Research article

Longitudinal antibody titer, avidity, and neutralizing responses after SARS-CoV-2 infection

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ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Antibody avidity
Neutralization
Humoral immunity
Longitudinal immunity

ABSTRACT

While waning immunity and SARS-CoV-2 variant immune escape continue to result in high infection rates worldwide, associations between longitudinal quantitative, qualitative, and functional humoral immune responses after SARS-CoV-2 infection remain unclear. In this study, we found significant waning of antibody against Spike S1 (R = −0.32, p = 0.035) and N protein (R = −0.39, p = 0.008), while RBD antibody moderately decreased (R = −0.19, p = 0.203). Likewise, neutralizing antibody titer (ND50) waned over time (R = −0.46, p = 0.001). In contrast, antibody avidity increased significantly over time for Spike S1 (R = 0.62, p = 6.0e−06), RBD (R = 0.54, p = 2.0e−04), and N (R = 0.33, p = 0.025) antibodies. Across all humoral responses, ND50 strongly associated with Spike S1 (R = 0.85, p = 2.7e−13) and RBD (R = 0.78, p = 2.9e−10) antibodies. Our findings provide longitudinal insight into humoral immune responses after infection and imply the potential of Spike S1/RBD antibody titer as surrogate correlates of protection.

1. Introduction

Since its first report in December 2019, SARS-CoV-2 has progressively mutated and displayed increasing infectivity [1, 2], resulting in an unparalleled global burden on health care systems and economies [3]. Despite high rates of vaccine coverage in the United States, new cases continue to increase due to the waning of vaccine-induced immunity and the emergence of immune escape variants [4]. As such, a short half-life of 3–5 months was observed in SARS-CoV-2-specific CD4+ and CD8+ T cells [5] and reduced responses of vaccine-induced memory B cells to variants of concern (VoC) were also found [4]. While the correlation between SARS-CoV-2-specific antibodies with their functional activities and the protection against SARS-CoV-2 infection is urgently needed [6], establishing this correlation in a comprehensive manner remains unmet [6].

Immune protection against SARS-CoV-2 is determined by not only the level of SARS-CoV-2-specific antibodies, but also by the binding strength of these antibodies to their cognate antigens (avidity), as well as their ability to neutralize the virus [7, 8, 9]. Upon exposure to a foreign antigen, B cells experience a whole process of proliferation, isotype switching, and affinity maturation, resulting in the generation of B-cell clones that can produce highly antigen-specific antibodies with strong avidity [10, 11]. Current widely administered SARS-CoV-2 mRNA vaccines (Pfizer-BioNTech and Moderna) induce the generation of Spike protein-specific antibodies that maintain protection [12, 13]. Previous studies have found a correlation between immune protection and the titer of neutralizing antibodies and/or antibodies able to block the interaction of receptor binding domain (RBD) and angiotensin converting enzyme 2 (ACE2) [14,15]. While blocking antibody titers declined over 6 months, we found reduced antibody responses and differential memory B cell cross-reactivity to SARS-CoV-2 VoC in vaccines [4]. Furthermore, B-cell clonal selection has been shown to result in higher avidity and better neutralizing antibodies in convalescent individuals [7, 8, 9]. In contrast, recent findings in literature analysis of increasing the affinity of RBD-ACE2 binding and/or decreasing the susceptibility of neutralizing antibodies by VoC suggested that quantitative avidity assay may be a better tool to characterize protective immunity against SARS-CoV-2 [16]. In fact, numerous studies repeatedly observed an association between antibody avidity and clinical outcomes of SARS-CoV-2 infection, in which severe disease and re-infection were found in individuals with SARS-CoV-2-specific antibodies of low avidity [17, 18, 19, 20]. To address these conflicting observations, herein we evaluated the avidity of SARS-CoV-2-specific antibodies and their neutralizing...
capacities in COVID-19convalescent. Further, we assessed potential in-
fluences of demographic/clinical characteristics of study subjects on
SARS-CoV-2 humoral immunity post-infection. Our data demonstrate a
strong positive correlation between antibody titer and neutralizing ac-
tivity, as well as an increase of antibody avidity over time following
infection.

2. Materials and methods

2.1. Study cohort

Serum samples from 32 subjects who were infected with SARS-CoV-2
(March 28 to November 21, 2020) as confirmed by PCR testing were
obtained from the Mayo Clinic COVID-19 Biobank. Based on the time of
PCR test and the time of collecting blood samples, these serum samples
were categorized into three timepoints: 10–20 days (T1, n = 29), 21–40
days (T2, n = 26), and >40 days (T3, n = 21).

