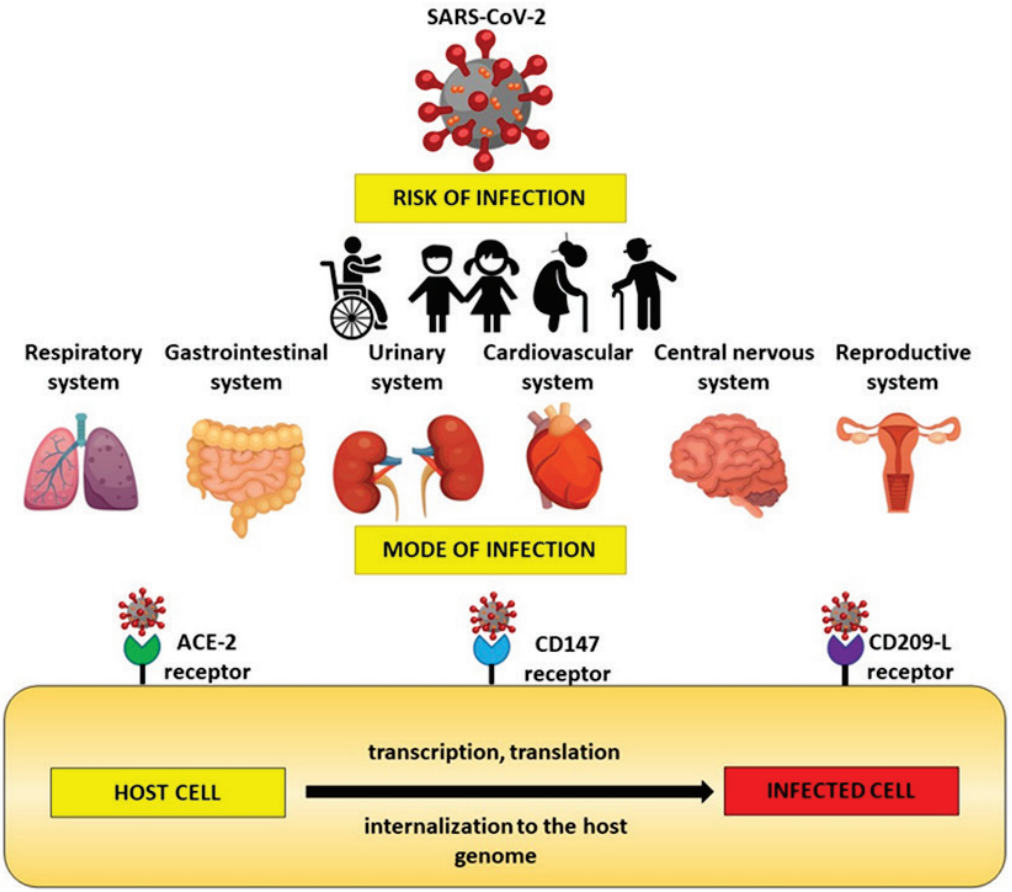

Fig. 2. Coronavirus infectivity and target receptors of interaction.

Innate immune response to SARS-CoV-2

The first defense against any pathogen is the innate immune response. After viral penetration of the airways, pathogen associated molecular patterns (PAMPs) are recognized by antigen presenting cells, mainly tissue macrophages and dendritic cells. PAMPs interact with the pathogen recognition receptors (PRRs) of cells during the innate immune response and produce inflammatory cytokines (TNF, IL-1b, IL-6, IL-8, and IL-12) and interferons (Kumar *et al.* 2009). Neutrophils and natural killer (NK) cells are involved in the amplification of inflammation. They migrate to the site of local inflammation and have the ability to phagocytose or act as "natural" killers. An important step in the subsequent initiation of an adaptive immune response and control of viral replication is the production of type I interferon. The antiviral state is induced in the cell through the stimulation of the synthesis of two enzymes: protein kinase and oligoadenylate synthetase. Protein kinase phosphorylates and inactivates translation factors to stop translation in the cell. The second enzyme, oligoadenylate synthetase, catalyses the formation of

special oligonucleotides which activate RNase L, degrading mRNA and rRNA (Kindler 2016). In interferon pathways, the signals from PRRs are transduced to the nucleus through the activation of a stimulator of IFN genes (STING) and a mitochondrial antiviral-signalling protein (MAVS). This leads to phosphorylation of interferon regulatory factors (IRFs), mainly IRF 3 and 7. Phosphorylated dimers of IRF3/7 translocate to the nucleus, where they can trigger expression of IFN and interferon-stimulated genes (ISGs). Interferon-stimulated genes and other IFN-I-induced molecules (including proinflammatory cytokines) have diverse functions (Schneider *et al.* 2014). The result of their action is to stop translation, increase the breakdown of nucleic acids of the virus, induce tissue repair, and trigger a prolonged adaptive immune response against viruses.

