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Innate and adaptive immune responses to SARS-CoV-2 in humans: relevance to acquired immunity and vaccine responses

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Summary

The factors responsible for the spectrum of coronavirus 19 (COVID-19) disease severity and the genesis and nature of protective immunity against COVID-19 remain elusive. Multiple studies have investigated the immune responses to COVID-19 in various populations, including those without evidence of COVID-19 infection. Information regarding innate and adaptive immune responses to the novel severe respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved rapidly. Data are accumulating defining disease phenotypes that aid in rational and informed development of new therapeutic approaches for the treatment of patients infected with SARS-CoV-2 and the development of novel vaccines. In this paper, data on important innate immune responses are summarized, including cytokines, specifically interleukin (IL)-6 and complement, and potential treatments are explored. Adaptive immune responses and derivative therapeutics such as monoclonal antibodies directed at spike proteins are also examined. Finally, data on real-time assessments of adaptive immune responses are explored, which include $CD4^+$/$CD8^+$ T cells, natural killer (NK) T cells, memory B cells and T follicular cells with specificities for COVID-19 peptides in infected and normal individuals. Data of two novel vaccines have been released, both showing > 95% efficacy in preventing SARS-CoV-2 infection. Analysis of humoral and cellular responses to the vaccines will determine the robustness and durability of protection. In addition, long-term assessment of SARS-CoV-2 memory B and T cell-mediated immune responses in patients recovering from an infection or those with cross-reactive immunological memory will help to define risk for future SARS-CoV infections. Finally, patients recovering from SARS-CoV-2 infection may experience prolonged immune activation probably due to T cell exhaustion. This will be an important new frontier for study.

Keywords: complement, humoral immunity, interleukin 6, SARS-CoV-2, T cells

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Introduction

Since the advent of coronavirus 19 (COVID-19) in Wuhan, China in December 2019, the virus has spread to virtually every country in the world, now accounting for approximately 84 million cases worldwide with 1 820 315 deaths [1]. Despite these stark reminders of the morbidity associated with severe respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia, there remain large numbers of individuals who exhibit no or minimal symptoms despite demonstrating viral polymerase chain reaction (PCR) positivity. The factors responsible for the spectrum of COVID-19 disease severity and the genesis and nature of protective immunity against COVID-19 remain elusive. There are now multiple studies which have investigated the immune responses to COVID-19 in various populations, including those without evidence of COVID-19 infection [2–4]. These studies have yielded valuable information on human immune responses to COVID-19 and have delivered insight into potential

paths forward in discerning who would be at greatest risk from the virus based on immunological assessments. In this paper, emerging studies are discussed that examine innate and adaptive immune responses to SARS-CoV-2 and how they might be modified to protect individuals from collateral tissue injury induced by excessive innate immunity and induce long-lasting immunity to SARS-CoV-2 canonical antigens that are capable of eliciting long-lived T and B cell immunity.

Despite efforts on many fronts, specific therapeutic approaches to treatment of SARS-CoV-2 have yielded variable or no significant benefit compared to standard of care [5–9]. These include remdesivir, immune plasma, monoclonal antibodies against spike protein and anti-inflammatory agents. Currently, emerging vaccines hold the most hope for saving lives and stemming the epidemic. However, in the early days of the epidemic the focus was upon therapies aimed at controlling the cytokine storm that emerged in patients with SARS-CoV-2 pneumonia. Efforts were aimed at controlling elements of innate immunity that probably contribute to the morbidity and mortality of SARS-CoV-2 pneumonia. In this paper, the relevant innate and adaptive immune responses developed by humans to SARS-CoV-2, and how they can be utilized to develop more rational therapeutic approaches to treatment of patients infected with COVID-19, are discussed.

Innate immune responses to COVID-19: interleukin 6

Early reports from patients with SARS-CoV-2 pneumonia identified interleukin (IL)-6 as a potential pathogenic factor in the initiation of acute respiratory distress syndrome (ARDS) [10]. IL-6 is a pleiotropic cytokine which functions as a mediator of both innate and adaptive immune functions. IL-6 has diverse immune and biological actions, including direction of immune cell differentiation, sentinel responses to invading pathogens and ischemic injury. IL-6 is also critical for plasma cell growth and immunoglobulin production. Excessive and unregulated IL-6 transcription is commonly seen in patients with autoimmune or inflammatory disorders [11]. Emerging data from patients with SARS-CoV-2 suggest that IL-6 transcription is initiated and sustained after respiratory epithelium is infected. The virus had a proclivity for the activation of alveolar and circulating macrophages, resulting in copious and sustained IL-6 production resulting in the cytokine storm, endothelial cell damage, capillary leak and clinical and pathological features of ARDS. These data suggest that inhibiting IL-6 production and/or blocking receptor binding could be an important therapeutic option for limiting morbidity and mortality [12–14].

