

Dynamics of SARS-CoV-2 and the Adaptive Immune Response

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Executive Summary

- SARS-CoV-2, the virus that causes COVID-19, is a beta-coronavirus that is closely related to SARS-CoV, the virus that caused Severe Acute Respiratory Syndrome (SARS).
- Manifestations of COVID-19 include: asymptomatic infection; a self-limiting illness comprised of fever, cough, and various non-specific symptoms; pneumonia, typically with ground-glass opacities on CT images; a variety of thrombotic or vasculitic syndromes; and life-threatening acute respiratory distress syndrome (ARDS), viral sepsis and cytokine storm.
- Antibodies to SARS-CoV-2 begin to appear during the first week after onset of symptoms. For a variable period of time (generally weeks 2-3 after onset of symptoms), rising antibody titers and declining levels of viral load in the respiratory tract are detectable.
- Virus-specific neutralizing antibodies and T cell responses emerge during seroconversion; however, the relationship between the presence of these immune responses, viral clearance, and the durability of this response are just beginning to be explored.
- In the early phase of COVID-19, there is a strong correlation between the presence of viral RNA detected by polymerase chain reaction (PCR) and the ability to isolate replication-competent, presumably transmissible, virus. Emerging data from a very small sample of patients suggest that although viral RNA may be detectable in the respiratory tract for a prolonged period, the ability to isolate replication-competent virus becomes increasingly difficult as antiviral antibodies appear. More data are urgently needed to determine whether shedding of viral RNA during convalescence represents infectious virus or non-infectious but detectable SARS-CoV-2 nucleic acid.
- The correlates of protective natural immunity to infection with SARS-CoV-2 are yet to be defined; however, a critical role for neutralizing antibodies is suggested by converging lines of evidence.
- A broad variety of serological tests for detection of antibodies to SARS-CoV-2 is available, including mostly rapid lateral flow assays and more conventional enzyme linked immunosorbent assays (ELISA). Interpretation of results is dependent on the sensitivity and specificity of the test, as well as the prevalence of COVID-19 in the population.
- Detection of antibodies to the S1 protein or the receptor binding domain (RBD) of the S1 protein of SARS-CoV-2 is the most specific indicator of past infection. Antibodies to other immunogenic viral components, such as the nucleocapsid protein, are also useful indicators of past infection. A confounding factor with regard to specificity of antibody tests is the degree to which they detect cross-reactivity with antibodies directed at the endemic human coronaviruses (HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63).
- The nature of the interaction between the virus and the host immune response may result in a variety of outcomes. Whereas mild infections may not stimulate a robust and durable immune response, in severe cases an over-exuberant immune response may lead to a spectrum of immunopathologic phenomena. Understanding these dynamics has important implications for the diagnosis, treatment and prevention of COVID-19.

Introduction

Generation of evidence-based guidance for COVID-19 requires a thorough understanding of the natural history of the disease as it relates to the dynamics of viral replication, emergence of the adaptive antiviral immune response, and the impact of these factors, in turn, on transmissibility of SARS-CoV-2 from an infected individual. In the case of diseases for which preventative vaccines are available (e.g., measles, mumps, rubella, diphtheria, tetanus, pertussis, seasonal influenza, hepatitis A, hepatitis B, and others), good data are available regarding the correlates of protective immunity that develop in patients who recover from the disease. In the majority of viral diseases, the appearance of neutralizing antibodies correlates well with viral clearance and clinical recovery. There are, however, notable exceptions. In this regard, infection with HIV gives rise to a wide array of immune responses, including neutralizing antibodies as well as virus-specific cellular immune responses; however, the virus is never cleared by these immune responses, and the natural history of the disease is almost uniformly fatal.

In the case of COVID-19, information is accumulating at an unprecedented rate. Although the outbreak began only at the very end of 2019, there are already many thousands of manuscripts and publications on the subject available at <https://connect.biorxiv.org/relate/content/181>, and <https://www.ncbi.nlm.nih.gov/pubmed/> respectively. On the other hand, gaining a deep understanding of the complex interplay between virus and host and how this translates to expression of disease and transmissibility takes time far beyond the initial scientific observations that have been made to date.

Lessons From Other Coronaviruses

SARS-CoV-2, the virus that causes COVID-19, is a betacoronavirus that is closely related to SARS-CoV, the virus that caused Severe Acute Respiratory Syndrome (SARS). It is the seventh coronavirus known to infect humans.