The type I IFN response is a crucial factor in the severity of disease, as observed in MERS infection (Shokri *et al.* 2019). SARS-CoV-2 has different tools to affect IFN-I induction and IFN-I signalling pathways. Viral ORFs and non-structural proteins can impair viral sensing, activation of IRF3, downstream signalling, and the expression of IFN-I. After the production of IFN-I, they can inhibit binding of IFN-I to its receptors on cell surfaces and disable JAK/STAT1 activation, which is needed for IFN-I actions (Kopecky-Bromberg *et al.* 2007, Lu *et al.* 2011, Ribero *et al.* 2020). The total result is impaired viral response, persistence of viral particles in cells, and induction of a cytokine storm. High serum levels of proinflammatory cytokines (IL-2, IL-6, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1α and TNF-α), neutrophilia and lymphopenia have been observed in patients with severe COVID-19 infection (Xu *et al.* 2020, Prompetchara *et al.* 2020, Bobcakova *et al.* 2021).

In patients with severe COVID-19 infection, the role of NLR family pyrin domain containing 3 (NLPR3) inflammasome should also be estimated. Inflammasomes are macromolecular complexes in macrophages. They react to different stimuli, and the result of their activation is the production of active IL-1β. Patients with a sustained and unregulated NLRP3-dependent inflammatory response can develop severe inflammation and severe clinical symptoms through pyroptosis induced by an NLRP3 inflammasome. SARS-CoV ORF8b is reported to activate NLRP3 by binding to the leucine rich-repeat (LRR) domain, suggesting a mechanism of coronavirus-induced NLRP3 direct activation (Shi 2019). Gene variants in genes which encode inflammasome proteins may also contribute to different disease outcomes and may be the cause of the variable response to SARS-CoV-2 (Freeman and Swartz 2020, Merad and Martin 2020).

Adaptive immune response to SARS-CoV-2

Antigen presenting cells (APCs), mainly dendritic cells, represent a key link in the adaptive immune response in viral infections. MHC class II molecules on APCs present viral peptides through the T-cell receptors (TCR) on naive T cells, with subsequent maturation and differentiation. T cells differentiate into T helper cells ($CD4^+$) and cytotoxic T cells ($CD8^+$). $CD4^+$T cells are crucial in the production of cytokines, which recruit phagocytes and activate other leukocytes. They also interact with B cells. $CD8^+$ T cells can find and kill virally infected epithelial cells through the release of cytotoxic factors. They are stored inside granules in cytoplasm. One of these mediators is perforin, a protein which can produce pores in cell membranes. Another is granzyme, which enters target cells through these holes produced by perforin. They can initiate apoptosis of infected cells. Another important component of antiviral response is B cell activation and antibody production. Antibodies can either neutralize the virus by binding to viral surface proteins or agglutinate viral particles together, thereby being more easily recognized by immune cells, or induce phagocytosis and activate the complement system (Felsenstein *et al.* 2020).

One important indication of SARS-CoV-2 infection in severe cases is lymphopenia and neutrophilia in peripheral blood. Lymphopenia (according to studies, total lymphocyte count $<1\times10^9$/l) is associated with a severe course of the disease and poorer outcome (Huang *et al.* 2020). The explanations for lymphopenia in COVID-19 infection differ between authors. The main reasons are presumably the increased destruction of lymphocytes induced by the virus or cytokines, or either the increased apoptosis of lymphocytes or redistribution of lymphocytes from peripheral blood to the infected lungs (Tan *et al.* 2020, Terpos *et al.* 2020, Song *et al.* 2020). In the present article, we have already mentioned that peripheral lymphocytes express CD147, which is one of the receptors for virus entry, and can thus increase direct entry of viral particles and the destruction of virally infected lymphocytes (Wang *et al.* 2020). A notable decrease in the total count of peripheral $CD3^+$ T cells and both subsets of $CD4^+$ T and $CD8^+$ T cells has been observed in patients with severe COVID-19 infection

