



Suboptimal Humoral Immunity in Severe Acute Respiratory Syndrome Coronavirus 2 Infection and Viral Variant Generation

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KEYWORDS

- SARS-CoV-2 • COVID-19 • Humoral immunity • Germinal centers
- T follicular helper cells • Variant generation • Immune pressure

KEY POINTS

- Severe COVID-19 generates neutralizing antibodies but with very little affinity maturation.
- In these patients switched memory B cells are generated extra-follicularly.
- Germinal centers do not properly develop in severe COVID-19.

The existence of novel coronavirus infection in Wuhan China was established in late December 2019.^{1,2} The severe acute respiratory disease first documented at that time and now called coronavirus disease (COVID)-19, was in terms of clinical features, virtually indistinguishable from SARS (severe acute respiratory syndrome) observed only in 2002 and 2003.³ Although there was a delay of many months before the viral etiology of the “original” SARS was finally identified in May 2003, the virus that caused the novel viral disease in Wuhan in 2019 was identified by many laboratories in a matter of days in the last week of 2019. The sequence of this positive orientation, single-stranded enveloped RNA virus bore strong similarities to the SARS coronavirus as well as to the coronavirus that causes a related disease, called Middle East respiratory syndrome or MERS.⁴ The virus of the 2002 to 2003 epidemic is now called SARS-CoV (or sometimes SARS-1) and the coronavirus that causes COVID-19 is called SARS-CoV-2.

Although SARS-CoV-2 is clearly more easily transmissible than SARS-CoV, and certainly far more extensive and sophisticated studies have been performed in COVID-19 than were undertaken in SARS, at this point, in terms of the “bigger picture”, it does not appear that there are major differences in the overall adaptive

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immune responses seen in COVID-19 than those that were seen in SARS, nor are the changes seen so far in the lung particularly different. However, draining lymph nodes, the sites of adaptive immune response induction, have rarely been systematically interrogated in the context of any human infectious diseases and our studies on COVID-19 focused on the site of severe infection (the lungs) and on secondary lymphoid organs including the draining thoracic lymph nodes and the spleen.

The nature of the T-dependent B cell response in viral infections determines the extent of the durability of the protective antibody response, but T-B interactions in the context of dysregulated inflammation may also contribute to pathologic alterations in B cells and thus to disease progression and disease sequelae. The emergence of B cells with a switched-memory phenotype is not in itself a marker for durability, (the length of time for which there is effective protection from reinfection by circulating antibodies). Memory B cells disappeared during a 6-year follow-up period in patients with SARS.⁵ In COVID-19, natural immunity (immunity generated by infection) has been documented to decline and does not effectively generate protection or herd immunity.⁶⁻¹⁰ We believe that suboptimal adaptive immunity against SARS-CoV-2 as documented by us in terms of the cells involved in humoral immunity,¹¹ but which also likely applies to cellular immunity, facilitates viral persistence, and variant generation. In this review, we will first discuss the durability of natural immunity in SARS, MERS, and COVID-19. We will then discuss the biology of germinal centers and the loss of germinal centers in severe COVID-19 and focus on the loss of Bcl-6+ T follicular helper cells in this disease. We will subsequently discuss the loss of germinal centers in other severe infections in animal models and examine what that might suggest in a mechanistic sense. Finally, we will consider the potential pathologic consequences of the extrafollicular B cell response in COVID-19.

NATURAL IMMUNITY IN SEVERE ACUTE RESPIRATORY SYNDROME, MIDDLE EAST RESPIRATORY SYNDROME RARELY GENERATED DURABLE HUMORAL IMMUNITY, AND THE DATA ON CORONAVIRUS DISEASE-19 ARE STILL EMERGING

Slifka, Antia, Ahmed, and colleagues have highlighted the dichotomy between the robust, virtually life-long, humoral immunity seen with natural infections with viruses such as measles, mumps, and rubella or vaccination with live-attenuated viral vaccines (with calculated antibody half-lives ranging roughly from 50 to 100 years) in comparison to the steadily declining humoral immunity seen in most of the infections with SARS and MERS and after immunization with killed parenteral vaccines such as the one used in influenza.¹²⁻¹⁵ In one of the deepest follow-up studies in SARS, a disease for which the virus was rapidly eliminated, and no vaccine was administered subsequently, patients were followed for up to 6 years after infection.⁵ Virus-specific IgG antibodies steadily waned and had completely disappeared in 21 of 23 patients; specific memory B cells had also become undetectable in all 23 by the end of the study, though about half preserved memory T cells.

