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Severe Acute Respiratory Syndrome Coronavirus 2 Antigens as Targets of Antibody Responses

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KEYWORDS

• SARS-CoV-2 • Coronavirus antigens • Antigen-antibody responses
• Humoral immunity

KEY POINTS

- Studies on humoral responses to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens have been reported and commonly focus on antibodies targeting spike (S), nucleocapsid (N), and the receptor-binding domain (RBD; anti-S, anti-N, and anti-RBD).
- During acute infection of COVID-19, anti-S and anti-N antibodies have been measured to monitor seroconversion in COVID-19 individuals.
- Anti-RBD immunoglobulin G (IgG), IgM, and IgA antibodies are highly specific to SARS-CoV-2 and show correlation with neutralization assays.
- Unique antibody responses to SARS-CoV-2 antigens show correlation with clinical outcomes and can be used as predictors for disease severity.
- Antibody levels (IgG) lasting several months after infection have been observed, and seropositive individuals (typically determined by anti-SARS-CoV-2 IgG enzyme-linked immunosorbent assays) exhibit protection from reinfection.

BACKGROUND

Severe Acute Respiratory Syndrome Coronavirus 2 Antigens

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China in December 2019.[1] SARS-CoV-2 belongs to the Coronaviridae family, which includes Letovirinae and Coronavirinae viruses, which are commonly known as coronaviruses. Coronaviruses are spherical envelope viruses (40–60 nm in

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diameter) that are encapsulated in a "corona" protein and contain a 27- to 32-kb single-stranded RNA (ssRNA) that encodes nonstructural polyproteins, accessory proteins, and structural proteins. The 4 major structural proteins of SARS-CoV-2 are spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins (Fig. 1).[2] S, E, and M proteins are anchored in the lipid bilayer membrane of the virus, whereas the N proteins are located inside the virion. M proteins maintain the shape and size of the viral envelope; E proteins facilitate viral release and assembly during pathogenesis. S proteins mediate the entry of the virus into the host cell, and N proteins stabilize ssRNA.

Severe Acute Respiratory Syndrome Coronavirus 2 Host-Cell Entry Mechanism

The entry of SARS-CoV-2 into a host cell is mediated by the ectodomain of the S protein, which includes a receptor-binding S1 subunit and a membrane-fusion S2 subunit. The S1 subunit contains a receptor-binding domain (RBD) that recognizes and binds angiotensin-converting enzyme 2 (ACE2) receptors on the host-cell surface. ACE2-RBD interactions trigger host cell transmembrane protease serine 2 (TMPRSS2) and endosomal cysteine proteases cathepsin B and L (CatB/L), leading to cleavage of the S protein at the S1-S2 boundary and release of the S1 subunit.[3] Subsequently, the S2 subunit goes through a structural change that facilitates fusion of host and viral membranes and results in entry of viral ssRNA into the host cell.[4]

Antibody Responses to Viral Infections

Antibodies play specific roles in either innate (natural) or adaptive humoral responses. Natural antibodies are defined as immunoglobulins present without past infection or exogenous antigen stimulation, whereas adaptive antibodies are produced in response to a trigger, such as an antigen. Any foreign substances, such as viral proteins, that elicit an immune response are defined as antigens. Upon viral infection, B-lymphocyte receptors recognize viral antigens, replicate, mature, and secrete antibodies with high-binding affinity to the triggering antigen.[5] An individual is defined as seroconverted when antibodies against a target antigen are detected in the blood. Although all antibodies are capable of binding antigens, only those that diminish or eliminate infectivity are categorized as neutralizing antibodies. In response to human coronaviruses, neutralizing antibodies typically bind to the S protein and disrupt viral entry by blocking interactions between viruses and ACE2 host receptors.[6]

