**COVID-19 and immunity: quo vadis?**

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**Abstract**

Understanding the precise nature and durability of protective immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is essential in order to gain insight into the pathophysiology of coronavirus disease 2019 (COVID-19) and to develop novel treatment strategies to this disease. Here I succinctly summarize what is currently known and unknown about the immune response during COVID-19 and discuss whether natural infections can lead to herd immunity.

Key words: SARS-CoV-2, T cells, B cells, herd immunity

## Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel single-stranded RNA virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of February2, 2021, approximately 100 million people have been infected with SARS-CoV-2, which has caused more than 2 million deaths worldwide, and these numbers are still increasing. COVID-19 is transmitted from person to person mainly via the respiratory route through droplets emitted by carriers of SARS-CoV-2. It is also transmitted in the form of virus-containing micro-droplets (< 5 μm) that can remain suspended for long periods and can be directly inhaled into the lungs (reviewed by [1, 2]).

Human angiotensin-converting enzyme 2 (ACE2) is the receptor for SARS-CoV-2 [3]. Although ACE2 is expressed to varying degrees in many organs, it is its expression in airway epithelia that plays a crucial role in COVID-19 pathogenesis, leading to pneumonia and acute respiratory distress syndrome in severe cases.

Although typical clinical symptoms of COVID-19 include fatigue, fever, dry cough and dyspnea, approximately 80% of the patients are either asymptomatic or have only mild symptoms, with the remaining showing severe (10-15%, requiring oxygen) and critical (5-10%, requiring artificial ventilation) forms the disease [4, 5]. Critical cases are often associated with dysregulated immune responses, the epitome of which is termed the cytokine storm, or multisystem inflammatory syndrome in the case of children, which is caused by the abrupt, massive release of inflammatory cytokines, such as IL-1, IL-6 and TNFα, by innate and adaptive immune cells [6]. Nevertheless, the fact that most people develop only mild symptoms indicates that the immune system can largely hold the virus in check, although the nature of the protective immune response to SARS-CoV-2 is not yet fully characterized. Here I discuss recent advances in the study of the innate and adaptive immune responses to SARS-CoV-2 and outline the questions remaining about SARS-CoV-2-induced immunity.



## Innate immune responses

Similarly to the innate immune responses in other viral infections, those of COVID-19 play a critical role in protection against SARS-CoV-2. In particular, X-chromosomal Toll-like receptor 7 (TLR7) has been implicated as one of the key pattern-recognition receptors (PRRs) involved in the recognition of SARS-CoV-2 single-stranded RNA. TLR7 deficiency due to loss-of-function variants has been identified in patients with severe COVID-19, who all

showed the suppressed production of type I interferons (IFN-I) and type II interferon (IFNγ) [7]. Because *TLR7* escapes X inactivation [8], it is more abundantly expressed in women, which might well be linked to the more prominent TLR-7 induced IFN-I responses in women [8] and their decreased susceptibility to develop severe COVID-19 compared with men. Besides TLR7, endosomal TLR3 as well as cytoplasmic retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) also sense SARS-CoV-2 single-stranded RNA (reviewed by [9]).

In SARS-CoV infections, signaling downstream of these PRRs induces the activation of nuclear factor κB (NF-κB) to produce inflammatory cytokines and phosphorylation of interferon regulatory factors, including IRF3 and IRF7, which leads to the robust production of IFN-I. Specific virus-derived proteins have been shown to induce activation of the nucleotide-binding oligomerization domain (NOD)-like receptor (NRL)-family pyrin domain containing 3 (NLRP3) inflammasome and the subsequent release of IL-1β and IL-18 in cells infected with SARS-CoV, and similar events are likely to occur in SARS-CoV-2-infected cells [9].