In COVID-19, vaccine availability may make conducting a similar study difficult, but all indications are that a trajectory for humoral immunity that is, similar to that observed in SARS is likely. Both antigen-specific switched memory B cells and plasma cells can, therefore, either be relatively short-lived, in the order of 2 to 4 years, after an infection such as SARS, or very long-lived after infection with measles or mumps or after yellow fever vaccination. Just as we recognize that short-lived plasma cells are of extrafollicular origin while long-lived plasma cells emerge from the germinal center, are there 2 different sites at which switched memory B cells are generated after different categories of human viral infections?

Is there an underlying immunologic explanation for why the durability of specific humoral immunity in SARS, MERS, and likely COVID-19 is so different from humoral immunity in infections such as measles, mumps, and rubella? What exactly is the relevance of antigen-specific switched memory B cells in COVID-19? It is also of some interest to ask whether the altered nature of the humoral immune response initiated in the draining lymph nodes in infections such as SARS, MERS, and COVID-19 (and described by us in COVID-19 as discussed in this review) may contribute to a possible increase in disease-related B cells in the end-organ and thus to the pathogenesis of COVID-19.

Most of our understanding of the pathogenesis of severe COVID-19 has been derived from generally nonquantitative studies of the blood, with few systematic studies of adaptive immune cells at the sites of infection and in draining lymph nodes. Although the virus attenuates type I interferon production by infected cells^{16,17} and the antiviral state is further compromised in some susceptible individuals by mutations or by preexisting antibodies to type I interferons,^{18,19} most of the initial tissue damage in the lungs is likely generated by excessive unregulated inflammation.

THE BIOLOGY OF THE GERMINAL CENTER RESPONSE

One of the most remarkable phenomena in adaptive immunity that has been of great interest to immunologists as well as molecular biologists is the germinal center response. This response is the key to protective responses against most pathogens and is central to the success of vaccination.

Over a century ago, pathologists had recognized the presence of proliferating cells in organized collections in lymph nodes (reviewed in ²⁰). It had originally been assumed, incorrectly, that these were the sites at which lymphocytes were generated, hence they were called “germinal centers.” Little was known then, however, as to what lymphocytes actually did. The function of lymphocytes in adaptive immunity would only be established in 1957 by Gowans.²¹ It soon became clear that there were 2 types of lymphocytes. B lymphocytes are generated in the bursa of Fabricius in birds (or the bone marrow in other vertebrates), whereas T lymphocytes are generated in the thymus.^{22,23} In the early 1960s, it was eventually recognized that germinal centers were not the sites at which lymphocytes are made. Over the next decade, it became apparent that germinal centers are induced structures that emerge after immunization, but their precise functional role remained mysterious for a while.

Although the increase in the affinity of antibodies after repeated immunization, a phenomenon called affinity maturation had been described well before the function of lymphocytes was appreciated, nothing was known about how this phenomenon occurred until the early 1980s (reviewed in^{24,25}). The theoretic possibility that antibody diversity might be caused by a process of somatic mutation had been entertained even in the 1960s by Burnet and others.²⁶ In the early 1970s, the sequencing of antibodies using Edman degradation started to reveal the theoretic possibilities of somatic mutation in antibody diversification, but whether this phenomenon occurred during lymphocyte development or after an immune response was initiated was not entirely clear. The recombinant DNA revolution in the early 1970s and the subsequent cloning of antibody genes started the process by which the function of the germinal center would begin to be understood in the early 1980s. The crucial molecular descriptions of the process of somatic hypermutation by the early 1980s also depended on the ability to generate single clonal B cells and monoclonal antibodies, a technology originally developed by Kohler and Milstein to better understand somatic hypermutation.