The study was approved by the Mayo Clinic Institutional Review
Board, and all study participants provided written informed consent.

2.2. Antibody avidity enzyme-linked immunosorbent assay

To characterize the progression of humoral immunity, both titer and
avidity of antibodies specific to receptor-binding domain (RBD), the S1
subunit of the Spike protein, and nucleocapsid (N) protein were assessed
using commercially available ELISA kits (RayBiotech COVID-19 human
IgG ELISA for N-protein, catalog no. IEQ-CoVNIgG-1; RayBiotech
COVID-19 human IgG ELISA for Spike S1 protein, catalog no. IEQ-
CoVS1RBD-IgG-1; Mabtech ELISA Path Total Antibody for RBD, catalog
no. 3890-1H-R1-1). Each serum sample was tested at four dilutions (for
N protein, 1/180, 1/540, 1/1620, 1/4860; for S1 subunit, 1/60, 1/180, 1/
540, 1/1620; for RBD, 1/60, 1/180, 1/540, 1/1620) following the in-
structions provided. The manufacturer’s protocol was slightly modified to
include parallel samples at the same dilutions that were washed with a
denaturing wash buffer (5 mM diethylamine) to determine antibody
avidity. Antibody avidity was calculated using the equation:

\[
\text{avidity} = \frac{\text{absorbance with denatured wash}}{\text{absorbance with regular wash}}
\]

A pool of plasma from convalescent subjects and the first WHO international standard (NIBSC code: 20/136; 1000 IU/mL stock solution) for anti-SARS-CoV-2 human immunoglobulin were included in each plate as positive controls. The RBD ELISA contained a negative control sample, while wells without sera were used as negative controls for the Spike S1 and N protein ELISAs. The concentration of serum antibodies was calculated with the reference of WHO international standard units (IU/mL). The coefficient of variation (CV) of the assays for the Spike S1, N protein, and RBD were calculated as 6.0%, 2.3%, and 5.7%, respectively.

2.3. SARS-CoV-2 pseudovirus/rVSV microneutralization assay

Neutralizing antibodies to the SARS-CoV-2 Spike protein of Wuhan-
Hu1 SARS-CoV-2 were quantified using a fluorescence-based micro-
neutralization assay, as described previously [4, 21]. Briefly, serum
samples were diluted two-fold serially, starting from 1/10 up to 1/5,120
(with 4 replicates for each dilution) in a 96-well microplate. An equal
samples were diluted two-fold serially, starting from 1/10 up to 1/5,120

We conducted a longitudinal observational study on a cohort con-
isting of 12 (37.5%) females and 20 (62.5%) males of mostly non-
Hispanic European descent (78.1%). The median age at first SARS-
CoV-2 positive PCR test was 59.5 years (interquartile range, IQR: 49.5–
64.3), and the median body mass index (BMI) was 25.25 (IQR, 22.96–
26.19). Of 32 subjects, 27 (84.4%) individuals were hospitalized for
COVID-19, including 24 individuals in the intensive care unit (ICU).

Lastly, correlations between log2-transformed N, S1, RBD, and ND50
were calculated using the ‘rmcorr’ function to account for multiple
measures within each subject. All statistical analyses performed with R
v.4.1.2.

3. Results

3.1. Study cohort

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COVID-19, including 24 individuals in the intensive care unit (ICU).

The median time in the hospital was 46 days (IQR, 32.0–57.5), and the
median time in the ICU was 24 days (IQR, 19.0–32.8). SARS-CoV-2 in-
fecions occurred from March to November 2020, prior to the emergence
of VoC (alpha/B.1.1.7 and beta/B.1.351) in North America.

3.2. Antibody responses over time

To characterize temporal dynamics following SARS-CoV-2 infection,
we measured the titer and avidity of antibodies specific to N protein, S1
Spike protein, and the RBD. As shown in Figure 1A, antibody titers
against each antigen decreased over time. Antibody titers against N
S1/RBD antibody titer (578, 803, and 408 IU/mL at T1, T2, and T3
were 960, 1066, and 534 IU/mL, respectively. The decrease in
mean antibody avidity of T1 samples (16.5%) (R

mean antibody avidity of T3 samples (24.1%) was ~1.5-fold higher than mean antibody avidity of T1 samples (16.5%) (R

Cell culture medium, instead of serum, was used as negative control
(n = 16 for each plate). Pooled positive serum, pooled negative serum,
and a human monoclonal neutralizing antibody (IgG1; Active Motif,
catalog no. 91361) were included in each test for quality control. The CV
of this assay was 7.38% [4]. The VSV virus expressing eGFP and the
SARS-CoV-2 Spike protein (Wuhan-Hu-1) was kindly provided by Dr.
Sean Whelan (Washington University School of Medicine, St. Louis, MO)
[21].