Recent studies on a variety of human coronaviruses (HCoVs), including SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), found that these viruses have evolved multiple mechanisms to impair the signaling components of the PRR–IFN-I pathways to allow their survival in host cells. SARS-CoV-2 also has some distinct mechanisms to antagonize proinflammatory signals, particularly IFN-I signaling (reviewed by [10]). Xia *et al*. [11] identified a number of SARS-CoV-2 viral proteins that can antagonize IFN-I production and signaling through distinct mechanisms; they found that the nonstructural proteins nsp6 and nsp13 bound to TNF receptor-associated factor (TRAF)-family member associated NF-κB activator (TANK)-binding kinase 1 (TBK1) to suppress IRF3 and TBK1 phosphorylation, respectively, whereas open reading frame 6 (ORF6) blocked IRF3 nuclear translocation, and all these events led to impaired IFN-I production. Moreover, nsp1 and other viral proteins suppressed IFN-I signaling by inhibiting the phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2.

Genetic analysis implicated the involvement of inborn errors of TLR3-dependent and IRF7-dependent IFN-I immunity in the exacerbation of COVID-19 [12]. In addition, anti-IFN-I autoantibodies have been identified in a substantial proportion of patients with severe COVID-19. Bastard *et al*. [13] reported that approximately 10% of patients with life-threatening pneumonia had neutralizing antibodies against IFN-I, whereas none of the asymptomatic patients or patients with mild COVID-19 had such antibodies. Because IFN-I is one of the most important anti-viral cytokines, it is not difficult to imagine that, in one way

or another, IFN-I deficiencies can promote uncontrolled viral replication and dissemination in patients with COVID-19.

It is also possible that abnormalities in the IFN-I pathway lead to excessive inflammatory responses in the lungs of COVID-19 patients, given that mice deficient in the IFN-I signaling pathway showed enhanced virus-induced lung inflammation [14]. The dysregulation of IFN-I production and signaling is thought to cause the delayed and aberrant production of proinflammatory cytokines that is often observed in patients with COVID-19 [15, 16]. The abortive IFN-I production and signaling are also likely to be causally linked to a frequently observed feature of this disease, i.e., the absence of 'flu'-like symptoms or asymptomatic disease progression in some patients.

**Adaptive immune responses**

Evidence from experimentally infected animals suggests that antibodies that neutralize the virus are the primary mode of protection against SARS-CoV-2, and both Th1-biased $CD4^+$ T cell responses and $CD8^+$ T cell responses can augment protection [17]. Clearly, various arms of the adaptive immune responses must act in coordination to limit disease severity, as described below.

i) *B cell responses*

Recent clinical reports indicate that neutralizing-antibodies to the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein are protective. Röltgen *et al*. [18] showed that anti-RBD responses were strongly associated with clinically milder infections. Moreover, Lucas *et al*. [19] showed that early neutralizing anti-spike IgG responses correlated with COVID-19 recovery, whereas delayed antibody production was associated with lethal COVID-19. In addition, the survival and recovery of COVID-19 patients were reportedly linked to early class-switching to anti-spike IgG and the ability of the antibodies to employ Fcγ receptors [20], suggesting that both IgG responses and Fcγ-related mechanism are important for virus elimination.

Nevertheless, patients who have X-linked agammaglobulinemia who developed COVID-19 pneumonia have been reported to recover [21]; therefore, antibody-independent mechanism(s) also appear to be important for virus clearance. In this regard, the study by Moderbacher *et al*. [22] convincingly demonstrated that coordinated SARS-CoV-2-specific adaptive immune responses, namely neutralizing-antibody responses by SARS-CoV-2-specific B cells as well as $CD4^+$ and $CD8^+$ T cell responses, were associated with milder

pathology, whereas the uncoordinated adaptive responses often observed in aged individuals were linked to greater disease severity.

One of the most important questions concerning adaptive immune responses to SARS-CoV-2 is how long the specific antibody responses to the virus last. Studies on seasonal coronaviruses show that anti-viral immunity to three out of four virus species (HCoV-NL63, HCoV-229E and HCoV-OC43) progressively declined over 6 to 12 months, and re-infection with the same virus occurred frequently at 12 months after infection [23]. Although there has been controversy over the longevity of the antibody response to SARS-CoV-2 [24], a recent study clearly indicated that the majority of convalescent subjects are 'decayers' who show a substantial decay or decline in their anti-SARS-CoV-2 IgG antibody response, whereas some are 'sustainers' showing stable or enhanced IgG production over the same period [25]. This study also showed that the sustainers harbored increased somatic mutations in virus-specific memory B cells and tended to have shorter and milder disease courses, confirming that appropriate B cell responses are indeed beneficial for protection against SARS-CoV-2, although there is still inter-individual heterogeneity.

