Insight into the Pediatric and Adult Dichotomy of COVID-19: Age-Related Differences in the Immune Response to SARS-CoV-2 infection

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Abstract

The difference in morbidity and mortality between adult and pediatric COVID-19 infections is dramatic. Understanding pediatric-specific acute and delayed immune responses to SARS-CoV-2 is critical for the development of vaccination strategies, immune-targeted therapies, and treatment and prevention of MIS-C. The goal of this review is to highlight research developments in understanding of the immune responses to SARS-CoV-2 infections, with a specific focus on age-related immune responses.

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Running title: Age-Related Differences in Immune Responses to COVID-19

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Introduction

In December 2019, a new coronavirus, SARS-CoV-2, led to a surge in cases of pneumonia in Wuhan, China.[1,2] This respiratory illness, coronavirus disease 2019 (COVID-19), subsequently spread rapidly, and on March 11, 2020, World Health Organization (WHO) classified COVID-19 as a world-wide pandemic.

COVID-19 is highly contagious with a transmissibility rate ranging from 1.4-6.9 in different case series,[3] meaning one infected individual will infect up to seven people, thus facilitating rapid spread of the infection. Case reports indicate about one in six children in the United States with confirmed SARS-CoV-2 infection are asymptomatic[4] and up to a half of infections are transmitted by people with no symptoms or mild symptoms.[5] Asymptomatic and mildly symptomatic children are a true concern in the propagation of this pandemic. Not only is COVID highly contagious, the disease carries a high mortality with an estimated case fatality rate (CFR) of a striking 1.38% with CFR reaching a staggering 13.4% in patients over 80 years.[6] In comparison, seasonal influenza usually has a CFR well below 0.1% with those at highest risk of severe complications having a bimodal distribution including adults over 65 years of age and children less than 5 years of age. Much of the burden of COVID-19, in terms of morbidity and mortality, is being carried by older adults.

Children less than 19 years, however, are less likely to become acutely ill from SARS-CoV-2 infection, and although COVID-19-related deaths have been reported in this age group, they make up less than 1% of COVID-19 associated ICU admissions and death.[7] Although children are equally likely to be infected by SARS-CoV-2,[8] 20% remain asymptomatic,[9] while others report mild symptoms of the upper respiratory infection, including fever, dry cough, rhinorrhea, ageusia, and anosmia.[10,11] Very few children progress to any significant respiratory distress.[12] In fact, only 1.7% of reported SARS-CoV-2 infections have occurred in children less than 18 years of age though this may reflect lower testing in this population.[13] In children 0-9 years old and 10-19 years old, the fatality rates have been markedly lower compared to adults at only 0.0026% and 0.0148% respectively.[6]SARS-CoV-2 is speculated to be contained in the upper airways of children by the innate and adaptive immune system; when the virus progresses to the lower airway, as in adults, SARS-CoV-2 is associated with high lethality.[14] However, ground glass opacities with surrounding halo on CT imaging in a majority of pediatric patients and clinical symptoms of pneumonia in some patients are strong evidence that, even in children, the virus is not always contained in the upper airway.[15,16] Children's ability to better contain the virus, therefore, is likely a function of the immune response rather than the viral exposure.

Although children tend to have milder presentations with COVID-19 acute infection,[17] a newly recognized SARS-CoV-2 associated syndrome called Multisystem Inflammatory Syndrome in Children (MIS-C) or Pediatric Multisystem Inflammatory Syndrome (PMIS) has been described worldwide.[18] Initially reported as hyperinflammatory shock[19] and "Kawasaki-like" illness,[20] this syndrome has been observed to have overlapping features with toxic shock syndrome, atypical Kawasaki disease, macrophage activation syndrome, cardiogenic and septic shock. Dilated coronary arteries have been reported in 17-25% of the patients with rare development of coronary artery aneurysms. Laboratory abnormalities include significant elevation of inflammatory markers (high CRP, ESR, D-dimer, ferritin, IL-6), neutrophilia, lymphopenia, elevated NT-proBNP with or without troponin elevation.[21] No acute or past significant respiratory illness has been seen in these patients. Early estimates report this syndrome occurs in between 0.011% and 0.31% of children with SARS-CoV-2 infection.[22] These reports likely underestimate and only reflect the cases with severe disease presentation. Figure 1 outlines the time course in adults and children infected with SARS-CoV-2.