In this regard, tocilizumab [anti-IL-6 receptor (anti-IL-6R)] monoclonal antibody is of interest due to its ability to reduce ARDS after chimeric antigen receptor T cell therapy (CART) cell therapy. Tocilizumab is a recombinant immunoglobulin (Ig)G1 humanized monoclonal antibody which inhibits the binding of IL-6 to the soluble and membrane-bound forms of IL-6R. Tocilizumab is Food and Drug Administration (FDA)-approved for the treatment of severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, giant cell arteritis and, more recently, for cytokine release syndrome occurring after CART-cell therapy.

Our group and others have reported on the benefits of anti-IL-6R therapy for treatment of SARS-CoV-2 pneumonia [10,13–20]. Press reports on two clinical trials of anti-IL-6R therapy, and a recently reported randomized clinical trial, failed to show benefit [16–18]. However, data from the Evaluating Minority Patients with Actemra (EMPACTA) trial showed that tocilizumab reduced the number of patients needing mechanical ventilation compared to placebo in a population of underserved and minority patients [19]. Also, very exciting and encouraging data released from the Remap-Cap international platform trial showed that tocilizumab significantly improved outcomes in the most severely ill patients with SARS-CoV-2 pneumonia [20]. Since tocilizumab was the first immune modulatory agent investigated in SARS-CoV-2 pneumonia, it has experienced many ups and downs in terms of results reported in real-world experience and clinical trials that are often diametrically opposed. Certainly, it appears that not all patients would benefit from anti-IL-6R therapy, but emerging data suggest it is clear that blocking early innate immune responses to COVID-19 infection can be beneficial in severe SARS-CoV-2 pneumonia.

Innate immune responses to COVID-19: complement

Little attention has been paid to potential role of complement activation in mediation of the severe manifestations of SARS-CoV-2 pneumonia. However, many symptoms could be attributed to systemic complement activation through the alternative, classic and possibly lectin-binding pathways. These include ARDS and propensity to a hypercoagulable state. In this regard, there is probably interaction between elevated IL-6 levels seen in SARS-CoV-2 pneumonia patients and activation of the complement system. IL-6 is a potent inducer of complement reactive protein (CRP), which has the ability to initiate complement activation. Recent reports have focused upon evaluating the association of COVID-19-related inflammation with activation of the C5a-C5a receptor (C5aR) axis [21]. This paper examined the role of complement activation and specifically generation of the potent anaphylatoxin C5a in patients with COVID-19 infection. Patients were divided into four categories: healthy controls, COVID-19 patients with minimal symptoms, patients with pneumonia and those with severe ARDS. Blood

levels of CRP, IL-6, C5a and chemokines associated with complement activation were examined. The investigators demonstrated a progressive and significant increase in all inflammatory markers evolving from minimal symptoms to ARDS. Importantly, they also examined lung samples from SARS-CoV-2 patients and found a significant increase in macrophage and neutrophil infiltration, with both cell types expressing high levels of C5a1 receptor. Bronchoalveolar lavage (BAL) fluid analysis showed increased levels of IL-6 and C-X-C motif ligand 8 (CXCL8), but C5a was detected in concentrations > 1000 pg/ml. The authors suggest that all three pathways to complement activation [classic, alternative and mannose binding lectin pathway (MBL/SP)] are involved in SARS-CoV-2-induced pathology. In this regard, reports suggest that patients with the most intense anti-COVID-19 antibody responses may develop more severe ARDS, probably due to classic pathway/alternative pathway complement activation by IgG/SARS-CoV-2 immune complexes [22]. Importantly, these investigators evaluated how inhibition of the C5a/C5aR1 axis would affect markers of inflammation. *In-vitro* experiments using a monoclonal antibody against C5aR1 with human cells showed that anti-C5aR1 inhibited C5a activation of neutrophils induced by high concentrations of C5a. Using a C5aR1 knock-in model of acute lung injury in mice, the investigators showed that anti-C5aR1 monoclonal markedly inhibited features of acute lung injury including neutrophil infiltration, IL-6 induction and albumin extravasation into alveoli. Pathological features were also markedly improved, with no evidence of ARDS in anti-C5aR1-treated animals. These observations suggest that modification of the C5a-C5aR1 axis could have benefit in treatment of patients with SARS-CoV-2 pneumonia [21].

Another important pathological consideration in patients with SARS-CoV-2 pneumonia is the proclivity for thrombotic events [23–25]. Intense complement activation is likely to cause activation of the coagulation system (with initiation of thrombotic events on the endothelium of blood vessels. Thus, inhibition of complement activation could prevent thrombotic complications of SARS-CoV-2 pneumonia.