Nevertheless, the story seems to be a little more complicated than anticipated, since a few studies have indicated that neutralizing-antibody responses were sometimes associated with severe disease outcomes in coronavirus infections. Using rhesus monkeys infected with SARS-CoV, Liu *et al*. [26] showed that the presence of anti-spike IgG prior to viral clearance abrogated the recruitment of wound-healing macrophages to the lungs, promoting proinflammatory cytokine-induced lung inflammation. Several clinical studies, including those from Europe [27] and China [28], showed that neutralizing-antibodies were more abundant in COVID-19 patients with severe disease than those with mild forms of the disease.

Woodruff *et al*. [29] showed that dysregulated antibody responses to SARS-CoV-2 frequently occurred in patients with severe COVID-19, apparently because of the formation of low-affinity antibodies by the extra-follicular B cells that reside outside germinal centers in secondary lymphoid tissues. Consistent with this observation, the germinal centers were absent in the lymph nodes and spleens of patients with severe COVID-19 and this correlated with the specific inhibition of the differentiation of Bcl-6-expressing T follicular helper T ($T_{FH}$) cells [30]. $T_{FH}$ cells are known to be indispensable for germinal center formation, affinity maturation and the development of high-affinity antibodies [31], and the elevated production of proinflammatory cytokines, including TNF$\alpha$, has been shown to abolish $T_{FH}$ cell differentiation [32]. Thus, the cytokine-induced loss of $T_{FH}$ cells and subsequent impairment of germinal center formation may, at least partly, account for the lack of durability and the lower quality of the B cell responses observed in severe SARS-CoV-2 infections.

The presence of 'enhancing antibodies' that can promote viral infection (i.e., antibody dependent enhancement of viral infection or ADE) has been reported for a number of coronaviruses [33]. Although it remains unknown how frequently ADE occurs in SARS-CoV-2 infections, a recent study showed that the enhancement of SARS-CoV-2 infection was commonly observed in plasma from elderly patients with severe symptoms and that it was associated with prolonged disease duration [34]. In addition, while a multitude of studies indicated that neutralizing antibodies that are abundant in patients with severe COVID-19 are mainly directed against the RBD of the spike protein S1 subunit [35, 36], a recent study by Liu *et al*. [37] showed that antibodies reactive to the N-terminal domain (NTD) of the spike protein S1 subunit were also abundant in hospitalized COVID-19 patients. The antibodies enhanced *in vitro* SARS-CoV-2 infectivity in a dose-dependent manner by enhancing the spike protein binding to ACE2, although the ratio of infectivity-enhancing antibodies to neutralizing antibodies was variable. These antibodies were invariably found in all the hospitalized COVID-19 patients in the study; therefore, an interesting possibility is that such antibodies are one of the exacerbating factors for COVID-19.

In this regard, it should be pointed out that all currently used COVID-19 mRNA vaccines encode the full-length spike protein; this raises the possibility that the administration of such vaccines might induce these enhancing anti-NTD antibodies in a certain proportion of vaccinated individuals.

*ii) T cell responses*

There is no doubt that SARS-CoV-2 induces T cell immunity. A group of $CD4^+$ and $CD8^+$ T cell clones that reduce in abundance after the patient recovers from a SARS-CoV-2 infection have been detected by longitudinal T cell antigen-receptor (TCR) repertoire sequencing [38]. Multiple distinct T cell response patterns have been reported in different patients, possibly as a result of the individuals' co-morbidities, genetic burden, immunological state at the time of infection and other variables [39, 40].