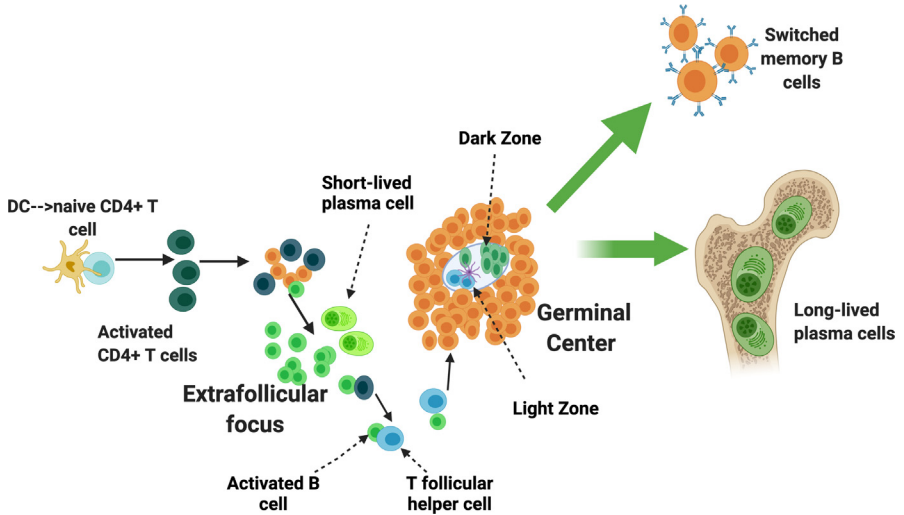


Fig. 1. A schematic overview of germinal center formation and the germinal center response.

The germinal center response is essential for the production of high-affinity antibodies and long-lived plasma cells. It may also contribute to the generation of memory B cells although the requirement for the germinal center for memory B cell generation is not absolute.²⁷ A current overview of germinal center biology is provided in **Fig. 1**. It summarizes detailed knowledge acquired over many decades about this Darwinian process. During an immune response to a protein antigen, helper T cells that recognize a linear peptide determinant within this protein antigen (and that is presented on MHC class II molecules), collaborate with B cells that see a conformational determinant on the same protein antigen. The initial interaction of these cognate helper T cells and B cells occurs at the interface between the T and B cell zones and leads to T cells activating B cells and inducing an extrafollicular B cell response. This extrafollicular response leads to B cell activation and proliferation, isotype switching to most human isotypes other than IgE, some memory B cell generation, and some differentiation of activated B cells into short-lived plasma cells (reviewed in 24, 25).

Naïve CD4+ T cells are initially activated by specific peptide antigens presented on dendritic cells in the T cell zone, and these cells then migrate toward the B cell zone to activate specific B cells that have migrated to the T–B interface.^{28,29} These activated helper T cells that drive the initial extrafollicular activation of B cells are sometimes referred to as pregerminal center T follicular helper cells or pre-GC T_{FH} cells. These cells are not in the follicle, so they are only precursors of “true” T_{FH} cells.^{28,29} At the time that the extrafollicular response is generated (or soon after), activated B cells that now express ICOS-L activate some of these previously activated CD4+ helper T cells and induce their polarization into Bcl-6-expressing T follicular helper cells – cells that are sometimes called GC- T_{FH} cells.¹¹ These cells express high levels of CXCR5 and enter the follicle, likely each with an interacting activated B cell and then set up the germinal center. In the germinal center, B cells proliferate rapidly and undergo somatic hypermutation in a dense region known as the dark zone. After some rounds of proliferation and genetic diversification of the antibody heavy and light chain genes mainly in the regions encoding the V domains, dark zone B cells migrate to the light zone whereby high-affinity B cells recognize antigen held up on follicular

dendritic cells. If they successfully capture the protein antigen and present it, they are selected in the light zone by T follicular helper cells. Selected cells return to the dark zone and there are repeated rounds of somatic hypermutation and selection. Unselected B cells die by apoptosis in the light zone. Early in the germinal center reaction memory B cells emerge, though they may also be generated extrafollicularly. After many rounds of selection, B cells are immortalized as plasmablasts that migrate to the bone marrow and differentiate into long-lived plasma cells.

THE LOSS OF GERMINAL CENTERS IN SEVERE CORONAVIRUS DISEASE -19

We have demonstrated the loss of germinal centers in thoracic lymph nodes in severe COVID-19, as shown in Fig. 2.¹¹ A pathologic description consistent with our data has also been reported,³⁰ Thoracic lymph nodes, such as Peyer's patches and mesenteric lymph nodes, constitutively contain germinal centers; age-matched elderly individuals who died of non-COVID-19 causes in the same time window (and were autopsied in a similar accelerated manner to those who succumbed to COVID-19) had robust germinal centers presumably induced by protein antigens from microbes or allergens constitutively present in the respiratory tract.¹¹ The presence of germinal centers in human thoracic lymph nodes is the norm. The loss of germinal centers in COVID-19 and in SARS^{11,30,31} is reminiscent of the loss of germinal centers in animal models of severe viral infection³² and is likely a general phenomenon when the lymph node milieu is altered by high levels of locally expressed cytokines in the context of a severe infection. However, in patients who survive, it is to be expected that with recovery, the lymph node cytokine milieu that prevents T follicular helper cell maturation and germinal center formation will dissipate. The restoration of affinity maturation in some convalescent individuals who have recovered from severe COVID-19 favors such a view.³³