Four endemic human coronaviruses, including 2 alphacoronaviruses (HCoV-OC43, and -HKU1), and 2 betacoronaviruses (HCoV-229E and -NL63) cause seasonal, generally mild upper respiratory infections. Endemic human coronavirus infections are common, especially among children and young adults, with a sharp decline in incidence after the age of 45 [Gao, *J Infect* 2015]. These infections generally resolve in association with emergence of antibodies; however, immunity is relatively short-lived, and individuals experience multiple repeated infections with the same or different endemic coronaviruses over time [Callow, *Epidemiol Infect* 1990; Galanti, *medRxiv* 2020; Edridge, *medRxiv* 2020]. In this regard, by 6 months after infection, most people have lost 50% of their endemic coronavirus-specific antibody levels, and by 1 year, most have lost 75% [Edridge, *medRxiv* 2020]. In a minority of cases (8-30%), antibodies generated in response to an endemic coronavirus infection demonstrate cross-reactivity with the spike and nucleocapsid proteins from SARS-CoV-2 (Edridge, *medRxiv* 2020; Ng, *bioRxiv* 2020). Similarly, in a study of patients with SARS, it was shown that all had pre-existing antibodies to HCoV-OC43 and -229E, and these antibody titers were boosted by infection with SARS-CoV [Chan, *Clin Diagn Lab Immunol* 2005]. Insofar as endemic coronavirus infections predominantly affect children and young adults, it is possible that immune responses to these viruses afford partial protection against SARS-CoV-2. This could in part explain the relative protection of people in these age groups from COVID-19.

SARS-CoV is the etiologic agent of Severe Acute Respiratory Syndrome (SARS), which emerged in 2002 as a severe respiratory illness, affecting 26 countries with over 8000 cases and nearly 800 deaths. Liu et al showed that the median time of detectability of viral nucleic acid in sputum was 21 days, and in stool it was 27 days. Prolonged shedding of PCR-detectable viral RNA was seen in some patients, particularly in stool; however, no culturable live virus could be isolated from stool after 6 weeks despite continued RNA shedding for more than 14 weeks (Liu, *Emerg Infect Dis* 2004). Antibody responses reliably develop in SARS patients, with a mean time to seroconversion of 11-15 days [Chan, *Clin Diagn Lab Immunol* 2005; Hsueh, *Clin Microbiol Infect* 2004]. The IgG response emerged 1-2 days before IgA and IgM, and importantly, neutralizing antibodies appeared in this same time frame [Hsueh, *Clin Microbiol Infect* 2004]. The durability of antibody responses in SARS is more robust compared with the endemic coronaviruses, however levels do decline significantly with time. Chan et al demonstrated that geometric mean titers of SARS-CoV-specific IgM declined by 93% by 7 months after onset of illness and IgA by 64%, although IgG levels were sustained over this time period [Chan, *Clin Diagn Lab Immunol* 2005]. Cao et al showed that 100% of SARS patients had detectable virus-specific IgG and neutralizing antibodies over a 16 month period. Although the geometric mean titer of IgG decreased significantly over this period, it remained stable for neutralizing antibodies; however, by 3 years after onset of illness, virus-specific IgG was no longer detectable in 25% of patients and overall geometric mean titers had decreased by 90%, and neutralizing antibodies were no longer detectable in 15% of patients and overall geometric mean titers had decreased by 97% [Cao, *N Engl J Med* 2007]. Most patients become seronegative for SARS between years 3-5 after infection [Tang, *J Immunol* 2011].

MERS-CoV causes middle eastern respiratory syndrome, which emerged in 2012 as a potentially severe respiratory disease with mortality rates among symptomatic patients as high as 35%. In patients who develop antibodies, the mean time to seroconversion is 18 days [Ko, *Diagn Microbiol Infect Dis* 2017], Among severe cases, failure to seroconvert is a very poor prognostic sign [Ko, *Diagn Microbiol Infect Dis* 2017]. For patients who do seroconvert, the durability of the antibody response is robust [Payne, *Emerg Infect Dis* 2016].

All 3 highly pathogenic human coronaviruses, SARS-CoV, MERS-CoV, and SARS-CoV-2 emerged as zoonotic infections, with bats as the primary species reservoir. The likely secondary species from which infections have “spilled over” into humans include civets (SARS-CoV), camels (MERS-CoV), and possibly pangolins (SARS-CoV-2).

Natural History of COVID-19

SARS-CoV-2 is closely related to SARS-CoV, and even more closely related to a bat coronavirus, BatCoV RaTG13 [Zhu, *N Engl J Med* 2020]. Bat coronaviruses circulate widely, and Menachery et al. reported in 2015 on one such isolate that had the potential to infect humans, by virtue of the ability of the viral spike protein to interact with a receptor on human cells [Menachery, *Nat Med* 2015]. SARS-CoV, SARS-CoV-2, and (theoretically) viruses such as BatCoV RaTG13, gain entry into human cells following a priming event by a host protease, TMPRSS2; interaction of the receptor binding domain (RBD) of the S1 region of the viral spike protein with human angiotensin converting enzyme-2 (ACE-2); and finally fusion of the viral and cell membranes facilitated by the S2 component of the viral spike protein [Li, *Nature* 2003; Matsuyama, *J Virol* 2010; Hoffman, *Cell* 2020]. Although it causes primarily a respiratory syndrome, the cellular and tissue tropism of SARS-CoV-2 is quite broad [Sungnak, *Nat Med* 2020], which likely explains some of the heterogeneous clinical presentations in COVID-19.