Gao *et al*. [26] have also suggested that the N protein of SARS-CoV-2 is a potent activator of the MBL/SP pathway and may be responsible for the rapid development of ARDS in SARS-CoV-2-infected patients. In this regard, C1 esterase inhibitor (C1-INH) regulates the intrinsic complement/coagulation pathway by inhibiting multiple pathways, including Factor XII activation. Deficiency or loss of function of C1-INH would probably result in enhanced coagulation and fibrinolysis. This is supported by elevated blood D-dimer levels in patients with hereditary angioedema (HAE) resulting from C1-INH deficiency. Thus, one could also surmise that use of C1-INH treatment could be of use in treating the manifestations of SARS-CoV-2 pneumonia, including inhibition of the innate immunity/coagulation pathway crosstalk [27]. A recent report detailed the use of C1-INH treatment in five patients with SARS-CoV-2 pneumonia. Four of five showed rapid improvement in oxygenation, reductions in fever and CRP levels. They also showed a decline in complement activation products after treatment [28].

Data presented in the studies evaluated above suggest that investigation of complement inhibitors, especially those that can inhibit coagulation pathway activation, hold promise in treatment of patients with SARS-CoV-2 pneumonia. In this regard, a recent paper by Vlaar *et al*. [29] examined the utility of an anti-C5a monoclonal IFX-1 for treatment of patients with SARS-CoV-2 pneumonia. This was a small study which examined the PAO2/FiO2 ratios on day 5 after treatment compared to placebo. The study did not meet the primary end-point, but of interest are the observations that pulmonary embolisms were reduced in anti-C5a-treated patients (13 *versus* 40%) compared to placebo. There was also lower mortality at 28 days. However, caution must be taken, as this is a small exploratory study not powered for those end-points. Other trials of inhibitors of C3, C5, C5a and C1INH are under way and should help to elucidate whether or not complement inhibition will have a role in treatment of patients with SARS-CoV-2 pneumonia [30].

Adaptive immune responses to COVID-19: B and T cells

Adaptive immunity involves the co-ordination of T and B cell immune responses to the SARS CoV-2 virus. In this regard, adaptive immunity is responsible for long-lasting and possibly sterilizing immunity to the virus. We now know that immune responses to the severe acute respiratory syndrome virus occurs within the first 7–10 days post-infection. However, understanding the key features of this is still a conundrum. It is very important in the long term to ascertain the nature of the B and T cell immune events and whether they result in long-lasting immunity with memory B/T cell development or dissipate over time, resulting in a risk for recurrent infection and disease. These are also prescient issues for development of vaccines to combat the SARS-CoV-2 epidemic. In this section, we will focus upon adaptive immune responses to SARS-CoV-2 and how to measure the strength and durability of the virus-specific immune responses.

Adaptive immune responses to COVID-19: antibodies

With rapid onset of the SARS-CoV-2 epidemic, critical information regarding immune responses to the virus

have lagged as efforts focused upon development of assays to detect antibody responses to the virus. We are now achieving a clearer understanding of the humoral immune responses to COVID-19. After the initial infection with COVID-19 early responses are IgM and IgA, but is unclear if these can modify the course of the disease [2,31,32]. Subsequent IgG responses occur within 7–10 days post-infection and would be expected to give sterilizing immunity to the virus, and with presumed development of memory B cells, result in recall of high-affinity IgG anti-COVID-19 responses should re-exposure occur. However, it is known that the intensity, character and duration of IgG responses may vary greatly. IgG titers usually peak at approximately 50–60 days post-infection and may last up to 10 months [33–35]. It is also not known if the disappearance of the antibody correlates with the disappearance of specific memory of the virus. There are now several cogent papers and that are beginning to address the nature and significance of IgG responses to the COVID-19 virus [8,34–36]. Is also known that intense antibody responses to the virus of the IgG class are likely to cause severe cytokine release syndrome and may be associated with increased risk of death [22–24].