Quantitation of B cells in the thoracic lymph nodes and the nodes and the spleen showed a dramatic decrease in the absolute numbers as well as percentages of Bcl-6+ CD19+ B cells – basically a loss of germinal center B cells. There was, however, preservation of AID + CD19+ B cells clearly indicating that there was T–B collaboration was preserved both in the follicle and outside the follicle though germinal centers failed to form.¹¹

Our previous studies also showed that SARS-CoV-2-specific switched memory B cells were identifiable in the blood of patients with severe COVID-19.¹¹ These data raised the possibility that the limited durability of humoral responses to some viral infections such as SARS and MERS on the one hand and the extremely long-lived

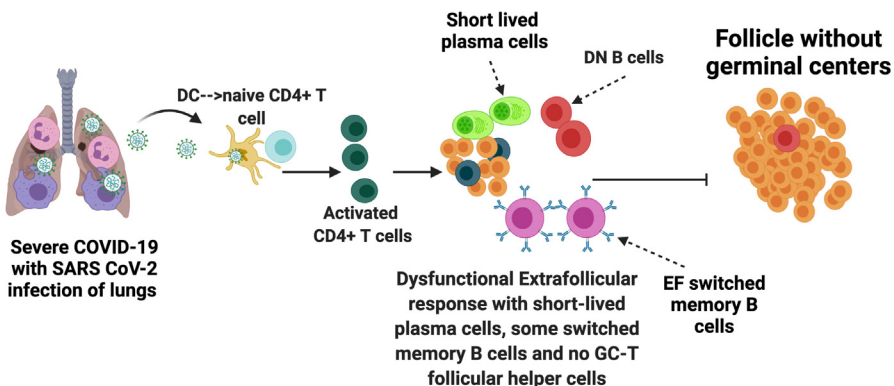


Fig. 2. An overview summary of the failure to generate germinal centers in severe COVID-19.

humoral immunity seen in infections such as measles, mumps, and rubella may have an underlying biological basis, related to the type of memory B cell populations generated in these contexts. In SARS, MERS, and COVID-19, the paucity or absence of proper germinal centers in lymph nodes could result in class-switched memory B cells that develop primarily outside germinal centers, a phenomenon well-established in rodents,²⁷ but not widely appreciated or studied. It is likely that the durability of memory B cells and plasma cells generated outside the germinal center at extrafollicular sites does not match the durability of their counterparts generated in germinal centers, though this has not been formally studied. Although germinal centers likely recover in a subset of survivors of severe infections as suggested from the studies of Wilson and colleagues,³³ and SARS-CoV-2 infection may persist in the gut in many for at least a few months,³⁴ long-lived plasma cell generation is likely somewhat compromised in COVID-19, as it likely was in SARS.⁵ In diseases such as mumps, rubella, and measles both class-switched memory B cells and long-lived plasma cells are likely generated in germinal centers thus generating much greater durability, typically extending for decades.¹³

LOSS OF BCL-6 EXPRESSING FOLLICULAR HELPER CELLS IN SEVERE CORONAVIRUS DISEASE-19

We showed that in patients with severe COVID-19 lymph node architecture was well preserved, there were well-defined follicles and T cell zones and that most CD4+ T cell subsets and regulatory T cells were well preserved.¹¹ There was, however, a striking loss of T follicular helper cells, especially Bcl-6+ T follicular helper cells. This loss of Bcl-6+ T follicular helper cells would suffice to explain the loss of germinal center B cells.¹¹

We showed that there were high levels of TNF- α expression in the thoracic lymph nodes in severe COVID-19¹¹ and we postulated that, as had been more mechanistically examined in murine models of severe intracellular infections discussed briefly in the next section, the high levels seen of TNF- α in lymph nodes might account for the loss of germinal centers in severe COVID-19.