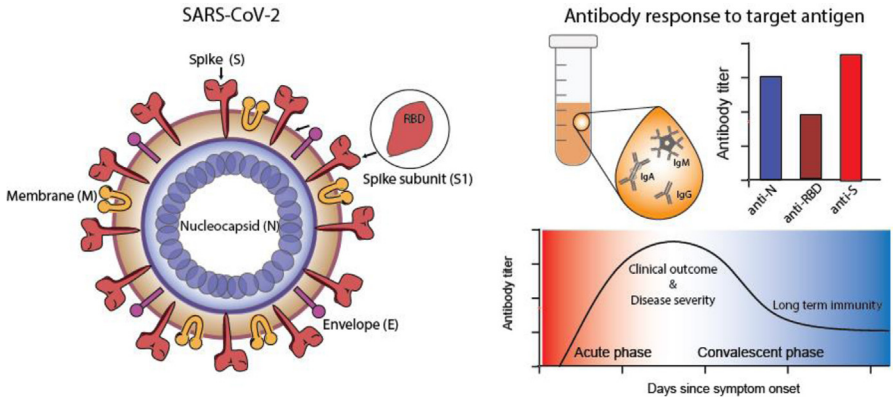

Fig. 1. SARS-CoV-2 containing S, subunit S1, RBD, N, M, and E proteins. Blood levels of antibodies against S, RBD, and N have been widely measured to study humoral immune responses in COVID-19 patients from acute infection through recovery and convalescence.

The 3 predominant antibody classes involved in immune responses against human coronaviruses include immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA). Immunoglobulin D (IgD) and immunoglobulin E (IgE) are other major antibody classes that are involved in antibody production in B cells and protection against parasites, respectively. IgD and IgE account for less than 1% of total serum immunoglobulins and are not widely studied in coronavirus serology: therefore, the discussion focuses on IgM, IgG, and IgA humoral responses. IgM is a pentameric antibody (900 kDa), accounting for 5% of total serum immunoglobulins,[7] that represents a major class of natural antibodies.[8] IgM exhibits low affinity and polyreactivity to nonprotein antigens (such as phosphorylcholine, phosphatidylcholine, and glycans) and protein antigens.[9] Therefore, natural IgM can recognize a wide range of viral antigens not previously encountered by the host before an adaptive immune response is triggered.[10] As a result, IgM is typically the first antibody to increase in concentration in response to a new virus. However, IgM antibodies are usually not neutralizing and only provide immediate control of a viral infection until the adaptive humoral response is triggered and produces specific, neutralizing, and long-lasting adaptive antibodies. IgG, a monomeric immunoglobulin (150 kDa), is the most abundant subtype in human serum (80% of total serum immunoglobulins)[7] and a major class of adaptive antibodies. Adaptive IgGs exhibit high specificity toward antigen targets and neutralization capacity. In addition, IgGs are produced at the highest concentrations compared with other immunoglobulins that can persist after an infection is resolved and therefore play a significant role in viral infection clearance and long-term immunity. IgG is further divided into 4 subclasses: IgG1, IgG2, IgG3, and IgG4, in order of decreasing abundance. IgG1 and IgG3 are predominantly involved in neutralization of viral infections, whereas IgG2 and IgG4 show negligible targeting toward viruses.[11] IgA (160 kDa), the second most abundant immunoglobulin class in blood circulation (10%–20% of total serum immunoglobulins),[7] is produced in response to upper respiratory viral infections and is the predominant antibody in mucosal immunity.[12] Although IgM, IgG, and IgA antibody responses were predicted to be similar between SARS-CoV-2 infection and other human coronavirus infections, the unprecedented clinical manifestations of COVID-19 pointed to unique antibody responses to SARS-CoV-2.

Studies on humoral responses to SARS-CoV-2 during early stages of infection (acute infection) and later stages when patients recover (convalescence) have been reported since March 2020. Here, the authors present a review of the literature on antibody responses targeting SARS-CoV-2 antigens (see **Fig. 1**). They first discuss antibody responses to S and N during acute infection, which are the 2 most common viral proteins used in SARS-CoV-2 antibody assays. Subsequently, the authors highlight antibody responses to the RBD epitope, which exhibit high specificity toward SARS-CoV-2 and high correlation with neutralization capacity. Review of SARS-CoV-2 antibody responses during acute SARS-CoV-2 infection includes discussion of specific antigen-antibody responses that correlate with clinical outcomes in COVID-19 patients, such as disease severity and survival. Finally, the authors present literature on antibody levels during convalescence and their impact on protection from reinfection.