One of the cardinal features associated with an effective vaccine is developing neutralizing antibodies directed at spike protein. This is a basis for multiple clinical trials and also the basis for development of monoclonal antibodies cocktails that have been important in COVID-19 therapeutics short of vaccines. However, until recently little was known about what constitutes an effective immune response to COVID-19. An important consideration is the nature of antibodies aimed at the receptor binding domain (RBD) of SARS-CoV-2. In this regard, recent papers have shown that antibodies binding to the receptor binding domain RBD are critical for long-term protective immunity to COVID-19 infection and are associated with better patient survival [34,35]. These authors conclude that measuring antibodies to specific epitopes of SARS-CoV-2 antigens offers a more accurate assessment of sterilizing and clinically significant immunity. Recently, Barnes *et al*. [34] reported on how the structure and specificity of neutralizing antibody to SAR-CoV-2 inform therapeutic strategies. Using structural, biophysical and bioinformatics analyses of SARS-CoV-2, the investigators analyzed approach angles of antibodies bound to RBDs on spike trimers. Their work provides a blueprint for designing antibody cocktails for therapeutics and potential COVID-19 spike-related immunogens for robust vaccine development. Thus, it is important to analyze the nature and specificity of the IgG responses to COVID-19. If antibodies are not directed at the RBD and cannot effectively bind spike trimers, they are likely to be ineffective in preventing infection. This should also be true of monoclonal antibody cocktails now being used for therapy in patients with SARS-CoV-2. Of interest in this regard is the use of convalescent plasma, which was shown to have no benefit in treatment of SARS-CoV-2 pneumonia in a controlled trial [8]. This is probably due to variations in subclass composition, titer and avidity of IgG responses in patients recovering from SARS-CoV-2. IgG antibodies directed at the RBD prevent spike adherence to the ACE2 receptor are likely to prevent infections. In a recent report by Ibarrondo *et al*. [36], the investigators examined the durability and robustness of anti-SARS-CoV-2 RBD-directed antibodies in 34 patients with known or suspected infection with SARS-CoV-2. The investigators reported on an observed rapid decline in IgG antibodies directed at the SARS-CoV-2 RBD indicating, in their opinion, that their observations 'raise concern that humoral immunity against SARS-CoV-2 may not be long lasting in persons with mild illness, who compose the majority of persons with COVID-19'. They also indicate that 'the results call for caution regarding antibody-based "immunity passports," herd immunity, and perhaps vaccine durability, especially in light of short-lived immunity against common human coronaviruses'. Given the information reported above, it is clear that a deeper understanding of the human immune response to COVID-19 is needed before such pronouncements can be made. First, early IgG responses emanate from germinal centers after T follicular cells activate naive B cells to mature into activated B cells that progress to B memory cells and IgG-producing plasmablasts. Plasmablasts are short-lived, and with dissipation the initial IgG responses are terminated. However, it should be understood that this does not mean that immunity has waned. This is because the persistence of B memory cells and long-lived plasma cells that reside in the bone marrow can reactivate antigen-specific responses to the SARS-CoV-2 RBD if re-exposed. In addition, this does not take into account the importance of T cell memory for COVID-19 antigenic determinates that can result in direct cytotoxic T cell immunity and help for B cell responses [33]. Thus, the comments of Ibarrondo *et al*. [36] need to be evaluated in the context of comprehensive immune responses to COVID-19 where redundancy, memory, diversity and durability are probably more important than initial IgG responses.

Despite the failure of convalescent plasma to improve SARS-CoV-2 pneumonia, monoclonal antibodies have emerged and are now being used for treatment of SARS-CoV-2 infection, primarily in outpatient settings. Initial reports from the Regeneron (Tarrytown, NY, USA) and Lilly Pharmaceuticals (Indianapolis, IN, USA) trials on

monoclonal IgG anti-spike protein monoclonals suggests that they may reduce symptoms and shorten the course of disease in patients who are not hospitalized [9,37,38]. However, the impact of these antibodies on severe SARS-CoV-2 pneumonia appears minimal, and possibly deleterious, in patients with high oxygen requirements. Is also suspected that the larger antigenic burden is a major driver of the magnitude of response to COVID-19; again, this may be associated with intense immune responses with cytokine release syndrome. The monoclonal antibody cocktail developed by Lilly Pharmaceuticals has recently been discontinued for adverse events and lack of efficacy in hospitalized patients. However, recent emergency approval was given for outpatient use [9]. In addition, the Regneron monoclonal REGN-CoV2 antibody cocktail recently showed that it also improved symptoms in non-hospitalized patients [38]. These therapies, when properly applied in the outpatient setting, offer hope for limiting SARS-CoV-2 pathogenesis and hospitalizations. This is a critical consideration, given the current burden on health-care systems worldwide.

Adaptive immune responses to COVID-19: T cells

With the rapidly evolving understanding of immune responses to the SARS-CoV-2 virus, information on T cell responses has taken center stage. In a series of interesting and extremely informative articles we have gained much knowledge that is likely to change the way we look at viral-directed immune responses, the risk and severity of infection in individuals naive to SARS-CoV-2 and the understanding of what constitutes an effective and sterilizing immune response. Importantly, it is critical to how we use this new information to improve the design of future vaccines.