(Chen *et al.* 2020, Diao *et al.* 2020, Song *et al.* 2020, Wang *et al.* 2020, Jiang *et al.* 2020). The total counts of B cells and NK cells in peripheral blood do not indicate any association with disease severity (Bobcakova *et al.* 2021, Jesenak *et al.* 2020). Another interesting fact is that T cells show a decrease depending on the age of the patient (the most severe decrease was seen in patients over 60 years) (Diao *et al.* 2020). This phenomenon in connection with physiological lymphocytosis, which is observed in differential leukograms in children between the fifth day and fifth year of age, might also explain the far better course of COVID-19 infection in children. Not only a decrease in total T cell count but also T cell exhaustion can contribute to a worse outcome in the disease. In our previous study (of 21 COVID-19 patients), in-hospital death was associated with a significantly higher proportion of both PD-1$^+$CD3$^+$CD4$^+$ and PD-1$^+$CD3$^+$CD8$^+$ cells than in a group of survivors (Bobcakova *et al.* 2021). Similar results have also been observed in other studies (Wang *et al.* 2020, Song *et al.* 2020).

Differences between children and adults with SARS-CoV-2 infection

Up to now, we can conclude that children generally have a better outcome from COVID-19 infection. Many different theories have been proposed as a possible explanation. In the present review, we examine the most interesting and plausible of them. The main proposed theories are (Table 2):

1) Differences in receptors and affinity to SARS-CoV-2 in children and adults;
2) Differences in type I interferon (IFN) production;
3) Differences in innate, trained immunity in children;
4) Differences in adaptive immunity and antibody production;
5) Associated comorbidities in adults;

Possibly protective effects of live attenuated vaccines and higher levels of vitamin D in children.

Differences in receptors and their affinity to SARS-CoV-2 in children and adults

As previously mentioned, the most important receptor for SARS-CoV-2 in the respiratory tract is ACE2. However, another two receptors for SARS-CoV-2 have been identified: CD147 and GRP78. Another factor required for entry of the virus into cells is TMPRSS2, a proteinase (required for cleavage of the S protein of the virus) associated with ACE2. Differences in the above-mentioned receptors and proteinase have been identified in children and adults. Expression of ACE2 in the nasal epithelium is different in children compared to adults. Bunyavanich *et al.* (2020) found age dependent ACE2 gene expression in the nasal epithelium. ACE2 gene expression was lowest in children (<10 years) and increased with age. The study was limited by not containing adults older than 60 years (Bunyavanich *et al.* 2020). The suggested mechanism is that fewer viral particles can attach to the respiratory epithelium, which is why children are affected with COVID-19 less frequently and with less severity than adults. Expression of ACE2 gene increases with age and can be modulated by environmental factors, for example, tobacco smoking increases ACE2 gene expression in bronchial epithelial cell samples (Aguiar *et al.* 2020, Leung *et al.* 2020). Another interesting fact is the different affinity of ACE2 to SARS-CoV-2 in adults and children. Children have a lower affinity of ACE2 receptors to SARS-CoV-2 (Muus *et al.* 2020). Expression of TPMRSS2 and CD147 also appears to increase with age, but more studies are needed to confirm this observation (Leung *et al.* 2020, Schuler *et al.* 2020, Aquiar *et al.* 2020).

Differences in type I interferon production

Type I interferon responses are required for early destruction of the virus and activation of host defense mechanisms. SARS-CoV-2 has a long incubation period and the ability to delay and decrease the production of interferon type I. Yoshikawa *et al.* (2010) studied the dynamics of innate immune responses to SARS-Co-V infection and observed that activation of the interferon regulatory factors (namely IRF 3/7) occurred not sooner than 48 h post-inoculation (Yoshikawa *et al.* 2010). Children have an advantage over adults. In the first years of life, they suffer from recurrent respiratory infections and thus have stronger and trained innate immune responses against viruses. It appears that children are capable of a quicker IFN response during the incubation period of a virus and have a lower threshold for an IFN antiviral response (Simon *et al.* 2015).This suggests that their immune systems can act more rigorously and quickly against SARS-CoV-2. Upregulation of type I and type III IFN-associated gene expression in the tracheobronchial epithelium has also been observed in children before infection (Simon *et al.* 2015). Higher levels of IL-17A and IFN-γ have also been found in children with COVID-19 (Pierce *et al.* 2020). Another

cause of difference and severity is pre-existing autoantibodies against type I interferons in adults. Bastard *et al.* (2020) found that the age-related increase in autoantibodies against type I interferon was associated with severe COVID-19 pneumonia (Bastard *et al.* 2020).