THE LOSS OF GERMINAL CENTERS IN ANIMAL MODELS OF SEVERE INTRACELLULAR INFECTIONS

There are a few murine models of intracellular infections in which germinal centers are lost, and in some, a block in T_{FH} cell differentiation has also been observed.³⁵⁻³⁷ In a murine malaria model, the loss of T_{FH} cells and germinal centers was observed and this was reversed by the blockade of TNF- α or IFN- γ . Genetic deletion of T-bet also prevented the loss of germinal centers. The T_{FH} cell precursors did not express high levels of PD-1 and CXCR5 but expressed genes such as T-bet and CXCR3, characteristic of T_{H1} cells.³⁵

In a study of a murine rickettsial infection caused by *Ehrlichia muris*, the loss of germinal centers was reversed by TNF- α blockade as well as by the use of mice that have an engineered deletion of TNF- α .³⁶ In another study involving *Salmonella* infection, IL-12 was shown to be responsible for the block in T_{FH} cell differentiation and the loss of germinal centers that was seen.³⁷ Given the known functional and sequential links between IL-12, T_{H1} cells and the downstream production of TNF- α , we suspect that signaling through TNFR2 on CD4+ T cells might cause a block in T_{FH} cell differentiation in these models and in severe viral infections.

There is a slightly artificial murine viral infection model in which germinal centers are lost.³² When regular inbred mice are first immunized with a specific LCMV peptide that

activates CD4+ T cells and then later infected with LCMV clone 13, they develop a severe viral infection that results in lymphopenia, the loss of germinal centers in lymph nodes, and an eventually lethal severe viral infection that involves the lungs and other organs. This disease resembles severe COVID-19 in many ways. Very high levels of IL-12 were observed in these mice. Overall a number of severe intracellular infections result in the loss of germinal centers, and this may involve in some poorly defined way, the sequential induction of high levels of IL-12, IFN- γ , and TNF- α .^{11,32,35-37}

THE EXTRAFOLLICULAR B CELL RESPONSE IN CORONAVIRUS DISEASE-19 AND ITS POTENTIAL CONTRIBUTION TO PATHOLOGY

Some activated B cell subsets seem to be key drivers of inflammatory and fibrotic diseases, many of which respond therapeutically to B cell depletion. Our previous studies revealed the presence of subsets of antigen-specific disease-related IgD⁻CD27⁻ double negative (DN) B cells that accumulate in the blood of patients with COVID-19 including those with severe disease¹¹ and similar overall B cell populations have been observed by others.³⁸ We have demonstrated that DN B cells in COVID-19 include SARS-CoV-2 specific cells, but there is no definitive evidence as yet that these cells actually produce the large number of autoantibodies now described in patients with COVID-19.^{39,40} The presence of these cells B cell in the blood correlates with immune dysregulation and a break in B and T cell tolerance in COVID-19. It has, however, never been established whether DN B cells actually accumulate in the lesions of inflammatory or fibrotic diseases, even in diseases that respond to B cell depletion or if they interact with CD4+ T cells in end-organs. Whether or not specific DN B cell subsets may be more relevant in a tissue context on inflammatory and fibrotic diseases has also not been investigated.

Although the contribution, if any, of B cells to the progression or sequelae of COVID-19 is unclear, the global lack of B cells, however, seems to correlate with less severe COVID-19, and this infection has been reported to be less likely to be lethal in patients with X-linked agammaglobulinemia.⁴¹⁻⁴³ This could be due to the paucity of B cells in these patients rather or possibly defective BTK signaling in myeloid and lymphoid cells. BTK inhibition seems to reduce hospitalization rates and disease progression in patients with COVID-19⁴⁴ though the results of randomized clinical trials are awaited, anti-CD20-mediated B cell depletion has been seen to be clinically useful in ameliorating severe progressive interstitial lung disease^{45,46} and also in reversing progression in severe combined immunodeficiency with associated granulomatous-lymphocytic interstitial lung disease.⁴⁷ Although there is no consensus view about B cell depletion in COVID-19 (in patients receiving anti-CD20 for other diagnoses), it has frequently been suggested to be beneficial. As no clear-cut negative outcomes have been observed with anti-CD20, trials using this therapeutic in various clinical contexts were allowed to resume in Europe in early 2021.

SUMMARY

Mild COVID-19 generates very poor antibody responses likely because the virus is contained by type I interferons generated by plasmacytoid dendritic cells, is rapidly cleared, and antigen does not accumulate in draining lymph nodes. Affinity maturation is rare with moderate and severe COVID-19, because viral infection results in an altered draining lymph node milieu that impairs GC-T_{FH} cell generation and germinal center formation. In addition, measurable T cell responses have only been observed late in convalescence and the virus persists. In the absence of affinity maturation,

immune pressure is undoubtedly suboptimal and most likely is a driver of variant generation.

CLINICS CARE POINTS

- Natural immunity is generated in severe COVID-19 but there is a defect in neutralization breadth.
- Natural immunity may best be supplemented by vaccination as recent studies now suggest.

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