One of the most interesting and provocative reports was by Braun *et al.* [3]. These investigators examined $CD4^+$ T cell responses to the spike glycoprotein in the peripheral blood of patients with known SARS-CoV-2 infections as well as in healthy controls. Spike-reactive $CD4^+$ T cells were detected in 83% of infected individuals. However, of greater interest was the detection of spike-reactive $CD4^+$ T cells in the peripheral blood of 35% of healthy donors. It was noted that spike-reactive $CD4^+$ T cells in healthy donors were directed against C-terminal epitopes of the spike protein. The investigators also noted that spike-reactive T cells against C-terminal epitopes have been identified in spike proteins of endemic coronaviruses which are responsible for seasonal upper respiratory tract infections. In unique and revealing experiments, the investigators showed that the SARS-CoV-2-reactive $CD4^+$ T cells from healthy donors also responded to the spike proteins of human endemic coronaviruses 229E and OC43. These findings suggest that the SARS-CoV-2-reactive T cells found in healthy donors probably arose from previous exposure to the seasonal coronaviruses. The impact of this finding is unknown; however, it raises many important considerations. If one assumes that these $CD4^+$ T cells exert cross-reactive immune responses to SARS-CoV-2 infection, they may contribute to our understanding of the varying clinical phenotypes of COVID-19 and the reported resilience of children and young adults to symptomatic SARS-CoV-2 infection. Children in day-care centers where respiratory infections are common may have more frequent exposure to seasonal coronaviruses and chances to develop effective cross-reactive immunity. Other reports have also shown that SARS-CoV-2 infections are extremely rare in school-aged children. The investigators showed that after the reopening of primary schools in the United Kingdom, only one of 23 358 nasal swabs taken from children in June 2020 had detectable SARS-CoV-2, giving an estimate of 3·9 cases per 100 000 students. These authors provide various reasons for this low infectivity rate, but do not mention the possibility of activation of SARS-CoV-2 cross-reactive $CD4^+$ T-cells as a possible factor in muting viral pathogenesis of SARS-CoV-2 [39]. Although these inferences remain to be proved, further investigations into the breadth and vigor of SARS-CoV-2 responses in younger individuals could help in identifying those at lower risk for severe disease.

Further evidence for this hypothesis was presented by Mateus *et al.* [4], who addressed the possible reasons for the reported detection of spike–protein cross-reactive T cell memory in unexposed individuals. Using blood samples collected before SARS-CoV-2 was discovered (2015–18), the investigators mapped 142 T cell epitopes across the SARS-CoV-2 genome and demonstrated a range of pre-existing memory $CD4^+$ T cells with comparable affinity to those identified in patients recovering from SARS-CoV-2 infection. They also identified the probable source of these memory responses to cross-reactivity with coronaviruses responsible for the common cold. These authors also conclude that these pre-existing memory responses to SARS-CoV-2 are probably responsible for the variation in clinical phenotypes seen in patients with SARS-CoV-2 infection. In summary, the authors provide direct evidence that numerous $CD4^+$ T cells that respond to SARS-CoV-2 epitopes actually cross-react with corresponding homologous sequences from many different commonly circulating human coronaviruses and that these reactive cells are largely canonical memory $CD4^+$ T cells. These findings of cross-reactive $CD4^+$ T cell specificities are in stark contrast to human coronavirus neutralizing antibodies, which are human

coronavirus species-specific and do not show cross-reactivity against SARS-CoV-2 receptor binding domains. These findings are remarkable, and point to the primacy of $CD4^+$ T cells in creating effective and durable and cross-reactive immune responses to human coronaviruses, including SARS-CoV-2.

Zhang *et al*. [40] examined the single-cell profiles of immune cell responses to SARS-CoV-2 in patients with moderate and severe symptoms. The authors examined single-cell RNA sequencing in peripheral blood of five normal patients and 13 patients with SARS-CoV-2 pneumonia. The patients with SARS-CoV-2 had moderate or severe symptoms, and some were convalescent cases. The authors looked at transcriptional profiles of T and B cells and also at determinants of the overall inflammatory response. Compared to normal individuals, most COVID-19 patients exhibited strong interferon (IFN)-α responses. The authors also identified a successful composition of $CD4^+$ effector-granulysin (GNLY), $CD8^+$ effector GNLY and natural killer (NK) T-CD160 (FcγR IIIb)$^+$ cells that was associated with successful convalescence in moderate disease patients. However, in patients with severe disease there were features of a deranged, excessive and persistent immune response. Persistent IFN-α responses resulted in T cell exhaustion with a skewed T cell receptor (TCR) repertoire and broad T cell expansion and the absence of NK T-$CD160^+$ cell responses. The absence of NK T-$CD160^+$ cells would suggest that the patients with more severe disease could not mediate viral elimination using antibody-dependent cell-mediated cytotoxicity (ADCC) which may contribute to the persistence of disease symptoms. This paper is important, as it is the first to show how co-ordinated and focused immune responses to SARS-CoV2 are necessary for successful viral elimination and convalescence. The rapid expansion of IFN-α-secreting cells in patients with SARS-CoV-2 pneumonia also suggests that the use of IFN-α therapy would not be advisable in excessively inflamed individuals.