Table 2. Differences in pathomechanisms between children and adults with COVID-19.

Differences in pathomechanisms between children and adults with COVID-19	
Receptors and their affinity to SARS-CoV-2	Lower expression of *ACE2* gene in nasal epithelium in children (Bunyavanich *et al.* 2020) Lower affinity of ACE-2 receptors to SARS-CoV-2 in children (Muus *et al.* 2020) Increasing expression of viral receptors TPMRSS2 and CD 147 with age (Leung *et al.* 2020, Schuler *et al.* 2020, Aquiar *et al.* 2020)
Type I interferon production	Lower threshold for IFN antiviral response in children (Simon *et al.* 2015) Upregulation of type I and type III IFN-associated gene expression in tracheobronchial epithelium in children before infection (Simon *et al.* 2015) Higher levels of IL-17A and IFN-γ in children with COVID-19 (Pierce *et al.* 2020) Pre-existing auto-antibodies against type I interferons associated with severe COVID-19 pneumonia in adults (Bastard *et al.* 2020)
"Trained" innate immunity	Higher exposure to respiratory viruses in children: the mechanism of cross-reactivity and „memory" of innate immune cells (Netea *et al.* 2020)
Associated comorbidities	Higher prevalence of risk comorbidities in adults
Inflammaging in adults	Higher capability of mounting the pro-inflammatory cytokine storm in adults Increased activity of NLRP3 inflammasome associated with severe COVID-19 in adults (Freeman and Swartz 2020)
Protective effects of live vaccines	The protective effect of live vaccines due to induction of „trained" immunity, heterologous lymphocyte activation and enhanced cytokine production observed after BCG, MMR and polio vaccines (Giamarellos-Bourboulis *et al.* 2020, Moorlag *et al.* 2019)
Vitamin D levels	Hypovitaminosis D is less frequent in infants and young children due to recommended supplementation in the first year of life Lower levels of vitamin D were correlated with increased severity of COVID-19 in adults (Panagiotou *et al.* 2020, Maghbooli *et al.* 2020)
T cell response	Developing T cell response in the first year of life: children less than 1 year have more severe disease course (Gallo *et al.* 2021). Immunosenescence in older adults: the age more than 75 years is connected with higher severity and mortality (O'Driscoll *et al.* 2021)

Differences in innate immunity and trained immunity in children and adults

The most important cellular particles of innate immunity are neutrophils and macrophages. Both are capable of phagocytosis, production of proinflammatory cytokines, and amplification of the immune response. They form an important connection between innate and adaptive immunity. However, overreaction of these particles is also dangerous, which we can observe in severe multisystem inflammatory syndromes associated with COVID-19 in children and young adults.

From the neonatal period into adulthood, the

immune response develops progressively. In the first few years, the most important mechanisms are the processes of innate immunity. The role of interferon type I is also significant. The term "trained immunity" refers to the epigenetic changes and metabolic reprogramming in innate immune cells after exposure to certain stimuli (infections, vaccinations, etc.), leading to certain types of "memory" (Netea *et al.* 2020). Children are in frequent contact with many respiratory infections (also less pathogenic coronaviruses), which can lead to a trained immune response to these diseases. The mechanism of cross-reactivity to these viruses and SARS-CoV-2 may be why the immune systems of children can kill the virus more effectively (Netea *et al.* 2020). In children with COVID-19, neutrophilia and neutropenia can be seen in only 6 % and 4.6 %, respectively, of cases (Henry *et al.* 2019). The other approximately 90 % of children infected with SARS-CoV-2 have normal neutrophil counts. In adults with COVID-19, neutrophilia on admission is associated with poor disease outcome (Wang *et al.* 2020). Neutrophils in patients with COVID-19 are capable of forming neutrophil extracellular traps (NETs). They are known as "traps" because they trap circulating platelets, red blood cells and neutrophils. The result of this process is vascular instability and hypercoagulability associated with endothelial damage and cytokine release and systemic inflammation. The differences between children and adults in the formation of NETs are not known (Zhuo *et al.* 2020). Another specific problem in adults and seniors is inflammaging, which refers to a chronic proinflammatory state of the organism which occurs with aging. In connection with comorbidities such as diabetes or cardiovascular diseases, adult patients may experience the development of severe hyperinflammation and a cytokine storm (Franceschi *et al.* 2018). Logically, children are less susceptible to the development of a proinflammatory cytokine storm because their innate immune response in the first few years of life is not so strongly competent. Interestingly, children with COVID-19 admitted to hospital have higher serum levels of IL-17A and IFN-γ, but not TNF-α or IL-6 (Pierce *et al.* 2020). A similar pathomechanism associated with ageing has also been observed in macrophages: severe COVID-19 is marked by the hyperactivation of macrophages. Studies of SARS-CoV and MERS-CoV have shown that acute infection induced by macrophage and neutrophil invasion results in high levels of proinflammatory cytokine, leading to acute lung injury, acute respiratory distress syndrome, and death (Channappanavar *et al.* 2017). In the most severe COVID-19 cases, a marked increase in IL-6 and ferritin levels was observed (Ruan *et al.* 2020). Macrophage hyperactivation in older adults perhaps accounts for increased morbidity and mortality seen in this type of acute infection.

