Will children reveal their secret? The coronavirus dilemma

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Epidemiological evidence shows that SARS-CoV-2 infection in children is less frequent and severe than adults. Age-related ACE2 receptor expression, lymphocyte count and trained immunity might be the keystone to reveal children's secret. https://bit.ly/2QWpWxK

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Introduction

On 11 March, 2020, a novel human coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became pandemic [1]. By 24 March, 372 757 SARS-CoV-2 confirmed cases and 16 231 related deaths have been reported worldwide [2]. In Italy, 62 844 cases and 5542 deaths have been reported, mostly in northern regions. Detailed data are updated by the Italian National Institute of Health [3].

Available reports suggest that SARS-CoV-2 infection in children appears to be unusual. Among 44 672 confirmed cases, a Chinese Centre of Disease Control and Prevention report showed 416 paediatric confirmed cases in the 0–9 years age group (0.9%) with no fatalities and 549 cases in the 10–19 years age group (1.2%) with one fatality (0.2%) [4]. The latest Italian report showed similar results with 318 (0.5%) confirmed cases in the 0–9 years age group and 386 (0.7%) confirmed cases in the 10–19 years age group. No children were treated in the intensive care unit and no deaths were reported [5].

Since respiratory viral infections are usually more common in children under 5 years of age compared to adults, experts started to question what could be the children hidden secret [6, 7]. A recent study seems to point out that children are just as likely adults to get infected with SARS-CoV-2 [8]. A report from the town of Vò Euganeo (Veneto, Italy), supposedly one of the two starting outbreak spots in northern Italy, showed opposite results. From 22 February to 5 March, 2020, 2778 people were tested for SARS-CoV-2 out of 3500 inhabitants. Swab tests were done in both symptomatic and asymptomatic inhabitants. Collected data showed that only two out of 316 swabs were returned positive in children under 14 years of age [9]. Data on susceptibility to SARS-CoV-2 according to age are conflicting. Dong *et al.* [10] retrospectively analysed the epidemiological characteristics of 2143 children affected by SARS-CoV-2 infection in China, supporting the evidence that children are as susceptible as adults to infection. They found an elevated vulnerability to SARS-CoV-2 among infants, with a proportion of severe and critical cases of 10.6% in this age group (40 out 379 infants). However, the majority of severe and critical cases in the study were not SARS-CoV-2 confirmed, opening the debate whether other untested pathogens could have been responsible for such clinical observations [11]. In fact, Sun *et al.* [12] showed that among eight

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children (age range 2 months to 15 years), who were admitted to the intensive care unit, only two (25%) were under the age of 12 months.

The reasons still remain unclear. The interaction between host immunological response and viral pathogenetic mechanisms might be the keystone.

The doorway

Angiotensin-converting enzyme 2 (ACE2) is a type I membrane protein expressed in many organs such as the lungs (type II alveolar epithelial cells), heart, intestine and kidneys, where it is physiologically involved in maturation of angiotensin II (AngII) [13, 14]. ACE2 has been proven to be the functional receptor of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and, recently, of SARS-CoV-2 [14, 15]. Xu *et al.* [16] found an almost identical three-dimensional structure in the receptor-binding domain of SARS-CoV and SARS-CoV-2 spike proteins. Full-length elucidation of the ACE2 structure also suggests a stronger binding affinity of SARS-CoV-2 to ACE2, along with a more efficient receptor recognition, which may have strong human-to-human transmission implications [16, 17]. Crucially, SARS-CoV and human coronavirus NL63 infections were shown to downregulate ACE2 protein expression [18]. A key role of ACE2 is the conversion of AngII to its metabolite angiotensin-(1–7) (Ang1–7), especially in the lung microenvironment, where ACE2 levels are intrinsically elevated. Ang1–7 has a homeostatic role in the regulation of the renin–angiotensin system (RAS), with anti-hypertensive and pro-fibrotic effects [19, 20]. As a matter of fact, elevation of ACE or low expression of ACE2 can lead to hypertension, chronic heart failure and lung injury [20]. Therefore, since ACE2 seems to act in a protective manner, SARS-CoV-2 could unbalance AngII/Ang1–7 levels and thus lead to inflammation and hypoxia [21].