Peng *et al*. [41] examined $CD4^+$ and $CD8^+$ T cell memory responses using IFN-γ responses to SARS-CoV-2 spike peptides in 42 individuals who were recovering from SARS-CoV-2 infection: 14 with severe disease, 28 with mild disease and 16 unexposed individuals as controls. These investigators showed that T cell responses were significantly higher in those with severe cases compared to milder cases. In this study, controls did not show any responses to COVID-19 spike peptides. T cell responses also correlated with antibody production to spike peptides. These investigators also identified 41 peptides associated with SARS-CoV-2 that contained either $CD4^+$ and/or $CD8^+$ specific epitopes, including six immunodominant regions that engendered responses in more than 50% of individuals. Of interest is the identification of $CD8^+$ SARS-CoV-2 specific cells that were specifically identified as central and effector memory cells in patients with mild disease. Of critical importance is the identification of multiple strong and immunodominant responses of T cells to non-spike (M and NP proteins) in 35 and 47% of patients, respectively. They conclude that this finding may define established protective immunity and probably renders protection from serious infection with SARS-CoV-2. As many of the immune responses were to non-spike proteins, this paper highlights the importance of including immunodominant T cell-reactive non-spike peptides in future vaccine development.

Swadling and Maini [42] elegantly summarized the findings of Peng *et al*. [41] in an editorial entitled 'T-cells in COVID-19 – united in diversity'. This succinct description of the complexities of the human immune response to SARS-CoV-2 and its relevance to identifying productive immune responses as well as implications for vaccine development are discussed. Functional $CD4^+$ and $CD8^+$ T cell responses to multiple regions of SARS-CoV-2 were identified and appeared to be sustained. The authors raise important points regarding the nature and durability of immunity to SARS-CoV-2. Certainly, those with more severe disease showed the most intense responses, but the presence of $CD8^+$ central and effector memory cells with multiple epitopic specificities, including promiscuous non-spike proteins in those with milder disease, are probably cross-reactive with other coronaviruses. This appears to represent long-lasting and recognizant immunity. The authors also raise the possibility that these $CD8^+$ cells may reside in the respiratory tract to take on any new invasion of SARS-CoV-2 and could rapidly initiate responses after initial invasion. Even though patients with the most intense responses are likely to retain them longer (i.e. neutralizing IgG antibodies and $CD4^+$/$CD8^+$ T cells), it is still important to consider that a small number of $CD8^+$ memory cells can rapidly expand upon re-encounter with the virus and probably initiate effective immune responses. Unlike antibody, which can result in a rapid sterilizing immunity, T cells have to wait for antigen presentation and re-initiation of memory response before elimination of virus can be accomplished. Thus, this could explain the variable phenotype of disease presentation seen currently and the fact that asymptomatic individuals may carry the virus until complete T cell responses and antibody are generated.

In terms of understanding the duration and efficacy of T cell responses to SARs-CoV-2 it is too early to determine this, as long-term studies will be needed in large populations. However, the data presented by Peng *et al*. [41] are encouraging, as T cells generated reacted with multiple epitopes on SARS-CoV-2. At this point, little information is available on the presence or duration of memory B

cells reactive to SARS-CoV-2. However, it is important to note that T cell memory specific to SARS-CoV-1 could be detected 17 years after initial infection [43,44].

Implications of human leukocyte antigenic (HLA) diversity and susceptibility to SARS-CoV-2 infection

Several studies have examined the HLA antigenic diversity and potential susceptibility to severe SARS-CoV-2 infections [45–47]. This is an important consideration, as individuals who express HLA class I and/or class II molecules that have poor affinity for SARS-CoV-2 peptides are likely to be more prone to severe infections and develop poor or non-sterilizing immunity after vaccination. The antigenic anatomy of antigen-presenting cell (APC)/T cell interactions is critical to the initiation of productive immune events. Nguen *et al*. [45] sampled the SARS-CoV-2 proteome for interactions with HLA antigens. They found that patients with HLA-B*46:01 had the least predicted binding sites for SARS-CoV-2 peptides. However, they also found that the individuals who were HLA-B*15:03 showed the highest capacity to bind SARS-CoV-2 peptides. They conclude that individual genetic variations may be critical to the generation of sterilizing immunity to SARS-CoV-2 as well as generation of responses to vaccines. Here, HLA class I phenotypical variations are important in directing $CD8^+$ T cell responses that mediate cytotoxicity. Poulton *et al*. [46] examined the role of HLA antigens in susceptibility to SARS-CoV-2 infection in 80 transplant patients who were previously HLA-typed. In this group of patients there was a significant association for risk for infection in patients that were HLA-DQB1*06. This may be relevant in assessing populations who are at increased risk for SARS-CoV-2 infection due to immunosuppression. In an interesting paper by Amoroso *et al*. [47], in an Italian transplant population, the investigators found that HLA-DRB1*O8 showed no peptide binding to SARS-CoV-2 peptides and, more importantly, was associated with increased mortality from SARS-CoV-2 [odds ratio (OR) = 2·9, 95% confidence interval (CI) = 1·15–7·21, P = 0·023]. The authors conclude that HLA antigen typing can identify individuals at higher risk for infection with SARS-CoV-2. This may identify individuals who could be 'super-spreaders' and also at risk for a severe disease phenotype and poor responses to vaccines.