The difference in morbidity and mortality between adult and pediatric COVID-19 infections is dramatic. Understanding pediatric-specific acute and delayed immune responses to SARS-CoV-2 is critical for the development of vaccination strategies, immune-targeted therapies, and treatment and prevention of MIS-C. The goal of this review is to highlight research developments in understanding of the immune responses to SARS-CoV-2 infections, with a specific focus on age-related immune responses.

The Virus

SARS-CoV-2 is classified in the *Coronaviridae* family and*betacoronavirus* genus and is the seventh coronavirus known to infect humans.[1] Whereas four coronaviruses, HKU1, NL63, OC43 and 229E, are associated with mild symptoms akin to the common cold, SARS-CoV, MERS-CoV, and SARS-CoV-2 have each had recent outbreaks associated with a more severe disease.[23]This positive-sense single-stranded RNA virus has a helical capsid formed by nucleocapsid (N) proteins as well as an enveloped coated with trimeric spike (S) proteins.[1] SARS-CoV-2 infection begins with viral entry through binding of the SARS-Co-V-2's S protein to the angiotensin-converting enzyme 2 (ACE2).[24,25]TMPRSS2, a transmembrane serine protease, assists in viral entry though cleavage of the S protein allowing for fusion of the viral membrane with the host cell membrane. Once intracellular, replication occurs via RNA-dependent RNA polymerase.

Epithelial Barriers and Viral Infection

ACE2, the receptor for the SARS-CoV-2, is expressed at low levels throughout the trachea, large, and small airways.[26]ACE2 is expressed in multiple epithelial type cells throughout the respiratory tract including alveolar type II cells with the highest expression occurring in clusters of goblet cells and ciliated cells of the nasal epithelium.[27,28] With the high expression of ACE II in the nasal epithelium, this location is not only accessible but welcoming to this destructive pathogen.

The older adult population is inarguably becoming sicker from SARS-CoV-2 infection, and differential expression of ACE2 receptor in adult epithelial cells to mediate viral entry could be a plausible explanation. Surprisingly, recent studies have shown no significant difference in ACE2 gene expression with age, with some studies showing reduced ACE2 gene expression in the older population.[26,29] In contrast to ACE2 receptors, TMPRSS2, the serine protease "sidekick" for viral entry, has been shown to have an increased expression with age.[30,31]Of note, CD147 and GRP78 are identified as other candidate receptors to allow entry of this viral invader.[26] Expression of CD147 has actually been shown to increase with age though more investigation is needed to elucidate the significance of this early finding; if the virus uses this back door for attack, this important vulnerability could help explain age differences.[32] Of note, CD147 is a glycosylated transmembrane protein of the immunoglobulin super family that acts as the main upstream stimulator of matrix metalloproteinases (MMPs).[33] Activation of CD147 could have important implications for disease progression. Likewise, GRP78 may be central to the disease process as it is a member of the heat-shock protein-70 (HSP70) family and involved in the folding and assembly of proteins in the endoplasmic reticulum.[34]

Paradoxically, infection with SARS-CoV-2 stimulates an interferon-mediated innate immune response upregulating ACE2, enabling more viral entry sites.[30] After SARS-CoV-2 has breached the upper airway through nasal epithelium, a systemic response with both cytokines and interferons may cascade to lower airway leading to increased expression ACE2 portals of entry that allow attack of the lower respiratory tract. An understanding of the gene expression of epithelial cells under stress are necessary to better understand how this invader breaches defenses. If a child's immune system fails to induce ACE2 receptors in the lower airway, then the virus may stay contained within the upper airway or have a delayed progression to pneumonia. Epithelial binding alone does not convincingly explain the stark age difference.