The above-mentioned SARS-CoV-2-induced impairment of the IFN-I pathway is thought to affect the early expansion and differentiation of anti-viral T cells, which may particularly affect elderly individuals with reduced and delayed T cell responses [41]. However, a recent study reported that high levels of SARS-CoV-2-reactive cells in peripheral blood were associated with protection from symptomatic SARS-CoV-2 infections [42], which is a promising sign that infection drives protective immunity in T cells. Consistent with this observation, most individuals with resolved infections have developed a robust and broad T

cell response targeting multiple structural and non-structural regions of SARS-CoV-2 [43], an observation that has been corroborated by others [44]. Such diverse T cell responses may provide multiple forms of protection that can help to mitigate against viral escape from immune responses.

Paradoxically, the above-mentioned study by Peng *et al*. [43] indicated that those with more severe COVID-19 have stronger T cell responses, raising the possibility that such responses may contribute to disease severity. Consistent with this, a study by Bacher *et al*. [45] showed that patients with severe COVID-19 developed a strong but rather unfocused $CD4^+$ memory T cell response and these T cells displayed lower functional avidity and clonality, despite increased frequencies, compared with mild cases. Thus, the role of SARS-CoV-2-specific T cells may not always be protective but may depend on the environment in which they are activated.

SARS-CoV-2 infection also appears to affect the differentiation and expansion of regulatory T cells (Tregs). Kalfaoglu *et al*. [46] examined $CD4^+$ T cells derived from the bronchoalveolar lavage fluid of COVID-19 patients and reported that Tregs were greatly diminished because of the impaired expression of the Treg-inducing transcription factor, FoxP3, although $CD4^+$ T cells were hyperactivated. On the basis of these results, they suggested that FoxP3-mediated negative-feedback mechanisms are impaired in the lungs, and possibly contribute to T cell hyperactivation and exacerbation of tissue damage there.

However, Tregs seem to be increased and hyperactive in the blood of patients with severe COVID-19. Galván-Peña *et al*. [47] reported that, in the peripheral blood of patients with severe COVID-19, both Treg proportions and intracellular levels of FoxP3 were increased, which closely correlated with poor outcomes. In addition, these Tregs showed a similar gene expression profile to tumor-infiltrating Tregs and they over-expressed a number of suppressive effector molecules. From these results, the authors suggested that blood Tregs are transformed into a phenotype similar to tumor-infiltrating Tregs; they strongly suppress ongoing protective T cell responses and contribute to tissue pathology in a similar manner to tumor Tregs. The pathophysiological role of Tregs in COVID-19 warrants further investigation.

Another notable observation with T cells in COVID-19 is that SARS-CoV-2-reactive T cells have been found not only in most convalescent individuals but also in a substantial fraction of blood samples from unexposed heathy individuals whose blood samples were collected before the outbreak of COVID-19 pandemic [44]. Numerous studies from geographically diverse areas have confirmed this observation and have reported significant $CD4^+$ T cell reactivity to SARS-CoV-2-derived peptides in substantial proportions (20–50%)

of unexposed individuals [48-53]. These T cells also cross-reacted with antigens derived from seasonal or 'common cold' coronaviruses (HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E), which share significant sequence homology with SARS-CoV-2. Because all HCoVs circulate widely in human populations, in which they mostly cause mild respiratory tract infections [54, 55], these observations have been interpreted to mean that HCoV infections confer cross-reactive immunity to SARS-CoV-2 [52].

To verify this idea, Sagar *et al*. [56] examined the clinical outcomes of COVID-19 patients who were positive or negative for HCoV infection as assessed by PCR. They found that COVID-19 patients with a history of HCoV infection had higher rates of survival than COVID-19 patients with no history of HCoV infection, although there were no differences between the two groups' susceptibility to SARS-CoV-2 infection. These results raise the possibility that a pre-existing HCoV memory contributes to the heterologous disease outcomes characteristically observed in COVID-19 patients. However, whether these cross-reactive immunities plays protective or exacerbating roles, or both, in immune responses against SARS-CoV-2 remains to be verified.