2.4. Statistical analysis

Associations between demographic variables BMI, sex, age, and
hospitalization status and antibody titers (N/S1/RBD/ND50) were
measured using a linear mixed-effects model applied via the ‘lmer’
function of the ‘lme4’ R package [25]. Intra-correlation effects from
different timepoint measurements (up to three per subject) were included
as random effect, time from first positive PCR test was included as a
covariate. Correlations between N/S1/RBD/ND50 and time were calcu-
lated using repeated measure correlation techniques via the ‘rmcorr’
function from the ‘rmcorr’ package [24]. This method accounts for
intra-correlation effects from multiple measurements. N/S1/RBD/ND50
were transformed on the log2-scale to account for skewed values.
Associations between ND50 and N/S1/RBD were measured using linear
mixed-effects models via the ‘lmer’ function, with subject as a random
effect and days between PCR test and timepoint included as a covariate.

Lastly, correlations between log2-transformed N, S1, RBD, and ND50
were calculated using the ‘rmcorr’ function to account for multiple
measures within each subject. All statistical analyses performed with R
v.4.1.2.
3.3. Correlations among antibody responses and associations with clinical outcomes

We further examined correlations between N/S1/RBD antibody titer and ND50 titer. We observed that N-specific IgG titers correlated with RBD antibody titers (R = 0.51, p = 3.7e–04, Figure 2A) and S1-IgG titers (R = 0.61, p = 9.96e–06, Figure 2B). We also observed a strong correlation between S1-specific IgG titers and RBD-specific total antibody titers (R = 0.71, p = 3.7e–08) (Figure 2C). With respect to neutralizing antibody titer, we observed correlations between ND50 titer and S1 antibody titer (R = 0.85, p = 2.7e–13), RBD antibody titer (R = 0.78, p = 2.9e–10) [25], and N antibody titer (R = 0.72, p = 2.3e–08, Figure 2D).

We next examined correlations between the avidity of the response to each viral protein. We did not find any correlation between N-specific IgG avidity and either S1 or RBD-antibody avidity; however, S1-antibody avidity did correlate strongly with RBD-antibody avidity (R = 0.59, p = 3.1e–05, Fig. S1).

We also assessed correlations between antibody avidity and neutralizing antibody titers in our subjects. We found negative correlations between the RBD and S1 (but not N protein) antibody avidity and ND50 titer (RBD: R = –0.323, p = 0.034; S1: R = –0.405, p = 0.006). However, using mixed effect models that adjusted for days post-first positive PCR test, we no longer observed the significant associations between S1/RBD antibody avidity and ND50 response.

Clinical and demographic factors, including sex [26], disease severity [27], and age [28] have been found to impact the immune outcomes for SARS-CoV-2; therefore, we examined associations between sex, age, BMI, and hospitalization status with ND50 titer, ELISA antibody titer, and antibody avidity (Table S1, Table S2). We found hospitalization status (i.e., in hospital, in ICU, or not hospitalized) to be significantly associated with S1-specific IgG titer (p = 0.05) and avidity (p = 0.003) and RBD-specific IgA/IgG/IgM titer (p = 0.02). We did not find any associations between sex and the antibody outcomes. After adjusting for time since positive PCR test, we found suggestive associations of age with N-specific IgG titers (p = 0.07). Likewise, we found a modest association with an increase in N-specific IgG titers and obese subjects (p = 0.06).

4. Discussion

As the COVID-19 pandemic continues, it is crucial to thoroughly understand the temporal dynamics and associations between quantitative and qualitative characteristics of humoral immunity following SARS-CoV-2 infection to determine factors contributing to immune protection. In this study, we observed that antibody titers specific to N protein and S1 Spike protein decreased over time— with the caveat that our starting point was 14–20 days after infection and our final measurements were 41–98 days after infection. We noted a similar, but non-significant trend for RBD antibody titer. Likewise, neutralizing antibody titers (ND50) decreased over the same timespan after infection and correlated most strongly and significantly with antibody titers specific to RBD and S1 Spike protein.