DISCUSSION

Antigen-Antibody Responses During Acute COVID-19 Infection

Antibody responses to spike and nucleocapsid

During the initial phase of the COVID-19 pandemic, it was shown that S and N proteins elicited an immune response that could be used to identify seropositive individuals.[13–17] Long and colleagues[15] provided one of the first reports of acute antibody

responses to SARS-CoV-2 in 63 symptomatic patients from hospitals in China. Upon COVID-19 diagnosis, patients were monitored for IgM and IgG using magnetic chemiluminescence enzyme immunoassays (MCLIA). Recombinant peptides from the SARS-CoV-2 S and N proteins served as targets for antibody detection. Of patients, 98.6% achieved seroconversion, as defined by the first positive test result for IgM or IgG. In a cohort of 63 confirmed COVID-19 participants, 26 initially seronegative individuals were monitored over time and reached seroconversion within 20 days of symptom onset via synchronous seroconversion (simultaneous seroconversion of IgG and IgM) or asynchronous seroconversion (seroconversion of IgM or IgG first, followed by seroconversion of the second immunoglobulin). Guo and colleagues[16] studied symptomatic patients composed of 82 confirmed COVID-19 cases and 58 probable cases (negative quantitative polymerase chain reaction [PCR] result but presented with COVID-19 symptoms) and observed asynchronous seroconversion for anti-N IgG, IgA, and IgM antibodies. IgM and IgA were detected on average 5 days after symptom onset, whereas IgG was detected 14 days after symptom onset. Asynchronous seroconversion is commonly observed for other respiratory viral infections, whereby IgM antibodies are produced first during infection, followed by strong mucosal IgA responses and production of IgG.[18] To assess seroconversion in asymptomatic cases, Long and colleagues obtained 2088 samples from COVID-19 individuals who traveled from Wuhan City or Hubei Province, received a positive reverse transcription (RT)-PCR test, and were quarantined.[19] Thirty-seven individuals were selected as asymptomatic cases, as defined by individuals with a positive RT-PCR test and no clinical symptoms preceding and during hospitalization, for further study, and plasma samples were collected 3 to 4 weeks after the initial positive RT-PCR test and analyzed by MCLIA. In these asymptomatic individuals, 81.1% tested positive for anti-S and anti-N IgG antibodies, and 62.2% tested positive for anti-S and anti-N IgM antibodies. However, anti-S and anti-N IgG levels in asymptomatic individuals were significantly lower than symptomatic individuals. Grzelak and colleagues[17] measured anti-S and anti-N IgG antibodies with 4 serologic assays: an anti-S enzyme linked immunosorbent assay (ELISA), an anti-N ELISA, an S-flow assay, and a luciferase immunoprecipitation system assay in 491 healthy controls, 51 hospitalized COVID-19 patients, and 209 suspected COVID-19 individuals with mild symptoms. The percentage of seropositive samples in hospitalized COVID-19 patients was on average 69% across the 4 serologic assays. Seroconversion in a subset of hospitalized patients was detectable between 5 and 10 days after symptom onset. In comparison, an average of 31% of suspected mild COVID-19 patients tested positive for IgG. The investigators attributed low seropositive rates to low viral loads in mild cases that elicited low antibody responses and analysis of samples collected before an individual seroconverted. Of note, determination of seroconversion is limited by assay sensitivity, which can account for discrepancies in reported seroconversion rates when different serologic methods are used. Nonetheless, these data demonstrate that anti-S and anti-N antibodies can be used to identify current and previous SARS-CoV-2 infections and present evidence that humoral responses are dependent on disease severity.