**Herd immunity**

There have been numerous discussions on how soon herd immunity against SARS-CoV-2 infection would be achieved [57-63] or whether herd immunity has already been achieved [64] in some parts of the world. Herd immunity, which is also known as population immunity or community immunity, is a key concept in the epidemiological control of pathogens and refers to the protection of susceptible individuals against infection when a specific proportion of the population (called the 'herd immunity threshold') becomes immune to the disease. In other words, when such a threshold is reached through natural infection or vaccination, susceptible individuals are protected from the endemic transmission of the pathogen, as the surrounding population becomes immune. However, in the case of naturally acquired immunity, there will always be the danger that it is accompanied by a substantial burden of illness and death.

Classically, herd immunity is calculated as $(1-1/R_0) \times 100$, where $R_o$ (the basic reproduction number) indicates the average number of persons infected by a single infected person. With SARS-CoV-2, the $R_o$ is currently estimated to be ~2.5 and the herd immunity threshold is thus calculated to be ~60% [62, 65], indicating that the virus might spread until 60% of the population becomes immune.

Although the concept of herd immunity is widely accepted, at least three distinct problems inherent in the classical concept of herd immunity are less appreciated. First, the classical concept assumes that herd immunity is mainly antibody-mediated, whereas emerging evidence indicates that anti-viral immunity requires both innate and adaptive immune responses and that antibodies are only one part of protective immunity. Second, the classical concept assumes that human populations mix homogenously and are equally susceptible and contagious. However, human populations are very heterogeneous because T cells recognize pathogen-derived antigen peptides in the context of extremely polymorphic MHC proteins. Hence, there is a great diversity of immunological competence among people in terms of their response to viral antigens [66, 67]. Additionally, local socio-demographic differences lead to some people having more exposure to a pathogen than others, hence, natural infection also does not occur randomly. Third, the classical concept assumes that infection confers durable immunity, but this is not always the case with many types of viral infections, including SARS-CoV-2. If individual immunity is transient, the herd immunity threshold would not be sustained and, hence, population immunity cannot be easily achieved. Taking variations in individual susceptibility, exposure to infection and social activity levels into consideration, Britton *et al*. [68] estimated the herd immunity threshold for SARS-CoV-2 to be approximately 43% across a broad range of populations, whereas Aguas *et al*. [69] suggested it could be as low as 10 to 20%.

So far, herd immunity has been mainly estimated using serological tests that measure anti-pathogen antibodies in representative samples from a community. However, a situation in Manaus, Brazil, indicates that seroprevalence is not necessarily an appropriate indicator for SARS-CoV-2 herd immunity. On the basis of seropositivity rates in Manaus, Buss *et al*. [70] reported that the cumulative incidence of SARS-CoV-2 infection had reached ~75% by October 2020, exceeding any of the putative herd immunity threshold so far calculated. Nevertheless, a recent report from Brazil [71] indicated that SARS-CoV-2 infection is resurging in this city. While it remains unknown whether the infection occurred in the remaining ~25% that had not been infected or in the individuals whose immunity had waned with time, it has to be kept in mind that the seroprevalence at a few time points may not be a reliable parameter for determining the development of herd immunity in a community.

Given that SARS-CoV-2-specific T cells are detected in the great majority of COVID-19 patients, irrespective of their clinical status [72], and that B cell responses decline over a timescale of months in a significant proportion of COVID-19 patients [25], T cell responses might be more sensitive indicators for SARS-CoV-2 infection prevalence than seropositivity. However, two recent papers showing durable memory formation in B cells as well as T cells in SARS-CoV-2 infection [73, 74] caution against drawing a hasty conclusion on this issue. It

should also be kept in mind that cross-reactive immunity with seasonal coronaviruses may affect the frequency of SARS-CoV-2-reactive T and B cells in the general population.

The current endemic wave is progressing much more rapidly and becoming more severe than the previous two peaks, at least in Japan, and SARS-CoV-2 seropositivity has consistently been <3% in a number of areas [75]; therefore, it is unlikely that Japan has achieved herd immunity to any functional degree. The fact there have been frequent large-scale infection clusters in Japanese high schools and universities [76-78] also supports this contention. On the basis of these observations, it is unlikely that SARS-CoV-2 can be eliminated from Japan through naturally acquired herd immunity. Rather, it is likely that we would continue to see multiple waves of endemic SARS-CoV-2 infection of varying magnitudes in the next couple of years, unless the currently developed vaccines can successfully provide durable immunity in large populations. Herd immunity is, therefore, only achievable through vaccination.