Interestingly, we noted that while antibody to Spike and N protein both decreased over time, the magnitude of the Ab response to these two proteins was quite different. This suggests that different factors, likely the availability or quality of T cell help, control the magnitude of the humoral immune response to these two structural proteins. Supporting this hypothesis, for SARS-CoV-2, it has been found that the...
immunodominance pattern of the T cell response corresponds to protein abundance [29], with differences in both the magnitudes and cytokine expression profiles of the Spike, N protein, and Membrane specific T cell responses [30]. It has been reported that N protein-specific T cells are detectable years after SARS-CoV-2 infection, whereas Spike protein-specific T cells are not [31]. Lastly, we found N/RBD/S1 Spike antibody avidity to increase significantly over time after infection. While we could not conclude avidity determination (i.e., incomplete vs complete) through the use of a high avidity control, we did not observe a plateau in avidity responses, suggesting incomplete avidity maturation during the sampling timespan, which was also observed by Struck and colleagues across the same timespan [32]. Although, it is logical to hypothesize that higher antibody avidity would lead to stronger neutralization of SARS-CoV-2 Spike-expressing viruses, we did not observe this association overall or at either timepoint (T1/T2/T3). Avidity did, however, correlate with disease severity (Supplemental able 1), with ICU patients having the highest avidity and those who were hospitalized having the lowest avidity. The exact role of antibody avidity in clinical outcomes remains to be elucidated and requires a larger sample size to be confidently addressed.

We acknowledge that antibody avidity/affinity binding measurements in vitro even against critical (for receptor interaction) viral antigenic domains such as the RBD, cannot always predict neutralization/protection (the ability of antibodies to prevent infection), e.g., many anti-RBD antibodies lack neutralizing activities [33]. Likewise, in vitro measurement of neutralizing antibodies to SARS-CoV-2 does not quantify the antibody avidity/affinity and does not fully reflect the in vivo potential of the neutralizing antibodies to compete with hACE2 for interaction/binding to RBD [8, 16, 34, 35].

In hospitalized patients or patients with severe disease, the rise of SARS-CoV-2 neutralizing antibody titer is not necessarily a maker of disease resolution or favorable disease outcome, but rather reflects the abundance of viral antigens that drive the neutralizing antibody response/avidity maturation to curb infection progression. Several studies have demonstrated that antibody affinity/avidity measures (rather than neutralizing antibody) are correlated with COVID-19 disease severity and clinical outcomes [17, 19]. This is indicative that antibody affinity/avidity plays an important part of protection, provided that high-affinity antibodies are targeting specific epitopes critical to protection.

On the one hand, neutralizing antibodies are highly protective against infection and symptomatic disease to SARS-CoV-2 and other viral pathogens. On the other hand, neutralizing antibodies are highly protective against infection and symptomatic disease to SARS-CoV-2 and other viral pathogens [14, 15, 16]. Thus, both antibody affinity/avidity and neutralization are causally related to protection and their measurements and interrelationships (associations) are worth exploring under different scenarios (i.e., hospitalized patients, vaccinees etc…). Other studies in COVID-19 mRNA vaccinated subjects and convalescent subjects have also failed to detect a relationship between neutralization and antibody affinity/avidity studied at different timepoints following antigen exposure [36, 37]. However, two recent comprehensive mechanistic studies on the evolution of humoral immunity to SARS-CoV-2 (using human monoclonal antibodies and a detailed assessment of memory B cell response/reertoire), have provided evidence for enhanced B cell somatic hypermutation, higher affinity, and increased neutralization potency and breadth of human antibodies 6 months after infection compared to 1 month, even though the antibody titers significantly decreased over time [9, 38]. It is likely that the increase in antibody affinity/avidity over time positively influences functional humoral immunity/neutralization to SARS-CoV-2; however, we assessed total neutralization antibody titers rather than neutralizing activity of specific antigens.
monoclonal antibodies. A future mechanistic study is needed to illuminate the relationship between antibody affinity/avidity and neutralizing activity.

Although our study cohort consisted mostly of individuals experiencing severe infection, our observations of polyclonal antibody titer and antibody avidity dynamics after infection are in line with other studies assessing individuals with more mild infection [39, 40, 41, 42]. In a large non-hospitalized cohort (n = 4683) that seroconverted in response to infection and did not experience reinfection, anti-Spike IgG levels peaked at 4–5 weeks after infection and subsequently waned [42]. Similarly, we observed the highest antibody titers to Spike S1 and RBD at our second timepoint (21–40 days). Other reports also document an increase in anti-Spike S1 IgG avidity over time after infection [39]. These reports examine non-hospitalized individuals experiencing mild disease (1–6 months) [27], mostly non-ICU patients (0–80 days) [40], and individuals >61 years of age (up to 6.5 months) [41]. In the latter cohort, neutralizing antibody titers decreased up to 6.5 months after infection [41]. Consistent findings across differing study cohorts (e.g., age, disease severity, cohort size) confirm the validity of our findings in defining quantitative and qualitative antibody trends following SARS-CoV-2 infection.