The Acute Immune Response

When a respiratory virus enters the airway, the innate immune response rapidly responds. Resident airway macrophages and dendritic cells are actively patrolling the airspace. Toll-like receptors reflexively trigger an inflammatory response when viral particles are identified. Macrophages, dendritic cells and epithelial cells release cytokines, including TNF, IL-1β, IL-6, IL-8, and IL-12 to activate the immune system.[36] Neutrophils swarm to the response, attempting to either phagocytose pathogens or kill invaders in the explosive web of extracellular trap. Natural killer cells and endothelial cells release type 1 interferon in an antiviral response to contain the virus.[1] Natural killer cells recognize infected cells by sensing the down regulation of Major histocompatibility complex (MHC) class I module. The natural killer cell then attempts to kill the infected cell by forming and immune synapse where perforin mediates the delivery of the granzymes into endosomes in the target cell, and finally into the target cell cytosol.[37] Key points of interest in this early immune

response that vary in the pediatric population are the release of interferon, response of neutrophils, the hyperactivation of macrophages and the resulting cytokine storm. Figure 2 highlights hypothesized areas of infection and immune responses that are altered between adults and children and could account for the age-related differences seen in morbidity and mortality related to COVID-19.

Innate immunity includes the early detection of coronavirus infection of the cell and the rapid generation of anti-viral mechanisms, such as the production of interferons (IFN). Specifically, type I IFN has an antiviral role via induction of interferon inducible genes and stimulation of apoptosis in infected cells. Notably, type I IFN decreases viral mRNA expression in SARS-CoV-2 infection, but SARS-CoV-2, like SARS-CoV, dodges this immune response via early antagonism of type I and type III IFN release.[38] This antagonism of IFN response aids in viral reproduction allowing for further aberrant inflammatory responses, as the IFN response is delayed by about 48 hours.[39,40] Children, however, have a lower threshold to inducing an IFN antiviral response compared to their adult counterparts[40] and upregulated type I and type III IFN-associated gene expression in tracheobronchial epithelium prior to infection.[41] This early IFN response in the incubation period of the virus theoretically prevents higher viral loads.[40]

Neutrophils are the initial cell responder to viral invasion, but cytokines, including IL-8, and eicosanoids, including leukotriene B4 (LTB4), continue to propagate a further neutrophilic inflammation. The vast majority of children with COVID-19 have normal neutrophil counts with only 4.6% of children with neutropenia and 6.0% neutrophilia.[42] In adults, however, severe COVID-19 infections in adults are associated with neutrophilia[43,44] although low neutrophil counts also portend poor outcomes,[45] perhaps related to neutrophil activation, extravasation, feed-forward hyperinflammation and resultant tissue destruction. Indeed, lung neutrophil infiltration has also been reported in multiple reports on the pathological findings from autopsied COVID-19 patients.[43,46] Although leukocytosis and neutrophilia are hallmarks of acute infection; neutrophils might be responsible for pathogenic inflammation in the setting of COVID-19. In SARS-CoV-2, neutrophils are believed to create neutrophil extracellular traps (NETs),[43] and these web-like structures of DNA, histones and proteases have the unintended effect of trapping, platelets, red blood cells, and neutrophils driving systemic inflammation, vascular instability, and hypercoagulability. In patients with COVID-19, extensive NET formation has been associated with endothelial damage and cytokine release.[43] It has not yet been assessed as to whether there is a difference in neutrophil activation between pediatric and adult COVID-19 patients.

Age-dependent monocyte and macrophage differences could also explain the differences between COVID-19 in pediatric and adult patients. Blood-circulating monocytes are recruited to the area by the potent CCL2 and CCL7 chemokines and differentiate to macrophages at sites of inflammation.[47] Monocytes from older adults have both increased CD11b[48] but decreased L-selectin,[49] resulting in aberrant monocyte transendothelial migration. Macrophages clean up the inflammatory battle site by phagocytosing both pathogen and cellular debris which in turn results in additional release of inflammatory molecules. T helper cells, specifically Th1 cells, release IFN-$\boldsymbol{\gamma}$ (type III IFN) to enhance the ability of macrophages activity. Phagocytosis and cytokine release has been shown to be altered in monocytes with age.[50]Impaired clearance of infected cells and elimination of activated macrophages ultimately results in end-organ damage.[51]Uncontrolled macrophage activation has been associated with decreased natural killer cell and cytotoxic T cell function which further limits the ability to contain infection.[52] These age-dependent inflammatory responses could partially explain clinical differences seen in COVID-19.