The main macromolecular component required to activate an immune response is an inflammasome. Inflammasome mutations are associated with certain autoinflammatory diseases (Gattorno *et al.* 2008). Uncontrolled or partially controlled activation of inflammasome leads to the production of excess amounts of proinflammatory cytokines, mainly IL-1β (Yang *et al.* 2012). Increased activity of the NLRP3 inflammasome is associated with severe COVID-19 in older patients (Freeman and Swartz 2020). Variations in genes which encode inflammasome proteins may also contribute to different disease outcomes and may be the cause of the variable response to SARS-CoV-2 (Freeman and Swartz 2020, Merad and Martin 2020).

Differences in adaptive immunity and antibody production

The most important cells for adaptive immune response are T cells. Decrease in $CD4^+$ and $CD8^+$ T cells and exhaustion of T cells are linked to severe courses of COVID-19 infection and poorer outcomes. T cell differentiation and selection (either positive or negative) occurs in the thymus. Immunosenescence observed in the elderly is a possible reason for ineffective clearance of SARS-CoV-2. Immunosenescence is the term used for the decline in innate immunity (mainly in reduced neutrophil and macrophage activation and cytotoxic activity of NK cells) and dysregulated T cell response (increase in anergic memory T cells, decline in naive T cells, exhaustion of T helper cells, T cytotoxic cells and B cells) due to thymic involution. An age of 75 years or more is associated with greater disease severity and higher mortality (O'Driscoll *et al.* 2021).

A very similar situation can be observed in children less than 1 year old. The immune system develops very quickly after birth, but in the first year, cellular immunity in children is not very competent. Neonatal T and B cells overexpress the surface features of naive cells (CD45RA). T cells are immature and do not provide sufficient assistance to B cells. $CD4^+$ cells with a T_h2 type immune response and relatively higher production of IL-4, IL-5, IL-13 predominate. After birth, the highest proportion of T cells is represented by

T regulatory cells (T_{reg}), which gradually decrease in number. Neonates also have a reduced B cell mediated humoral response, which is delayed and shorter, and the produced antibodies have lower affinity. During the neonatal period, B1 lymphocytes predominate, producing IL-10 and TGF-β and stimulating a T_h2 response. Suppression of the T_h1 response may reduce the risk of an exaggerated proinflammatory response upon massive exposure to post-birth antigenic stimuli to protect its own structures (Ygberg and Nilsson 2012, Simon *et al.* 2015, Jeseňák *et al.* 2012). Immaturity of the immune system is the reason for increased susceptibility to infections during the perinatal period. The greater the degree of immaturity due to premature birth, the higher the risk of serious infection and sepsis. The components of non-specific immunity in infancy mature first, while the functions of neutrophils, macrophages and dendritic cells remain low. Around the first year, the T_h1 response and production of memory T cells increases (Ygberg and Nilsson 2012, Simon *et al.* 2015). A very young age (less than 1 year) is associated with a severer course of disease (Gallo *et al.* 2021).

It appears that the most significant components in the immune response to SARS-CoV-2 are T cells and an innate immune system. Antibodies against SARS-CoV-2 are also important components in the immune response, the most significant being IgG antibodies, which target the S protein of the virus. A study by Weisberger *et al.* (2021) found the presence of anti-spike (S) IgG, IgM and IgA antibodies and anti-nucleocapsid (N) IgG antibodies in adult COVID-19 cohorts. Children with or without MIS-C showed a lower breadth of anti-SARS-CoV-2-specific antibodies, predominantly IgG antibodies generated to target the S protein but not the N protein. Children with or without MIS-C had a lower neutralizing activity than both adult COVID-19 cohorts, indicating a reduced protective serological response (Weisberger *et al.* 2021). Another factor in the production of antibodies against SARS-CoV-2 is that children often have limited upper respiratory tract infections associated with shorter durations of antibody production (Galanti and Shaman 2020). Children are therefore probably more susceptible to reinfection with SARS-CoV-2.