Our study poses several questions to explore and a couple of limitations to consider. First, we selected sera based on biohazard availability and time since the first positive SARS-CoV-2 PCR test; therefore, the exact time of infection may differ between subjects. Because most of our cohort was hospitalized, subjects might not have been tested until hospitalization, potentially resulting in the underestimation of timepoints. Second, although we found a strong correlation between antibody titer and neutralization capacity, antibody subclasses and other isotypes should be assessed in detail. With respect to anti-RBD titer and avidity, we assessed total antibody (IgG/IgA/IgM) rather than only IgG; however, concentrations of serum IgM and IgA represent only a small proportion of circulating immunoglobulins in convalescent COVID-19 individuals [43]. Therefore, a total concentration of anti-RBD antibodies and their avidities should largely represent IgG titer/avidity kinetics after infection. Our study poses interesting questions about the interplay between time and characteristic of the antibody response. Given that the virus enters at mucosal surfaces, a study evaluating mucosal- and epithelial-derived IgA and characteristic of the antibody response may provide important additional insights into humoral immunity to SARS-CoV-2 and infections.

Our study included patients who had SARS-CoV-2 infection, and the data were analyzed to examine the dynamics of antibody responses over time. The qualitative analysis of antibody responses following SARS-CoV-2 infection showed strong correlations between RBD/Spike S1 protein IgG titer and neutralization capacity in the cohort consisting mostly of hospitalized individuals. We found strong correlations between RBD/Spike S1 protein IgG titer and neutralization antibody titer (ND50) over time, highlighting a potential use of RBD/Spike IgG titers as an easier-to-measure surrogate of protection. Further, we found N/RBD/Spike S1 antibody avidity to increase significantly following infection despite antibody waning, suggesting the ongoing occurrence of clonal selection and affinity maturation of memory B cells. Our findings contribute to the assessment of antibody temporal dynamics following SARS-CoV-2 exposure and highlight the need to further examine antibody avidity and neutralizing capabilities at the monoclonal level.

5. Conclusions

We assessed longitudinally the titer, avidity, and neutralization capacity of SARS-CoV-2-specific antibodies following SARS-CoV-2 infection in the cohort consisting mostly of hospitalized individuals. We found strong correlations between RBD/Spike S1 protein IgG titer and neutralization antibody titer (ND50) over time, highlighting a potential use of RBD/Spike IgG titers as an easier-to-measure surrogate of protection. Further, we found N/RBD/Spike S1 antibody avidity to increase significantly following infection despite antibody waning, suggesting the ongoing occurrence of clonal selection and affinity maturation of memory B cells. Our findings contribute to the assessment of antibody temporal dynamics following SARS-CoV-2 exposure and highlight the need to further examine antibody avidity and neutralizing capabilities at the monoclonal level.

Declaration

Author contribution statement

Richard B. Kennedy: Conceived and designed the experiments; Wrote the paper.

Jonathan M. Monroe: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Iana H. Haralambieva, Huy Quang Quach: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Richard B. Kennedy was supported by the Mayo Clinic Department of Medicine Coronavirus Research Initiative Award. This work was supported by the Center for Individualized Medicine, Mayo Clinic, and National Institute of Health (R01 AI48793).

Data availability statement

Data included in article/ supp. material/ referenced in article.

Declaration of interest's statement

The authors declare the following conflict of interests:

Dr. Kennedy holds patents related to the identification of epitopes from vaccinia virus, zika virus, and SARS-CoV-2 and has received grant funding from ICW Ventures for preclinical studies on a peptide-based COVID-19 vaccine. Dr. Kennedy has received funding from Merck Research Laboratories to study waning immunity to mumps vaccine and has consulted with Merck Research Laboratories and Sanofi Pasteur on MMR and influenza vaccine development. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. This research has been reviewed by the Mayo Clinic Conflict of Interest.

Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e11676.

Acknowledgements

We gratefully acknowledge the Mayo Clinic COVID-19 Biobank for providing access to COVID-19 patient sera samples and the associated demographic and clinical data.

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