After observing that SARS-CoV-2 anti-N and anti-S antibody responses differed between symptomatic and asymptomatic individuals, antibodies and their role in clinical outcomes were widely studied. Guthmiller and colleagues[20] studied SARS-CoV-2 anti-S and anti-N antibody responses in 35 acutely infected and 105 convalescent individuals using ELISA. Severe infection was associated with enhanced anti-S and anti-N antibody responses, and high anti-N titers were especially observed in patients who were hospitalized for long durations. Legros and colleagues[21] observed that disease

severity, indicated by hospital admission status: outpatient, hospitalized floor, or hospitalized intensive care unit (ICU), strongly correlated with high levels of anti-S1 titers in a cohort of 140 patients. In addition, anti-S IgG antibody measurements and neutralization activity measurements showed that no neutralizing antibodies were present in 3% of hospitalized ICU patients, 34% of hospitalized floor patients, and 70.7% of outpatients. Atyeo and colleagues[22] used bead-based assays to investigate COVID-19 serologic markers in a discovery cohort (N = 22) from Seattle and in a validation cohort (N = 40) from Boston and observed divergent humoral responses between convalescent and deceased individuals. Anti-S IgG1, IgA1, and IgM levels were elevated among convalescent patients, whereas anti-N IgG levels were enhanced in deceased patients (**Fig. 2**A). Deceased patients also showed a less coordinated humoral response (**Fig. 2**B) compared with convalescent individuals who showed coordination between antibodies, natural killer cells, and phagocytic activity. The relationship between antibody responses and COVID-19 severity shows the potential of humoral immunity as a factor in patient care or therapeutic development.

Antibody responses to the receptor-binding domain

As high-resolution data on SARS-CoV-2 epitopes were obtained, RBD showed highly divergent sequences from other human coronaviruses and was identified as a primary target for antibody neutralization in animal models.[23] Premkumar and colleagues[24] developed a sensitive antibody ELISA against the SARS-CoV-2 RBD that had negligible cross-reactivity with antibodies against other human coronavirus RBD. Anti-RBD ELISAs for IgG and IgM were validated in sera from 63 symptomatic COVID-19 patients collected at least 9 days after symptom onset, demonstrating 98% and 81% sensitivity for IgG and IgM, respectively. The investigators then measured anti-RBD IgG, IgM, and IgA antibodies in 48 longitudinal serum samples and showed that most individuals seroconverted for IgG between days 7 and 9 after symptom onset. In addition, anti-RBD titers strongly correlated with SARS-CoV-2 luciferase neutralization assays, whereby 91% of patients showed detectable levels of neutralizing antibodies 21 days after symptom onset. Corroborating studies by Cao and colleagues,[25] Rydyznski Moderbacher and colleagues,[26] and Garcia-Beltran and colleagues[27] showed that neutralizing antibodies are predominantly specific to RBD, and a recent report demonstrated the potential of using commercial anti-RBD assays as a surrogate marker for neutralization.[28] Anti-RBD antibodies also serve as optimal targets in serologic diagnostics, providing high sensitivity and specificity in the detection of immunoglobulins.[6,29,30]

Because of the high correlation between anti-RBD antibodies and SARS-CoV-2 neutralization,[27,31,32] anti-RBD antibodies can provide more sensitive measurements of disease severity compared with anti-S and anti-N antibodies.[27,33,34] Li and colleagues[35] measured anti-S, anti-N, and anti-RBD IgG and IgM antibodies using MCLIA in 1850 patients with severe and mild COVID-19 progression. Recovered COVID-19 patients showed high anti-RBD and anti-S IgG levels. Lower anti-S, anti-RBD, and anti-N levels were associated with longer duration of infection as determined by persistent viral shedding. Röltgen and colleagues[31] similarly observed elevated anti-RBD-to-anti-N ratios in mild individuals compared with severely ill patients. In contrast, Ravichandran and colleagues[33] detected high IgA antibody titers in all patients with severe COVID-19. Anti-RBD IgA was especially high in patients who succumbed to the disease, compared with patients who recovered. Discrepancies in reported antigen-antibody correlations with clinical outcomes may be due to differences in cohort characteristics, whereby age, ethnicity, and geography can influence humoral immunity in a cohort. In addition, time of sampling is critical to determine the