In those who develop symptomatic disease following infection with SARS-CoV-2, the median incubation period is 4-5 days, and 97.5% develop symptoms by 12 days [Li, *N Engl J Med* 2020; Guan, *N Engl J Med* 2020; Chan, *Lancet* 2020; Lauer, *Ann Intern Med* 2020]. In most symptomatic cases (>80%), the disease presents as a non-specific upper respiratory tract infection with fever, dry cough and sore throat. Uncomplicated disease resolves without sequelae in 1-2 weeks. More severe disease, with dyspnea, hypoxemia, or extensive pulmonary involvement demonstrated by imaging, develops in approximately 14% of cases. Critical disease, with respiratory failure, shock, or multi-organ dysfunction, occurs in approximately 5% of cases [Wang, *JAMA* 2020; Huang, *Lancet* 2020; Chen, *Lancet* 2020].

Risk factors for severe disease and poor outcomes include demographic factors, including age and male sex; comorbid conditions, such as hypertension, congestive heart failure, coronary artery disease, chronic lung disease, diabetes, obesity, chronic kidney disease, and immunosuppression; and laboratory parameters, including hypoxemia, elevated levels of inflammatory markers (i.e., interleukin-6, C-reactive protein, D-dimer, ferritin and procalcitonin), elevated neutrophil-to-lymphocyte ratio, and elevated red blood cell distribution width [Zhou, *Lancet* 2020; Wu, *JAMA* 2020; Petrilli, *medRxiv* 2020; Zhang, *Nature* 2020; Kim, *medRxiv* 2020; Chen, *J Clin Invest* 2020; Liu Y, *J Infect* 2020; Ebinger, *medRxiv* 2020; Wei, *J Infect* 2020; Foy, *medRxiv* 2020; Cummings, *medRxiv* 2020]. In otherwise mild-to-moderate cases of COVID-19, there can be sudden onset of ARDS and respiratory decompensation that tends to occur during the second week after onset of symptoms [Berlin, *N Engl J Med* 2020]. This syndrome has characteristics of cytokine release syndrome [Wang, *JAMA* 2020; Xiong, *Emerg Microbes Infect* 2020; Mehta, *Lancet* 2020]. Although the pathogenesis of this delayed onset decompensation is not well defined, preliminary data suggest that it is an immunologically-driven phenomenon, rather than a result of uncontrolled viral replication [Lescure, *Lancet Infect Dis* 2020]. Estimates of overall mortality vary widely from country to country, and are driven by a large number of factors; however, age remains the strongest factor, with case fatality rates in excess of 15% for patients over the age of 80 years [Dowd, *Proc Natl Acad Sci* 2020].

An expanding spectrum of complications is being described as part of COVID-19. This is likely due to a combination of direct effects of viral infection in the broad variety of cell types that express viral entry factors, as well as inflammatory sequelae of the antiviral immune response. Some of these complications include meningoencephalitis, stroke, hypogeusia and hyposmia [Whittaker, *Acta Neurol Scand* 2020]; acute kidney injury [Hirsch, *Kidney Int* 2020]; arterial and venous thrombotic events, endothelialitis and microangiopathy [Thomas, *Thromb Res* 2020; Ackermann, *N Engl J Med* 2020]; and a Kawasaki-like syndrome in children [Verdoni, *Lancet* 2020; Riphagen, *Lancet* 2020]. A number of these disease manifestations may have a common underlying pathogenesis related to expression of viral entry receptors on endothelial cells and/or pericytes [Teuwen, *Nat Rev Immunol* 2020; He, *bioRxiv* 2020]. In this context, seemingly disparate vascular complications may be underpinned by an antiviral immune response that results in an inflammatory vasculitis.

Viral Dynamics and Adaptive Immune Responses

Acute Infection

PCR testing for the presence of viral nucleic acid is the most reliable and practical method for identifying individuals who are infected with SARS-CoV-2. A number of caveats pertain to PCR testing in patients with COVID-19. Firstly, it is difficult to sample the critically affected tissue (i.e., lung), and sampling the throat, nasopharynx and nares may not serve as adequate surrogates for a number of reasons (e.g., operator variability in swab technique and the relatively spartan ability of the epithelium in the upper airway to support viral replication compared with the lower respiratory tract). It is also difficult to normalize the PCR signal to the amount of the sample collected on the swab. A commonly employed method for semi-quantitation involves recording the cycle number of the PCR at which the sample produced a signal above background – the greater the viral load, the fewer PCR cycles needed for the sample to become positive.