Immune responses to SARS-CoV-2 in immunocompromised patients

Among the myriad unanswered questions regarding risk for and severity of SARS-CoV-2 infection is the additional risks imposed by immunosuppressive medications or anti-cancer therapies with immunomodulatory effects. To date, only a few reports have looked at this question. However, it is of critical importance in determining the nature of immune responses to infection and vaccines. Aydillo *et al*. [48] reported on viral shedding after immunosuppressive therapies. In general, 'normal' individuals shed virus for up to 10 days, but these investigators found that patients may shed virus for at least 2 months. These investigators did not examine antibody titers or T cell responses in these patients. Another study examined SARs-CoV-2 nasal and blood PCR levels in kidney transplant patients with SARS-CoV-2 infection. This study showed that 25% displayed persistent viral shedding and that all patients developed antibodies that persisted for 2 months. Importantly, they observed that those with viremia had an increased mortality. Again, the nature of the immune response was not examined. It is possible that low titers of non-neutralizing antibodies were produced in those with poorer outcomes. One also has to question the role of the HLA phenotype in effective antigen presentation, as discussed above [49]. Pendecki *et al*. [50] performed an extensive evaluation of SARS-CoV-2 IgG antibodies in 38 immunocompromised kidney transplant recipients, all of whom had tested positive for SARS-CoV-2 by PCR. In this cohort, only three of 38 (7·9%) failed to generate antibodies after infection. The authors also examined a population of 822 kidney transplant patients and found a prevalence of IgG anti-SARS-CoV-2 antibodies using RBD peptides of 10·4%, which is higher than that seen in normal populations. They did not determine outcomes of these patients or comment upon the durability of IgG responses. They also determined that antibody assays using RBD peptides as targets are more sensitive than those using NP peptides.

We have had the opportunity to examine IgG antibody responses and COVID-19 T cell ($CD4^+$/$CD8^+$) in a kidney transplant patient who developed and recovered from COVID-19 infection. At 4 months post-infection, IgG antibodies were at a low level of detection and no responses of $CD4^+$/$CD8^+$ T cells to spike proteins were seen. This raises many questions regarding the efficacy and durability of immune responses in this individual. It was assumed that he would probably benefit from vaccination. Clearly, there is much to do before we truly understand the immune responses to this virus in immune compromised individuals.

Understanding the composition and durability of immunological memory to SARS-CoV-2

An interesting and informative paper recently published by Dan *et al*. [33] explored the constituents of immunological memory that developed after confirmed SARS-CoV-2 infection in 185 patients, with 41 patients having more than one determination at approximately 6 months after initial infection. This study attempted to improve our understanding of the full complement of immunological

memory which has not yet been performed. The authors simultaneously examined spike- specific IgG, IgA, IgM responses, spike-specific B memory cells (B_m) and $CD4^+$ and $CD8^+$-T cell responses specific for SARS-CoV-2. Patients studied exhibited the full range of clinical manifestations of SARS-CoV-2 infection. The value of this study is that it revealed real-world information on the kinetics of humoral and cellular immune responses to COVID-19.

Spike-specific IgG responses (including IgG to RBD) were present in 'almost all' individuals at 5 months post-COVID-19 infection. Due to lack of sampling frequency, the authors could not precisely determine the rate of decay of spike-specific IgG, but found a broadly heterogeneous initial spike-IgG response that did not configure into a stable or assessable memory profile. Thus, diversity in antibody responses to SARS-CoV-2 was the most consistent feature of humoral immune responses.

From my standpoint, the most interesting and novel aspect of this paper is the examination of B_m cell responses. The authors found that spike-specific B_m cells ($CD19^+$, $CD27^+$ IgD^-) were found in 'almost all' patients and did not demonstrate a determinable half-life. In fact, they appeared to increase up to 5 months post-SARS-CoV-2 infection. The importance of this observation cannot be over-estimated. If confirmed, it could represent a long-lived recognizant B cell and IgG response capacity. In fact, B_m responses have been detected up to 60 years after smallpox vaccination and greater than 90^+ years after infection with the 1918 H1N1 influenza A virus [51,52].

To further understand the composition of B cell immune responses, we have to return to the T cell compartment. T follicular helper cells (T_{fh}) constitute a subset of $CD4^+$ T cells that are critical in activating naive B cell immune responses to antigens (SARS-CoV-2) in the germinal centers. In this regard, cytokines (IL-6 and IL-21) are critical to drive naive $CD4^+$ T cells to T_{fh} cells. Dan *et al.* [35] examined circulating T_{fh} cells (cT_{fh}) specific for SARS-CoV-2 in their aforementioned patient population. Memory cT_{fh} cells were detected in 100% of patients infected with SARS-CoV-2. This memory appeared to be robust and persisted for more than 6 months.