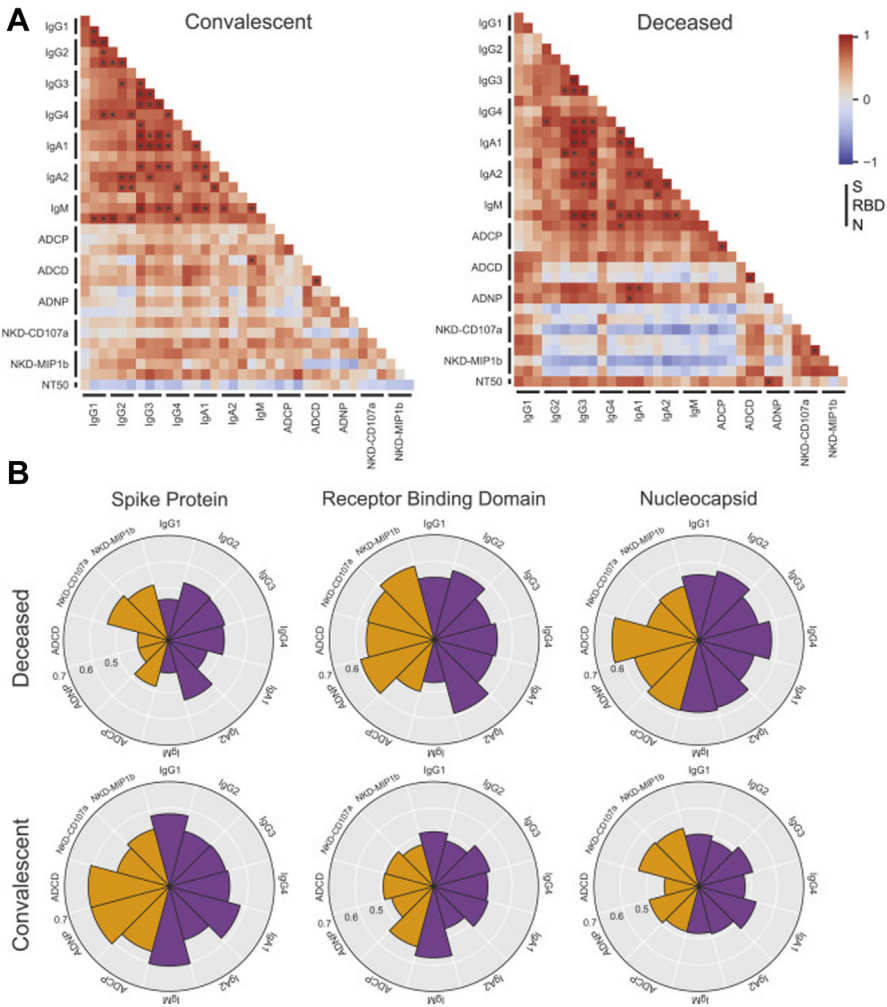

Fig. 2. Deceased individuals showed less coordinated and N-directed antibody responses. (*A*) The correlation heatmap shows pairwise Spearman correlation matrices of antigen-specific antibody titers and effector functions for convalescent (*left*) and deceased (*right*) patients. For each feature analyzed, the bar covers the S, RBD, and N antigens, shown in the legend on the right. Statistical significance is indicated by gray asterisks with Holm-Bonferroni correction for multiple hypothesis testing (P<.001). Negative correlations are indicated in blue, and positive correlations are denoted in red. (*B*) The Nightingale Rose plots show the mean percentile of antibody features within the deceased (*top*) and convalescent (*bottom*) groups. Plots represent the S-, RBD-, and N-specific responses across deceased (*top*) and convalescent (*bottom*) individuals. Each wedge represents an SARS-CoV-2 antibody feature. The size of the wedge depicts the magnitude of the value. The colors represent the type of feature: orange, antibody functions; purple, antibody isotypes and subclasses. (*From* Lumley, S. F. *et al.* Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. *N. Engl. J. Med.* 384, 533–540 (2021); with permission)

sensitivity of antibody tests, as recent analysis of patients who presented early or delayed seroconversion showed no correlation between antibody levels and disease prognosis.[36] Therefore, if individuals exhibit a wide range of seroconversion kinetics, SARS-CoV-2 antibody measurements in samples collected at a single time point after symptom onset may be challenging to interpret.