However, the effect of RAS derangement is not clear. Low levels of ACE2 has been detected in patients with underlying chronic conditions, which normally do not affect the paediatric population [20–22]. In a study by Xie *et al.* [23], ACE2 was seen to dramatically decrease with ageing in rat models. The report of Chen *et al.* [24], encompassing ACE2 genomics, epigenomics and transcriptomics data, supports the evidence that young people seem to be less susceptible to virus detrimental effects, suggesting a negative correlation between ACE2 expression and SARS-CoV-2 severe outcomes. Furthermore, according to their analysis, both oestrogens and androgens, decrements in which are well known with ageing, have shown to upregulate ACE2 expression [24, 25]. Together, this evidence may suggest that the increased concentration of ACE2 receptors in lung pneumocytes in children may have a protective effect from severe clinical manifestations of SARS-CoV-2 infection.

The crux

The SARS-CoV-2 viral genome has been sequenced and it is 75 to 80% identical to SARS-CoV [26]. Genetic and clinical evidence suggests that SARS-CoV-2 has similar pathogenetic mechanisms to SARS-CoV and Middle East respiratory syndrome-related coronavirus (MERS-CoV) [27, 28].

Innate immune cells recognise pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) that include Toll-like receptors (TLRs) and other cytosolic pathogen sensors. PRRs set off the activation of the downstream signalling cascade that lead to the production of type I and III interferons (IFNs) and other proinflammatory mediators, which initiate the host innate and adaptive immune response. Type I IFNs activate the JAK/STAT pathway which plays a critical role in regulating immune response; IFNs can also directly activate immunity through dendritic cells stimulation and they also increase cytotoxic T and NK cell activity [29]. Moreover, NK cells migrate to the infected sites and respond to viruses producing IFN-γ, killing virus-infected cells and boosting the adaptive immune response [29]. Cytokines and IFNs facilitate inflammation, but they are also answerable for lung injury during acute viral infection. In SARS-CoV-2 severe cases, patients have high levels of innate pro-inflammatory cytokine and type I IFNs. Similarly to SARS-CoV and MERS-CoV infections, several reports show increased neutrophil and reduced lymphocyte counts in SARS-CoV-2 patients with the onset of the so called "cytokine storm", supporting the hypothesis of the importance of innate immune response as both a protective and a destructive mechanism [27].

Milder disease presentation in children might be linked to "trained immunity". "Trained immunity" represents an innate immune memory and it is formed by innate immunity cells that became "memory cells" after antigen exposure [30]. Mitroulis *et al.* [31] demonstrated that systemic antigens determine transcriptomic, metabolomic and functional changes in haemopoietic progenitor, leading to the generation of myeloid cells with a faster responsiveness to infections. These modifications not only occur in bone marrow but also in NK cells and innate lymphoid cells group 2 (ILC2). Lung ILC2 were shown to be able to remember their activation status if stimulated by inhaled allergens [32]. Cytomegalovirus and influenza A can trigger a stronger NK mediated secondary innate immune response on reinfection [33]. It is demonstrated that common epigenetic mechanisms determine memory cell development, both in the