In the early stages of disease, viral nucleic acid is readily detectable in the oro- and nasopharynx, saliva, as well as sputum from the lower respiratory tract [Zou, *N Engl J Med* 2020; Pan, *Lancet Infect Dis* 2020; Yu, *Clin Infect Dis* 2020; Rothe, *N Engl J Med* 2020; Williams, *J Clin Microbiol* 2020]. Less frequently, viral nucleic acid can be detected in stool, serum, and urine [Tan, *medRxiv* 2020; Hogan, *medRxiv* 2020; Zheng, *BMJ* 2020; Fang, *J Infect* 2020; Huang J (1), *medRxiv* 2020]. In contrast to the situation in SARS, wherein the appearance of symptoms precedes the peak of infectiousness, pre-symptomatic individuals infected with SARS-CoV-2 have detectable levels of virus in their respiratory tract, and transmission from such individuals has been documented [Rothe, *N Engl J Med* 2020; Kimball, *Morb Mortal Wkly Rep* 2020; He, *Nat Med* 2020; Li, *Nat Med* 2020]. He et al. estimated that 44% of secondary cases of COVID-19 resulted from infection by pre-symptomatic individuals. The authors inferred that the period of infectiousness began 2-3 days prior to the onset of symptoms, and peaked 0.7 days prior to the onset of symptoms [He, *Nat Med* 2020]. Asymptomatic individuals have lower levels of viral nucleic acid and shorter durations of viral shedding compared with patients who subsequently become symptomatic [Zhou, *Int J Infect Dis* 2020; Xiao, *medRxiv* 2020].

The ratio of symptomatic to asymptomatic infections with SARS-CoV-2 remains largely unknown at this time. Near the peak of the COVID-19 outbreak in New York City in March - April 2020, Sutton et al. adopted universal PCR testing for SARS-CoV-2 for all women admitted to 2 hospitals' labor and delivery wards. Over a 2-week period, 215 women were admitted for delivery. Four (1.9%) women had symptoms suggestive of COVID-19 and all 4 tested positive for SARS-CoV-2. Of the 211 women without symptoms, 29 (13.7%) tested positive for SARS-CoV-2; thus, 29/33 (88%) of women who tested positive were asymptomatic [Sutton, *N Engl J Med* 2020].

Higher levels of SARS-CoV-2 RNA in the respiratory tract and/or delayed viral clearance are associated with more severe disease [Lescure, *Lancet Infect Dis* 2020; Liu, *Lancet Infect Dis* 2020; Huang, *Am J Respir Crit Care Med* 2020; Zheng, *BMJ* 2020; Fang, *J Infect* 2020; Tan, *medRxiv* 2020; Lui, *J Infect* 2020]. In addition, the presence of viral nucleic acid in serum is an indicator of severe disease and poor prognosis [Fang, *J Infect* 2020; Hogan, *medRxiv* 2020].

Humoral Immune Response

Antibody responses to SARS-CoV-2 infection are directed at a variety of viral proteins, including the spike (S) protein, the membrane (M) protein, and the nucleocapsid (N) protein. Because the receptor binding domain (RBD) of the viral spike protein mediates viral entry into human cells via binding to ACE-2, it is not surprising that the RBD, and the S protein more generally, are quite immunogenic.

When detecting antibodies to SARS-CoV-2, it is important to ensure that the assay (whether it is immunofluorescence, enzyme-linked immunoassay [ELISA], lateral flow assay, or other) is both sensitive and specific. In this regard, the positive predictive value of a positive test from an assay that has 95% specificity in a population with a 5% true prevalence of infection is only 50%. Sensitivity depends on selecting immunogenic proteins or peptides that perform well under the technical conditions of the assay. Specificity is largely dependent on the ability to discriminate between SARS-CoV-2 and other antigens, including those from other human coronaviruses (i.e., SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63).

Okba et al. showed that sera from a small number of COVID-19 patients tested positive in an ELISA using the S protein of SARS-CoV and SARS CoV-2, and to a lesser extent, MERS-CoV. With the S1 protein as a target, the sera cross-reacted minimally only with SARS-CoV and not at all with MERS-CoV [Okba, *Emerg Infect Dis* 2020]. Using an in-house ELISA, the authors showed that ELISA assays for antibodies to SARS-CoV-2 S1, RBD and N proteins yielded negative results from healthy blood donors (N = 45), patients with non-coronavirus respiratory infections (N = 76), and patients with endemic

human coronavirus (OC43, HKU1, 229E, and NL63) infections (N = 75). Stored sera from 2 patients with SARS did show cross reactivity in all 3 assays. Using a commercially available ELISA for detection of antibodies to SARS-CoV-2 S1 protein, 4/75 (5.3%) of the sera from the endemic coronavirus panel (OC43, HKU1, 229E, and NL63) had a positive result for IgG antibodies, and 5/75 (6.7%) for IgA antibodies. The IgG assay otherwise only showed false positive results with SARS sera, whereas the IgA assay also had 1 (2.2%) false positive among the healthy blood donors and 3 (3.9%) false positives among the sera from patients with non-coronavirus respiratory infections [Okba, Emerg Infect Dis 2020].