High-resolution kinetics of antigen-antibody responses

Sensitive antibody detection at early stages of infection is critical for assessing seroconversion and understanding kinetics of the humoral response. Norman and colleagues[37] demonstrated high-resolution detection of 12 SARS-CoV-2 antibody-antigen interactions during early infection (between zero and 14 days after a positive RT-PCR test) using ultrasensitive single molecule array (Simoa) assays. Simoa enables extremely low limits of detection such that plasma samples were diluted 4000-fold for serologic measurements, which reduced nonspecific binding compared with typical 200-fold dilutions used in ELISA formats. The investigators measured anti-S, anti-S1, anti-RBD, and anti-N IgG, IgM, and IgA responses in longitudinal plasma samples from 4 COVID-19 patients, which showed distinct immune responses. Some patients showed elevated antibody levels 5 days after a positive RT-PCR test[37] (**Fig. 3**A and **3**B), whereas others did not mount antibody responses to any antigens after 2 weeks (**Fig. 3**C and **3**D). Observation of diverse antibody kinetics among COVID-19 patients was later corroborated by Ogata and colleagues[38] in longitudinal studies of 39 COVID-19 patients. Ogata and colleagues showed that SARS-CoV-2 S, S1, and N antigens were detectable in the blood of severe COVID-19 patients. Antigens were detectable within zero to 10 days after a positive RT-PCR test. Antigen-positive patients showed seroconversion on an average of 7 days after the first positive RT-PCR test, and production of anti-S, anti-S1, and anti-N IgGs, IgAs, and IgMs correlated with S1 and N viral antigen clearance. Although an abundance of antibodies correlates with disease severity, timing of antibody production has also been demonstrated as an indicator of clinical outcome. Lucas and colleagues[39] analyzed 209 patient serum samples and demonstrated that patients who produced anti-RBD antibodies within 14 days of symptom onset correlated with lower mortality compared with those who seroconverted after 14 days. Mechanisms that cause delayed seroconversion remain to be studied. Nonetheless, these data highlight the potential for antibody kinetics and timing of seroconversion as a guide for patient care.

Antigen-Antibody Responses in COVID-19 Pediatric Patients

Clinical manifestations of SARS-CoV-2 differ in adult and pediatric patients. Adults present severe respiratory symptoms, whereas children are often asymptomatic during acute infection. Although many adults and children with COVID-19 become seropositive, children can develop a novel disease known as multisystem inflammatory syndrome in children (MIS-C) weeks after acute COVID-19 infection and present life-threatening symptoms, such as fever, myocardial disfunction, and cardiogenic shock. Such discrimination between clinical manifestations has been correlated with distinct antibody responses in adult versus pediatric patients. A recent study by Bartsch and colleagues[34] compared SARS-CoV-2 antibody titers in 25 mild COVID-19 pediatric patients, 17 pediatric patients who developed MIS-C, 34 mild COVID-19 adults patients, and 26 severe COVID-19 adult patients. Anti-S, anti-RBD, and anti-N IgM, IgG, and IgA levels were highest in adults with severe COVID-19 and significantly lower in mild COVID-19 adults and both pediatric cohorts. Interestingly, pediatric patients seroconverted earlier than adults with mild illness. However, less pronounced antibody levels suggest that children might not make an effective