**Vaccines**

Bacillus Calmette-Guérin (BCG) induces long-term stimulation of innate immunity, which is now termed trained immunity [79]. BCG vaccination has been reported to correlate with reduced COVID-19 case fatality rates [80, 81]. Publicly available resources also indicate that both COVID-19 incidence and total deaths are strongly associated with the presence or absence of national mandatory BCG vaccination programs, raising the question of whether BCG vaccination and reduced COVID-19 mortality are causally related [82]. A recent study with healthcare workers in Los Angeles showed that BCG vaccination was associated with reduced IgG responses and clinical symptoms in SARS-CoV-2 infection [83]. A non-randomized observational study in the United Arab Emirates with hospital staff aged between 21 to 80 years old showed that a group of 71 individuals who received the booster BCG immunization (the first one was administered at birth) had no positive PCR cases whereas a group of 209 individuals with newborn vaccination alone had 18 positive PCR-detected COVID-19 cases [84], indicating the potential usefulness of the booster BCG vaccination in preventing COVID-19 infections. Currently, trials assessing the efficacy of BCG vaccination to protect against COVID-19 are being conducted in Australia and the Netherlands [85].

A number of recent reports indicate that messenger RNA (mRNA) vaccines, particularly those released from Pfizer and Moderna, are highly effective for inhibiting

COVID-19 illness, achieving >90% efficacy among the >70,000 clinical trial participants (half of 43,000 people in Pfizer's case and half of 30,000 people in Moderna's), after two-time intramuscular injections 3 to 4 weeks apart [86-88]. The frequency of unsolicited adverse events and severe adverse events related to the vaccination was apparently very low. The primary mode of protection appears to be neutralizing-antibodies, although $CD8^+$ T cell responses also appear to be involved in protection as judged by the data obtained from vaccinated monkeys [89]. Currently, the following questions remain unknown: (1) whether these vaccines can protect against asymptomatic SARS-CoV-2 infection, (2) how long the protective immune response to SARS-CoV-2 lasts, (3) to what extent these vaccines are protective against SARS-CoV-2 mutant strains, (4) whether these vaccines can confer herd immunity, and (5) how safe these vaccines are in the long run.

## Conclusions

Although both innate and adaptive immune responses clearly play critical roles in generating protective immunity to SARS-CoV-2, they may also have detrimental effects on the clinical outcome of COVID-19. Although IgG responses are important for virus elimination, the fact that antibody responses are stronger in patients with severe forms of the disease indicates that their internal environment antagonizes the function of neutralizing-antibodies. Indeed, antibodies that can promote viral infection have been found in patients with severe COVID-19, indicating that anti-viral antibodies are a double-edged sword. Pre-existing T cell immunity, probably derived from prior infection by common-cold coronaviruses, appears to significantly impact the pathophysiology of COVID-19, but it remains unresolved whether it has any protective or detrimental roles. Finally, it is unlikely that we have reached herd immunity through natural infection in any part of the world and vaccination seems to be the only realistic path to population immunity against SARS-CoV-2. It is now our task to urgently obtain a much more complete understanding of the mechanism by which immune responses limit or promote COVID-19, as it is likely that the worst of this pandemic is yet to come.

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**Conflicts of Interests statement**

I declare that I have no competing interests regarding the article.

**Abbreviations**

ACE-2: angiotensin-converting enzyme 2; COVID-19: coronavirus disease 2019; HCoV: human coronavirus; IFN-I: type 1 interferon; IFNγ: interferon gamma; MDA5: melanoma differentiation-associated protein 5; NF-κB: nuclear factor κB; NLRP3: NRL family pyrin domain containing 3; NTD: N-terminal domain; PRR: pattern recognition receptor; RBD: receptor-binding domain; RIG-I: retinoic acid-inducible gene 1; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; $T_{FH}$ cell: follicular helper T cell; Treg: regulatory T cell; TLR7: Toll-like receptor 7

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