adaptive and innate immune system [34]. Trained immune memory is mediated by epigenetic modifications in haemopoietic progenitor and in cells of the innate immunity; it represents a cross protection against various pathogens and it can also be activated by vaccines [30]. After pathogen exposure, increased activation of antigen-presenting cells leads to a nonspecific resistance of the host to reinfection, providing cross-protection to other infections. It is also assumed that vaccines could induce cross-reactivity, training the innate immune system. A growing body of evidence suggests that measles-vaccinated children have a reduction in mortality rates that cannot be explained only by the prevention of measles-related deaths [35]. Several papers have examined the immunomodulating effect of influenza vaccination through the elicitation of NK cytotoxic response. Mysliwska *et al.* [29] investigated the relationship between NK activity in the vaccinated population and specific immune protection against influenza virus and non-specific immune protection against other infections. Monitoring NK activity before and after immunisation, they found it was still significantly elevated 1 month later. They concluded that NK cell activation may confer protection against influenza and other respiratory viral infections. Both frequent viral infections and vaccines in children could induce an innate immune system with an enhanced state of activation, which would result in more effective defence against different pathogens [35]. A relatively low benefit from trained immunity (partial immunisation status and underexposure to viral infections) may explain the epidemiological evidence of more severe clinical presentation among SARS-CoV-2 infected infants compared to older children [10]. Moreover, human neonatal antigen-presenting cells and plasmacytoid dendritic cells have impaired production of type I IFNs and present a bias against the production of Th1 cytokines [36]. Such polarisation, which allows beneficial microbial colonisation, leaves newborns more susceptible to pathogenic infections. The age-dependent maturation of the immune response occurs with repeated stimuli and results in an enhanced innate function (trained immunity), which may protect older children as discussed above.

Adaptive immune response plays also a crucial role in SARS-CoV-2 infection; proinflammatory mediators activate Th1-type immune response (CD4+ and CD8+ T cells) and B lymphocytes that cause an effective virus-specific antibody response [37]. Adults infected by SARS-CoV-2, especially those with a severe disease, usually have decreased lymphocyte count and lymphocytopenia [27, 38, 39]. In children with SARS-CoV-2, peripheral blood lymphocytes remain mostly in the normal range, suggesting less immune dysfunction [30, 40]. In healthy children, this could be related to the fact that lymphocytes, especially NK cells, are constitutionally in a greater amount than in healthy adults. Lymphocyte count is very high in the first months of life and decreases in later childhood and in adolescence [41]. Moreover, lymphocytes could be higher in children even due to frequently experienced viral infections in childhood, as the result of an everlasting immune system activation in the first years of life.

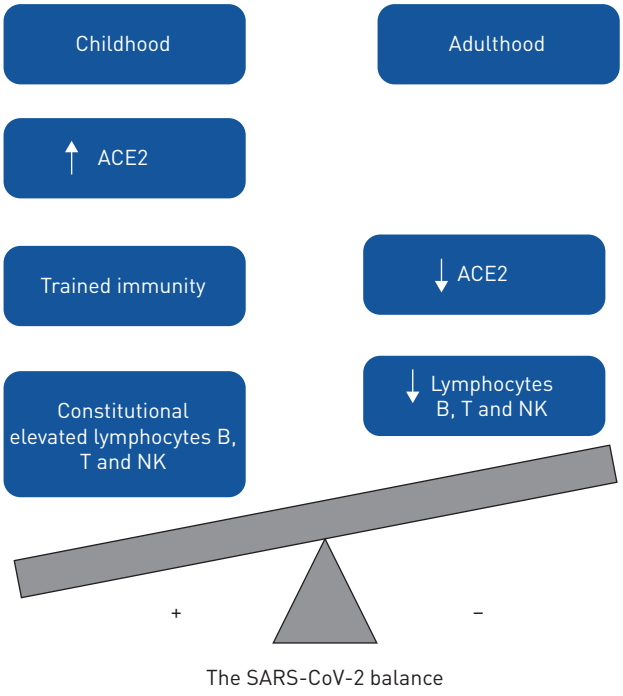

FIGURE 1 The figure illustrates the theoretical constructs and putative immunological and pathogenetic differences between children and adults relative to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Unlike adults, children show constitutionally elevated angiotensin-converting enzyme 2 (ACE2) expression and lymphocyte count. Moreover, they undergo several viral infections and scheduled immunisations, which may boost their innate and adaptive immunity.

Conclusion

We can speculate that high ACE2 receptor concentrations, trained immunity and a constitutional high lymphocyte count in children may partially explain the mild disease observed in this group of patients (figure 1). The real reasons will probably remain a mystery, fortunately because the number of infected children is too low to allow good-sized immunological studies.

Conflict of interest: None declared.

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