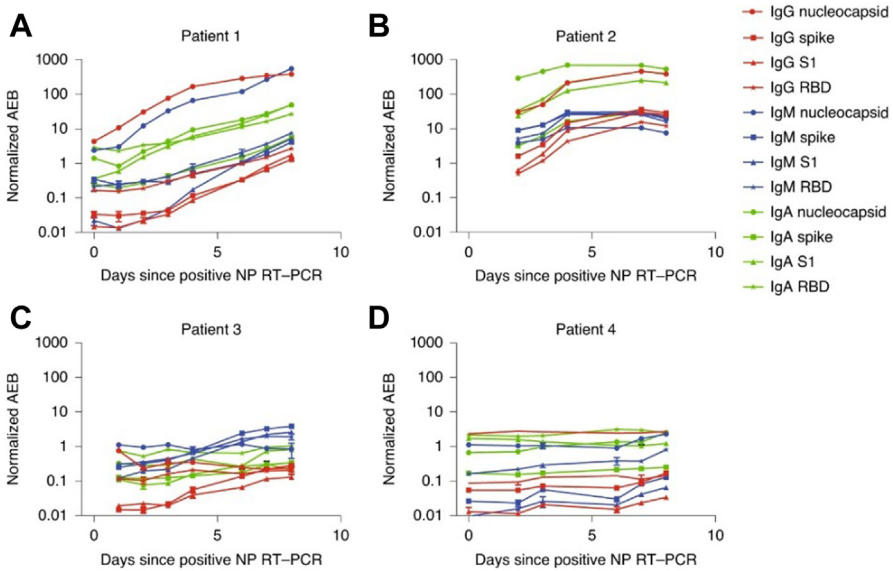

Fig. 3. Profiling the seroconversion time course in COVID-19 patients. (*A–D*) Normalized average number of enzymes per bead (AEB) over the 10 days since a positive nasopharyngeal RT-PCR for patients 1 (*A*), 2 (*B*), 3 (*C*), and 4 (*D*). Patient 1 was a 67-year-old man who recovered 10 days after diagnosis with COVID-19. Patient 2 was a 50-year-old man with multiple comorbidities who died of ARDS 20 days after diagnosis with COVID-19. He received remdesivir from days 1 to 5. Patient 3 was a 50-year-old man with pancytopenia and B-cell acute lymphoblastic leukemia. He died of ARDS 8 days after diagnosis with COVID-19. Patient 4 was an 89-year-old man who died of hypoxemic respiratory failure 8 days after diagnosis with COVID-19. He received hydroxychloroquine from days 1 to 5. The circle, square, triangle, and star represent the mean of 2 replicate measurements, whereas the error bars represent the standard deviation. (*From* Norman, M. *et al.* Ultrasensitive high-resolution profiling of early seroconversion in patients with COVID-19. *Nat. Biomed. Eng.* 4, 1180–1187 (2020); *with permission.*)

humoral response compared with adults. To assess COVID-19 pediatric patients who later develop severe MIS-C, Weisberg and colleagues[40] measured anti-S and anti-N IgG, IgA, and IgM antibody responses in 13 adults (median age 62) with COVID-19 and acute respiratory distress syndrome (COVID-ARDS) and 47 children (median age 11) with and without MIS-C. Although anti-S IgG, IgA, and IgM antibodies were elevated in adult patients with ARDS compared with patients without ARDS, all antibody classes showed similar levels for pediatric patients with and without MIS-C. Anti-N IgG responses were lower in pediatric patients compared with adult patients and were not dependent on disease severity in either adult or pediatric cohorts, suggesting that anti-N IgG production is age-dependent. In contrast, other studies have shown that antibody responses correlate with MIS-C severity. Pierce and colleagues[41] observed that patients with MIS-C had a larger ratio of IgG1 versus IgG3 compared with non-MIS-C patients. In a study with 192 enrolled pediatric participants, Yonker and colleagues[42] similarly reported that anti-RBD IgM and IgG antibodies were significantly increased in MIS-C patients compared with non-MIS-C patients. Children with acute COVID-19 and MIS-C had detectable IgM and IgG anti-RBD antibodies, as expected. Mild MIS-C pediatric patients showed low anti-RBD IgM and IgG levels. Notably, severe MIS-C cases showed elevated IgG levels against other coronaviruses

(229E, NL63, HKU1, and OC43), whereas mild MIS-C pediatric, COVID-19 adult, and recovered adult patients did not show such nonspecific antibody responses. These studies suggest that antibody subtype, antigen target, and humoral kinetics play a role in immunity in children. Future studies on significantly large cohorts are critical to further understand the relationship between humoral responses and COVID-19/MIS-C pathologic condition in children.