The kinetics of the humoral immune response to SARS-CoV-2 are atypical, in that the early peak in the IgM response is contemporaneous with IgG and IgA responses rather than preceding them. The largest data sets, using a variety of immunoassays, demonstrate that the median time to seroconversion is approximately one week after onset of symptoms, with nearly 100% seroconversion by week 3 [see table below].

Time after symptom onset	Seroconversion rate	Reference
2-4 days	30%	Long, medRxiv 2020
5-7 days	60%	Long, medRxiv 2020
6-10 days	50%	Liu, J Clin Microbiol 2020
8-10 days	75%	Long, medRxiv 2020
11-14 days	50%	Zhao, Clin Infect Dis 2020
11-13 days	90%	Long, medRxiv 2020
11-15 days	89%	Liu, J Clin Microbiol 2020
≥14 days	100%	To, Lancet Infect Dis 2020; Xiao, J Infect 2020

IgG titers rise and plateau after 3-8 weeks, and IgM titers begin to decline after week 3-5 [Liu, J Clin Microbiol 2020; Xiao, J Infect 2020; Zeng, J Infect 2020; Li, medRxiv 2020]. Using the viral S protein, or its component RBD is more sensitive for detection of antibodies to the virus compared with the nucleocapsid (N) protein [Liu, J Clin Microbiol 2020; To, Lancet Infect Dis 2020].

The kinetics of the humoral immune response are quite different in mild-to-moderate cases of COVID-19 compared with severe-to-critical cases. For both S-specific and RBD-specific IgG, patients with mild-to-moderate disease have a high early peak in these antibody levels followed by a steep decline. In contrast, patients with severe-to-critical disease have a delayed rise in these antibody levels, which then remain significantly higher compared with the less severe patients [Li, medRxiv 2020]. The steep decline in SARS-CoV-2-specific antibodies in patients with mild-to-moderate disease raises the possibility that early peak levels protect these patients from developing severe disease, but that they do not develop durable immunity. In this sense, such cases may resemble the transient immune responses associated with infection with the endemic coronaviruses.

Several investigators have probed the B cell receptor repertoire from patients with COVID-19. Clonally expanded virus-specific B cells emerge, with skewing toward utilization of several heavy chain variable regions. Virus-specific antibodies were shown to be predominantly IgG1 and IgA, and were mostly specific for RBD and S. Interestingly, convergent responses were seen in which antibodies with virtually identical amino acid sequences were isolated from multiple individuals [Robbiani, bioRxiv 2020; Galson, bioRxiv 2020; Nielsen, Nat Res 2020].

Neutralizing antibodies, which are able to prevent the virus from infecting susceptible cells, emerge concurrently with the rise in antiviral antibody titers [Okba, Emerg Infect Dis 2020; To, Lancet Infect Dis 2020; Wolfel, Nature 2020; Dong, medRxiv 2020]. In one study, levels of antiviral IgA correlated somewhat better than IgG with levels of neutralizing antibodies [Liu, J Clin Microbiol 2020], and there is a suggestion from a small dataset that an early, robust IgA response may be associated with more mild disease [Dahlke, medRxiv 2020]. Wu et al. reported a number of interesting findings related to the emergence of neutralizing antibodies in 175 COVID-19 patients who had all recovered from a mild disease course [Wu F, medRxiv 2020]. In 6 of these subjects, serial analysis of plasma samples was possible, demonstrating contemporaneous appearance of SARS-CoV-2 neutralizing antibodies with antibodies directed against the viral S1 and S2 proteins as well as the receptor binding domain. These antibody responses mostly peaked between the first and second weeks after onset of illness. Samples from all 175 patients at the time of hospital discharge demonstrated that 165/175 (94%) had developed SARS-CoV-2 neutralizing antibodies. There was a positive correlation between neutralizing antibody titer and age, and stability of the titer was demonstrated in a subset of 47 patients who had paired

samples available from the time of hospital discharge and 2 weeks later. The 10 patients who did not develop detectable neutralizing antibodies were disproportionately female (80% vs. 53% for the full study population) and young (mean age 35 years vs. 50 years for the full study population). Other studies have also found strong correlations between neutralizing antibody titers and S- and RBD-specific antibody titers [Amanat, *Nat Med* 2020; Ni, *Immunity* 2020; Juno, *medRxiv* 2020].