Associated comorbidities in adults

Pre-existing **comorbidities**, such as cardiovascular disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, immunosuppression, obesity, and sickle cell disease all predispose patients to an adverse clinical course and increased risk of intubation and death. Cancers, particularly hematological and pulmonary malignancies, are also thought to be major comorbidities associated with poor COVID-19 outcomes (Gallo *et al.* 2021). All can lead to pre-existing cellular stress with hyperinflammatory states and the over-production of proinflammatory cytokines. Logically, this can lead to greater predisposition to a cytokine storm, hypercoagulability, ARDS, and multiorgan dysfunction syndrome. These comorbidities, of course, have a higher prevalence in adults than children. In children, other comorbidities have been identified and include primary immunodeficiencies, syndromological diseases, autism, neurological diseases, and autoimmune diseases. These conditions and very young age (less than 1 year) are associated with a severer course of disease (Gallo *et al.* 2021).

Immunological effects of live vaccines and higher levels of vitamin D in children

To complete the present review, another two factors which may contribute to a less severe course of COVID-19 in children should be mentioned. These two factors play an important role in every viral infection and are not specific to COVID-19. Children are vaccinated according to the national immunization programme in each country: in most of these countries, they receive two doses of the MMR (measles, mumps and rubella) vaccine up to the age of five, and in some, also mandatory vaccination against tuberculosis (BCG). Both of these vaccines are live attenuated vaccines. Previous reviews have shown that vaccination against tuberculosis protects against viral pathogens (experimental studies have shown that the BCG vaccine protects against various DNA and RNA viruses, including herpes and influenza viruses). These effects are thought to be mediated through the induction of trained immunity and heterologous lymphocyte activation, resulting in enhanced cytokine production, macrophage activity, T-cell responses and antibody titres (Giamarellos-Bourboulis *et al.* 2020, Moorlag *et al.* 2019). With the same mechanism, the MMR and oral polio vaccines can also contribute to differences in the severity of COVID-19 (Franklin *et al.* 2020, Chumakov *et al.* 2020).

Vitamin D deficiency appears to negatively correlate with disease severity and radiological findings. Toddlers and infants are supplied daily with vitamin D to

prevent rickets; vitamin D deficiency is therefore less frequent in this group. WHO recommendations for daily vitamin D intake in infants less than 1 year of age, based on randomized controlled trials, are 5 to 10 µg daily (Bouillon 2017). Vitamin D has multiple effects on the innate and adaptive immune response. Vitamin D affects the proliferation of T cells, regulates the T_h1/T_h2 cellular response, induces the formation of T_{reg} lymphocytes, and stimulates immune tolerance. It also affects the production of proinflammatory cytokines, mainly by reducing the production of T_h1 type proinflammatory cytokines and promoting the production of T_h2 type cytokines (Arnsonet *et al.* 2007, Holick 2007). Vitamin D levels are lower in older age groups, especially males (Mosekilde 2007). De Smet *et al.* (2020) found lower vitamin D levels in patients with COVID-19 than controls matched according to sex, age and season (De Smet *et al.* 2020). Vitamin D levels negatively correlate with the severity of radiological findings. Two other studies have found a correlation between low vitamin D levels and COVID-19 severity and mortality (Panagiotou *et al.* 2020, Maghbooli *et al.* 2020). The risk factors in vitamin D deficiency are very similar to the factors associated with a severe course of COVID-19, highlighting the importance of the vitamin D.

Conclusions

COVID-19 infection is still largely a mystery. More knowledge has offered many answers and different therapeutic strategies for specific groups of patients, but many questions remain unanswered and unresolved. All the proposed differences in the pathomechanism of the immune response to SARS-CoV-2 need to be verified in larger patient cohorts. We can conclude that despite all the various differences in the immune response to SARS-CoV-2, the most important component is the innate immune response. The differences in response and the capabilities of the organism to develop a proinflammatory state and the cytokine storm are directly related to the severity of the disease. Perhaps COVID-19 is another important lesson in learning about trained innate immunity and interferon pathways and a challenge to immunologists and researchers to define new and effective therapeutic and prevention strategies.

Conflict of Interest

There is no conflict of interest.

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