Additionally, severe COVID-19 is marked by hyperactivation of macrophages either through direct viral sensing or cytokine exposure, hyperinflammation and coagulation.[47,51] Cytokines are released in early SARS-CoV-2 infection; viral invasion leads to increases in inflammatory IL-1β, IL-6, IL-12, TNF-α.[1]As the adaptive response develops, a second peak in cytokine occurs. This peak is modest in mild infection or exaggerated in severe presentation. Both SARS-CoV and MERS-CoV infection result in macrophage and neutrophil invasion resulting in high levels of pro-inflammatory cytokine-induced acute lung injury, ARDS, and death.[53]Critically ill adult patients with COVID-19 frequently have features of a similar cytokine storm. The most severe COVID-19 infections are associated with a similar cytokine profile with a marked increase IL-6 and ferritin

levels.[54] The inflammatory markers, such as IL-6, IL-10, myeloperoxidase, and p-selectin, increase with age during critical illness[50,55] suggesting that perhaps the macrophage hyperactivation in older adults accounts for the increased morbidity and mortality seen in this acute infection.

The Delayed Immune Response

In viral infections, the dendritic cells serve as the key link to the adaptive immune response. MHC class II molecules on the cells present viral peptides to T cell receptors on naïve T cells allowing for the maturation of T cells. Activation of the T lymphocyte leads to massive cell proliferation and generation of T helper cells (CD4+), cytotoxic T cells (CD8+), and antibody-producing B cells to recognize the antigen. T helper cells are crucial in generating cytokines that recruit phagocytes and activate other leukocytes. In established lung infection, the major mechanism for eliminating the virus from the body is by CD8+ T cells killing virally infected cells. CD8+ T cells can detect and selectively kill virally infected epithelial through release of specialized lytic granules which in turn induce apoptosis of the infected cell. Antibodies produced by B cells neutralize the virus by binding to viral surface proteins, thus preventing viral entry into further host cells.[56] These three roles of the adaptive immune system – generation of cytokines by helper T cells, cell-mediated immunity, and generation of antibodies – differ in pediatric and adult populations.

As SARS-CoV-2 is a novel virus, no specific IgG memory antibodies are present, and the host is reliant on the innate immune response until IgM and IgG seroconversion at day 12 and day 14 of the virus respectively.[14,57] In a study of 285 patients, within 19 days of infection, all patients had antiviral IgG.[58] Specifically, these antibodies were shown to bind a recombinant antigen containing the nucleoprotein and a peptide from the spike protein of SARS-CoV-2.[58] If limited to the upper respiratory tract, the systemic immune response is hypothesized to be less robust, antibody production may be of a shorter duration. As children often have limited infections, children may be more vulnerable to recurrent infections.[59] This short-lived immunity was specifically noted in endemic coronavirus where an upper respiratory infection with endemic coronavirus left children vulnerable to reinfection within one year.[59]

In general, the best immune strategy for prevention of a viral infection is to prevent initial entry into the host cell, which can effectively stop the infection. However, once a high intracellular viral load is reached, there is a release of a large numbers of viral particles which overwhelm antibody neutralization capacity which further limits the body's ability to contain viral spread. This is the typical situation in SARS-CoV-2, where the level of antibody does not correlate with effective immune control. However, if neutralizing antibodies persist after a patient recovers from COVID-19, and the virus is eliminated, then the patient should be protected from reinfection for an unknown duration.[60] Although production of neutralizing antibodies to the S protein would theoretically limit SARS-CoV2 infection, the virus can escape. The RNA-dependent polymerase of RNA viruses, such as coronaviruses, is intrinsically error-prone and inserts wrong nucleotide into the RNA products every 10,000 bases. Some of these nucleotide substitutions may change the amino acid sequence of the S protein[61] leaving the antibody binding impaired but not viral attachment.