Most neutralizing antibodies isolated from COVID-19 patients bind to RBD, although some bind to S and not to RBD. Among RBD-specific neutralizing antibodies, some compete with ACE-2 for binding, some partially compete with ACE-2, and others do not compete with ACE-2 [Wu, *Cell Host Microbe* 2020; Wan, *bioRxiv* 2020; Cao, *Cell* 2020; Seydoux, *bioRxiv* 2020; Wu Y, *medRxiv* 2020; Brouwer, *bioRxiv* 2020]. Even amongst RBD-specific neutralizing antibodies that compete with ACE-2, electron microscopy shows that different antibodies bind to distinct epitopes [Wu Y, *medRxiv* 2020; Brouwer, *bioRxiv* 2020]. These data indicate the possibility of combining monoclonal antibodies for synergistic inhibition of viral entry, and also suggest multiple candidate vaccine targets.

One of the concerns about vaccine candidates is the theoretical possibility of antibody-dependent enhancement, or ADE. In the case of Dengue virus, some virus-specific antibodies deliver bound virus into macrophages by virtue of their Fc receptor. This “Trojan horse” phenomenon leads to infection of macrophages, hence the term ADE [Halstead, *J Exp Med* 1977; Dejnirattisai, *Science* 2010]. This phenomenon was at least theoretically possible with SARS-CoV as well [Jaume, *J Virol* 2011; Wang, *ACS Infect Dis* 2016]. Reassuring data come from Quinlan et al who have shown that immunization with the SARS-CoV-2 RBD in an animal model leads to a potent neutralizing antibody response without ADE [Quinlan, *bioRxiv* 2020].

Cellular Immune Response

All COVID-19 patients have detectable virus-specific CD4+ T cell responses, and most have CD8+ T cell responses as well. S- and RBD-specific T cell responses predominate; however, responses are also detectable against multiple other viral components [Grifoni, *Cell* 2020; Juno, *medRxiv* 2020; Ni, *Immunity* 2020]. Relationships between cellular and humoral responses have also been mapped. Grifoni et al. found a strong correlation between the frequency of S-specific CD4+ T cells and titers of RBD-specific IgG titers [Grifoni, *Cell* 2020]. Juno et al. found a negative correlation between the frequency of S-specific CCR6+CXCR3- (TH17-like) T cells and neutralizing antibody titers [Juno, *medRxiv* 2020]. Further evidence of the importance of the cellular immune response in COVID-19 comes from the observation of a strong correlation between convalescence and reappearance of effector/effector memory (CD45RA- CD62L-) and central memory (CD45RA- CD62L+) T cells [Odak, *medRxiv* 2020].

Viral Clearance: Convalescence and Beyond

As antibody titers rise during weeks 2-3 after the onset of symptoms, levels of virus decrease and become more difficult to detect (see Figure 1). Estimates of the median time from the onset of illness to having a negative viral PCR test (nasopharyngeal or throat swabs, or sputum) range from 9.5 - 24 days [Ling, *Chin Med J* 2020; Lo, *Int J Biol Sci* 2020; Xu, *Clin Infect Dis* 2020; Chen X, *medRxiv* 2020; Huang J(1), *medRxiv* 2020; Xiao, *Clin Infect Dis* 2020]. Consistent with this range, Du et al. found that in secondary cases of COVID-19, the median time between contact with an infected individual and a negative PCR test during convalescence was 26 days [Du, *J Infect* 2020]. Risk factors for prolonged shedding of viral RNA include older age, male sex, comorbidities, delayed hospital admission, and invasive mechanical ventilation [Xu, *Clin Infect Dis* 2020; Xiao, *Clin Infect Dis* 2020]. Although Xu et al found that essentially all 113 patients with confirmed COVID-19 became PCR-negative by day 35 from the onset of illness [Xu, *Clin Infect Dis* 2020], others have found that in approximately 10% of patients, prolonged shedding of viral nucleic acid (median 53.5 days) may occur [Li, *J Med Virol* 2020]. Interestingly, prolonged shedding in this latter study occurred predominantly in patients with mild disease. In addition to the study by Xu et al. noted above, other studies have also found prolonged viral shedding in patients with severe disease [Lescure, *Lancet Infect Dis* 2020; Liu, *Lancet Infect Dis* 2020; Huang, *Am J Respir Crit Care Med* 2020; Zheng, *BMJ* 2020; Fang, *J Infect* 2020; Tan, *medRxiv* 2020]. Importantly, in a small study of 9 COVID-19 patients from Germany, Wolfel et al. found that infectious, replication-competent virus (by RT-PCR analysis of VERO E6 cell co-cultures) could not be detected after day 8 even when direct PCR of sputum was positive. Similar results were found by analyzing samples for subgenomic SARS-CoV-2 RNA, which indicates active viral replication. Subgenomic RNA was found in nasopharyngeal swab samples and sputum taken early in the course of disease, but could not be detected after day 5 from nasopharyngeal swabs and after day 9 from sputum. Although 2 stool samples had very low levels of detectable subgenomic viral RNA, no replication-competent virus could be detected in culture with VERO E6 cells [Wolfel, *Nature* 2020]. A similar single case was reported in which SARS-CoV-2 could not be cultured beyond day

18 after the onset of symptoms despite persistently positive PCR tests [Liu WD J Infect 2020]. These data, which must be replicated on a larger scale, provide some evidence that prolonged positivity for viral RNA detected by RT-PCR is not necessarily indicative of active viral replication and infectivity.