$CD4^+$ and $CD8^+$ T cell responses to SARS-CoV-2 peptides were also examined. The investigators found that most patients developed $CD4^+$/$CD8^+$ responses to SARS-CoV-2. There was a slow decay observed over 6 months. However, the investigators felt the responses were similar to those seen with yellow fever vaccines, where the long-term durability could be ~ 10 years. This is similar to a recent report describing $CD4^+$ T cell responses to SARS-CoV-1, 17 years post-infection [51,52].

The authors offer several considerations of their work, suggesting that there are few certainties regarding our understanding of how effective or how long our immune responses to SARS-CoV-2 or vaccines will last. However, reasonable assumptions can be made. First, sterilizing immunity requires the presence of high-titer IgG anti-spike (RBD) antibodies. Short of this, it is not yet known how those with memory T and B cell responses would handle subsequent encounters with the virus. As memory B and T cells have to be reactivated by antigen-presenting cells, they cannot deliver sterilizing immunity immediately. This process has to develop over time. Thus, an initial infection event with SARS-CoV-2 is needed, but is probably rapidly dissipated as immune activation events progress [44]. This would probably limit SARS-CoV-2 to a URI or 'cold'-like illness. Again, we cannot be sure of this, and one must consider that analysis of cells from the peripheral blood probably does not represent resident SARS-CoV-2 reactive memory T and B cells in lymphoid tissues of the upper respiratory tract and lungs, which could result in more rapid and effective immunity. The authors conclude that immune memory to SARS-CoV-2 consisting of at least three of five immunological compartments (IgG, B_m, $CD4^+$, $CD8^+$ T cells) was measurable in ~ 90% of individuals more than 5 months after SARS-CoV-2 infection, indicating that durable immunity against COVID-19 disease is probable for most individuals. However, some individuals who exhibit poor memory responses, as is likely in immunocompromised individuals or those with poor antigen presenting capabilities, may be susceptible to reinfection. More data are needed to completely understand the complexities and integration of immune responses to SARS-CoV-2.

Summary and conclusions

In this paper, we have attempted to address the cogent issues regarding innate and adaptive immune responses to the novel SARS-CoV-2. As noted, data regarding these issues are rapidly accumulating and helping the development of new therapeutic approaches for treatment of patients infected with SARS-CoV-2 and development of novel vaccines. At the time of writing, data of two novel vaccines have been released, both showing > 95% efficacy in preventing SARS-CoV-2 infection [53,54]. This is extremely important to all of us, especially to determine on a large scale if exposure to these vaccines can prevent disease. It will be of interest to see data on the ability of these novel vaccines to initiate and sustain both antibody and T cell-mediated immune responses, as described above. Fortunately, we now have the tools to analyze both antibody and B and T cell immune responses to SARS-CoV-2 antigens.

There was a surge in interest in analyzing and monitoring antibody responses as a measure of the presence and duration of immunity to SARS-CoV-2. However, data from several studies discussed here suggest that antibodies can rapidly dissipate, lasting no more than 10 months. It is

important to understand that this does not mean that those infected have lost immunity to SARS-CoV-2, as T cells and possibly memory B cells have the capacity to recall and initiate sterilizing immune responses. The nature of immune responses to SARS-CoV-2 has also been discussed, and it is noted that investigators have identified productive immune responses that consist of granulysin producing T cells and $CD160^{+}$ NK T cells + antibody. Investigators have also identified patients with severe infection who develop T cell exhaustion and senescence, resulting in ongoing dysfunctional T cell activation that may account for the manifestations of the post-viral inflammatory syndromes and possibly autoimmune manifestations seen in some patients [55]. This will be a new frontier for scientific investigation and analysis of post-SARS-CoV-2 infection-related immune dysregulation.

This review started with an examination of the innate immune responses which included cytokines and complement activation. The interventions directed at the IL-6/IL-6R Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway have shown variable, but mostly disappointing, results in treating patients with active SARS-CoV-2 pneumonia; however, recent data are encouraging for the use of tocilizumab in the most severely ill patients [22]. Despite demonstration of pathogenicity, anti-complement therapies have also not gained favor, although clinical trials with anti-C5a are still under way [30]. Modification of innate immunity was the first attempt to modify the pathogenicity of SARS-CoV-2 but, as noted, above the focus has rapidly moved to a clearer understanding of adaptive immune responses and how this information can be used to design more effective and durable vaccines. Ultimately, this is the last best hope for controlling the pandemic and arming ourselves against future assaults from as-yet unknown viral pathogens.

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Data Availability Statement

Not applicable to this paper.

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