Humoral Immunity During Convalescence

Mounting evidence suggests that high antibody responses persist for several months after COVID-19 patients reach convalescence.[25,27,43–45] In a longitudinal cohort of 250 convalescent patients, Boonyaratanakornkit and colleagues[43] observed high anti-S1 IgG antibodies lasting several months ($t_{1/2} \sim 66.2$ days) that correlated with neutralization titers. Similarly, a study of 43 COVID-19-positive patients showed 95% maintenance of anti-RBD IgG levels and neutralization titers at 6 months after symptom onset ($t_{1/2} \sim 140$ days).[46] A large-scale study in 30,082 COVID-19 patients showed high anti-S1 IgG and neutralization titers lasting 5 months after infection.[44] However, studies have also shown that antibody levels decline after infection. In a longitudinal study of anti-N and anti-S IgG, anti-S1 and anti-RBD IgA, and anti-S1 and anti-RBD IgM levels in 26 health care workers, Marot and colleagues[45] observed waning of IgA antibodies 2 months after disease onset, whereas IgG and IgM levels persisted for 3 months. Decline of anti-S, anti-N, and anti-RBD IgG, IgA, and IgM were similarly observed 3 months after infection by Seow and colleagues,[47] which correlated with a decline in neutralizing antibody responses.

The risk of reinfection in convalescent COVID-19 individuals with or without maintenance of antibody titers remains a major unknown in the COVID-19 pandemic. Lumley and colleagues[48] measured anti-S and anti-N IgG antibodies across 12,541 health care workers in the United Kingdom over 7 months and defined reinfection as the first positive PCR test at least 60 days after an initial positive antibody test. Individuals who were initially seropositive for anti-S and anti-N IgG antibodies presented a substantially lower rate of reinfection than individuals who were initially seronegative. These results agree with work from Hall and colleagues,[49] who found that 30,625 health care workers with previous SARS-CoV-2 infection were at 84% lower risk of reinfection than workers without previous infection. Of note, Lee and colleagues[50] presented a case study of one patient who mounted robust anti-RBD and anti-S1 IgG responses to SARS-CoV-2 but, despite maintaining antibody seropositivity, was reinfected with SARS-CoV-2 26 days after initial infection. Evidence of SARS-CoV-2 reinfection in previously infected individuals indicates that antibody markers may not be sufficient to predict immunity to SARS-CoV-2. Although it is now possible to perform serologic studies in individuals over 1 year after SARS-CoV-2 infection, these studies are yet to be reported, and mechanisms of long-term humoral immunity remain unknown.

SUMMARY

Studies on humoral responses to SARS-CoV-2 have been reported with unprecedented speed and with resolution that enables analysis of specific antigen-antibody interactions during all stages of infection and recovery. The authors present a review of literature on antigen targets for antibody responses in the context of acute immunity, clinical relevance, and convalescent immunity. S, N, and RBD proteins have been identified as key antigens in IgG, IgA, and IgM antibody responses during COVID-19 infection. Analysis of antigen-antibody responses continues to be critical in understanding COVID-19, especially in the context of vaccine development and

viral variants. As COVID-19 vaccines are distributed, the ability to detect antigen-specific antibodies will be key in identifying individuals who have seroconverted from natural infected versus vaccination. For example, messenger RNA vaccines encode for S protein, and therefore, detection of antibodies against N protein would signify seropositivity from natural SARS-CoV-2 infection. Another key question is whether individuals with SARS-CoV-2 antibodies, either by vaccination or by natural infection, will be immune from reinfection by SARS-CoV-2 variants. Mutations for several variants have been identified on the S protein, specifically the RBD region.[51] New assays for detection of antibodies against antigen variants will be key to further understanding humoral responses to SARS-CoV-2 as the virus evolves.

CLINICS CARE POINTS

- Point-of-care SARS-CoV-2 antibody testing is effective in identifying antibodies from previus infections in mildly and severely symptomatic individuals.
- Monitoring patient seroconversion can effectively guide clinicians to assess COVID-19 disease severity and implementent suitable therapeutics.
- Quantifying antibody levels via point-of-care serology tests as a screen for prior infection can enable clinicians to evaluate effective timepoints for administration of vaccines and boosters for healthy and immunocompromised individuals.
- Monitoring overall seroconversion in a population provides data for disease epidemiology and outbreak containment measures.

DISCLOSURE

D.R. Walt has a financial interest in Quanterix Corporation, a company that develops an ultrasensitive digital immunoassay platform. He is an inventor of the Simoa technology, founder of the company, and serves on its Board of Directors. Dr D.R. Walt's interests were reviewed and are managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict-of-interest policies.

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