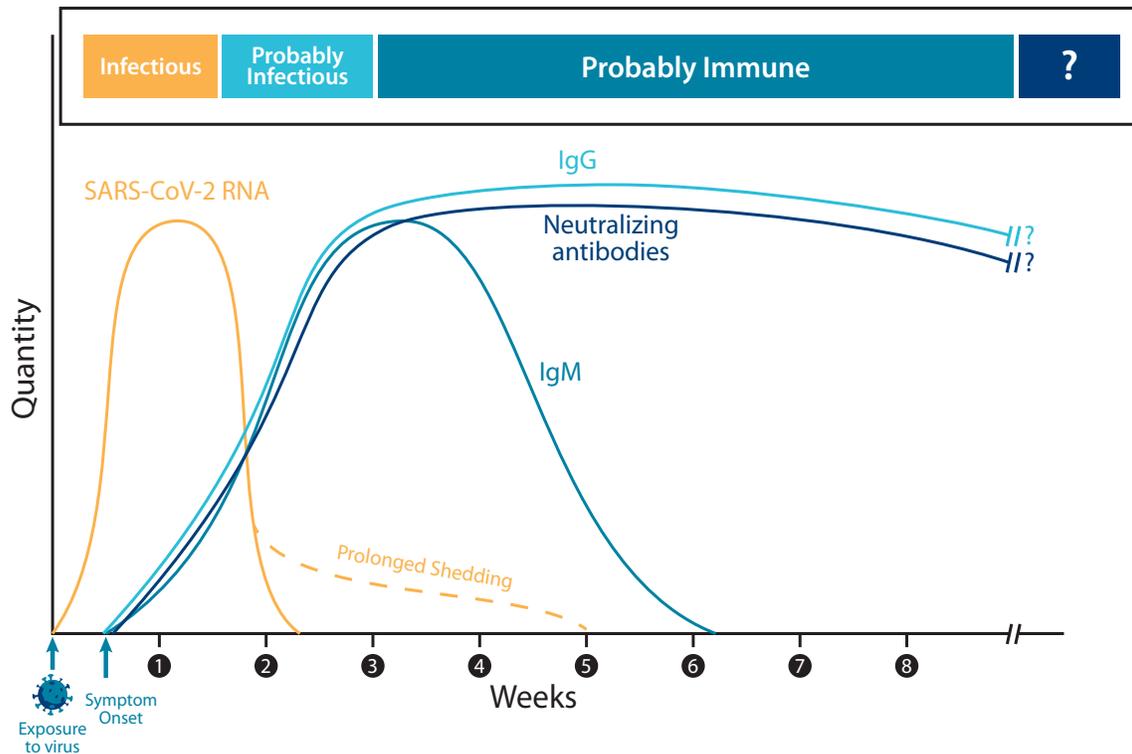


Figure 1. Kinetics of viral replication and the humoral immune response.

Finally, there is a phenomenon of PCR tests becoming positive again following at least 2 negative tests during convalescence [Tang, Infect Control Hosp Epidemiol 2020; Jiang, J Infect 2020; Huang J(2), medRxiv 2020; Ye, J Infect 2020; Chen, Int J Infect Dis 2020; Cao, J Med Virol 2020; Chen J, medRxiv 2020]. This phenomenon is not rare, and it has variously been referred to as reinfection, recurrence, reactivation and recrudescence. Among the case descriptions, most patients appear well or have minor non-specific symptoms along with benign laboratory parameters. However, there are a few cases in which more significant symptoms have occurred, or that involve modest increases in inflammatory biomarkers or worsening of chest imaging studies. Some authors have reported a small number of cases in which recurrent positive PCR tests were associated with low levels of virus-specific antibodies [Li, medRxiv 2020; Hu, medRxiv 2020].