In the elderly, immune evasion might be exacerbated by reduced myeloid cell antigen-presenting cell (APC) function or availability.[62] Aging is also associated with a decline in the adaptive immune function. Since T cells are necessary for virus clearance in infected animals, prior studies have focused on virus-specific T cell response in the elderly. Oligoclonal expansion of virus specific T cells which decrease diversity of the T cell repertoire in aged hosts have been previously shown to be associated with increased susceptibility to viral infections.[63,64] Decreased T cell function with age is also caused by progressive involution of the thymus, a central immune organ that instructs T cell maturation and specificity.[65,66] Thymic involution leads to a decline in naïve T cell output while existing memory T cells are relatively preserved.[65,66] This might explain why the capacity to respond to a novel/changing antigen is particularly poor in older individuals. Since the immune evasion by SARS-CoV-2 is exacerbated and since the T cells may be dysfunctional,[67] it is conceivable that late T cell response may amplify pathogenic inflammatory outcomes in the presence of sustained high viral loads in the lungs.[68,69]

MIS-C is an emerging disease and very little is understood about its pathophysiology. Early case reports

of COVID-related inflammatory disease include a classical "Kawasaki-like" illness in a six-month old girl who was admitted due to Kawasaki disease and tested positive for COVID-19 prior to discharge.[70] Other reports include a 14-year-old boy with hyperferritemia, cytokine storm, acute respiratory distress syndrome (ARDS), and hypotension requiring inotropic support.[71] Further reports from Italy and London also describe this picture of multi-organ involvement with hypotension often refractory to intravenous fluid resusitation.[19,20,72]This picture of a cytokine storm in the setting of SARS-CoV-2 exposure is similar to the cytokine storm in adults, yet the presentation is roughly a month delayed from the peak of cases in each city. Belhadjer et al. reported 90% positive testing for SARS-CoV-2 infection in children with MIS-C.[18] In their case series, positive antibody assays were seen in 86%, positive nasopharyngeal PCR in 34% and positive fecal PCR in 6% of the patients. This suggests that the virus has been cleared from the upper respiratory tract and the clinical presentation is likely a post-viral syndrome.[22]

Of note, as one ages, thymus hypoplasia leads to a decrease in function and number of both T cells and T regulatory cells leaving elderly more susceptible to viral infection and immune dysregulation such as that seen in cytokine storm.[73] Serological signatures also differ vastly between healthy children and elderly, with higher cross-reactive SARS-CoV-2 IgA and IgG observed in elderly, whereas children displayed elevated SARS-CoV-2 IgM. Theoretically, the less-experienced humoral immunity in children, as evidenced by the higher IgM, may induce more potent antibodies upon SARS-CoV-2 infection.[74]

Conclusion

Our understanding of how children are affected by SARS-CoV-2 has evolved since the beginning of the pandemic, however, much remains to be learned. It is now clear that children are not as spared from this pandemic as originally thought. Children are often asymptomatic carriers that play an unfortunate role in the spread of this disease, and they are also more likely to become ill from SARS-CoV infection that previously thought. The increase in cases of MIS-C reveals that the delayed inflammatory response of COVID-19 can cause significant illness in children; Understanding how to prevent this hyperinflammatory response is critical and could have important implications for vaccine development. Because they are less likely to be affected by the acute infection, children may offer critical insight in immune modulation and containment of the acute phase of illness. Importantly, children have many years ahead of them; it remains to be seen whether SARS-CoV-2 infection or MIS-C have long-term health implications for these children. Many essential questions remain to be answered regarding the pediatric impact of COVID-19 and research remains critical as we continue to fight this pandemic.

Figure 1: Time course of symptoms and disease severity related to SARS-CoV-2 infection in adults (top) and children (bottom).

Figure 2: Summary of hypothesized age-related differences in SARS-CoV-2 infections in adults as compared to children. Areas of interest include viral entry, interferon and cytokine immune response, neutrophil activation, macrophage hyperstimulation, antibody production and T-cell responses.

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