Recurrence of positive PCR tests following convalescence has several possible explanations. In some cases, it is possible that the negative tests were actually falsely negative due to inadequate sampling or imperfect test sensitivity: in this scenario, low levels of viral replication may be continuous for a prolonged period of time, but may falsely appear to be recurrent. If the negative tests preceding the “recurrent” positive test are true negatives, it is still possible that ongoing low levels of virus replication are occurring in certain compartments (i.e., the lower respiratory tract), and that viral load in the upper respiratory tract is around the limit of detectability. This could give rise to intermittently positive PCR test results. A case reported from China supports this possibility. The case involved a 78-year-old woman who recovered from COVID-19 and was awaiting discharge from the hospital after 3 consecutive negative nasopharyngeal swab PCR tests for SARS-CoV-2. On the day she was to be discharged, she experienced sudden cardiac arrest and died. At autopsy, PCR was positive for SARS-CoV-2 nucleic acid in lung tissue, and electron microscopy demonstrated coronavirus particles in bronchiolar and alveolar epithelial cells. Histopathological examination showed alveolar damage, hyaline membranes, microangiopathy and inflammatory infiltrates consistent with COVID-19 [Yao, Cell Res 2020]. It is also possible that there is no ongoing viral replication, and that nucleic acid from neutralized virus is shed slowly or intermittently at around the limit of detectability. These different scenarios have different implications with regard to potential infectiousness of such patients. A recurrent positive test associated with low levels of virus-specific antibodies (as noted above) again raises the question of whether a subset of people who have been infected with SARS-CoV-2 may have inadequate or insufficiently durable immunity in a manner that is somewhat analogous to infection with the endemic coronaviruses.

Transmissibility

SARS-CoV-2 clearly replicates in the upper and lower respiratory tract in the asymptomatic/pre-symptomatic and early stages of COVID-19. Transmission of COVID-19 by asymptomatic and pre-symptomatic SARS-CoV-2-positive individuals has been documented, and may be a significant factor in the rapid spread of the pandemic [Rothe, *N Engl J Med* 2020; Kimball, *Morbidity and Mortality Weekly Report* 2020; Sutton, *N Engl J Med* 2020]. Most transmission from symptomatic individuals is believed to occur by respiratory droplets or contact with contaminated surfaces. The theoretical possibility has been raised that SARS-CoV-2 may be transmitted in airborne fashion by aerosol [van Doremalen, *N Engl J Med* 2020], although the experimental conditions from this publication are far from physiologic. In a study from the University of Nebraska Medical Center, Santarpia et al. found viral RNA in the rooms of patients with COVID-19 in places such as the window ledges and the floor underneath the patients' beds, as well as in air samples more than 6 feet from the patients' beds [Santarpia, *medRxiv* 2020]. Although neither of these papers has demonstrated the presence of replication-competent, transmissible virus in aerosol form, the theoretical possibility has been highlighted.

Conclusion

SARS-CoV-2, the etiologic agent of COVID-19, has had an enormous impact on health, work and life across the globe in recent months. The virus is a formidable adversary. A considerable amount of viral replication can occur prior to the development of any symptoms, and transmission from asymptomatic individuals is likely quite common. In most cases, infection causes a self-limited respiratory illness; however, in some cases very severe multi-organ disease develops after a week or more of otherwise unremarkable illness. The later, severe manifestations of COVID-19 may all be variants of an over-exuberant immune response directed against ACE-2-expressing cells that have been infected by the virus. An inflammatory vasculitis may manifest in a number of different ways, including microangiopathy, and thrombosis.

The adaptive immune response against the virus involves humoral and cellular elements. Virus-specific IgG, IgM, and IgA antibody responses all emerge during the first and second week after infection, and are useful from a diagnostic standpoint. Virus-specific T cell responses also develop early. Clearance of virus and convalescence is strongly correlated with appearance of neutralizing antibodies (which are generally specific for the spike and RBD elements of the virus) and certain CD4+ T cell responses. Although the precise elements of protective immunity are not yet definitively known, it is likely that a robust neutralizing antibody response is essential, and likely provides immunity for most convalescent individuals for at least some months. Reassuring data in this regard come from infection and re-challenge studies in rhesus macaques. In two independent studies, macaques were partially or completely protected against re-infection with SARS-CoV-2, and this protection correlated extremely well with neutralizing antibody titers generated following the first infection [Bao, *bioRxiv* 2020; Chandrashekar, *Science* 2020].

Multiple outcomes appear possible based on the dynamics between SARS-CoV-2 infection and the host immune response. In asymptomatic or mild disease, it is possible that innate immune mechanisms are very successful in limiting viral replication. Adaptive immune responses develop, but at a low amplitude, providing only transient protective immunity. This situation may be somewhat analogous to infection with the endemic coronaviruses. In most cases, high levels of viral replication are dampened by the emergence of a robust humoral and cellular immune response. Clearance of the virus is associated with S- and RBD-specific neutralizing antibody responses as well as certain CD4+ T cell responses, and protective immunity (of uncertain durability) results. Finally, in some cases, an over-exuberant immune response can trigger life-threatening immunopathological sequelae. These different virus-host dynamics have implications for vaccine development and therapeutic paradigms. More than 100 vaccine candidates are already in development, and these studies will be tremendously valuable in advancing our understanding of protective immunity in COVID-19. In addition, as more is understood about qualitative and quantitative aspects of the antiviral immune response, it will become possible to rationally design treatment strategies that include antiviral agents, and in certain cases, immunomodulators to address the immunopathological syndromes that emerge in some patients.

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Dynamics of SARS-CoV-2 and the Adaptive Immune Response

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