Rapid decline of neutralizing antibodies is associated with decay of IgM in adults recovered from mild COVID-19

Highlights
- After mild COVID-19, anti-S-trimer, RBD, and NP IgG are stable for up to 6 months
- Neutralization activity against the virus rapidly decays over time
- Neutralization is most strongly correlated with anti-S-trimer IgM titers
- Antibodies are initially higher in those with fever but reach similar nadirs

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In brief
Harrington et al., report that in adults with mild COVID-19, IgG responses are maintained for >6 months and are correlated with fever. Neutralization activity rapidly decays, correlating with the loss of anti-S-trimer IgM titers. Thus, neutralizing antibodies fade quickly after mild COVID-19 infection, despite the long-term maintenance of IgG.
Report

Rapid decline of neutralizing antibodies is associated with decay of IgM in adults recovered from mild COVID-19

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SUMMARY

The fate of protective immunity following mild severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection remains ill defined. Here, we characterize antibody responses in a cohort of participants recovered from mild SARS-CoV-2 infection with follow-up to 6 months. We measure immunoglobulin A (IgA), IgM, and IgG binding and avidity to viral antigens and assess neutralizing antibody responses over time. Furthermore, we correlate the effect of fever, gender, age, and time since symptom onset with antibody responses. We observe that total anti-S trimer, anti-receptor-binding domain (RBD), and anti-nucleocapsid protein (NP) IgG are relatively stable over 6 months of follow-up, that anti-S and anti-RBD avidity increases over time, and that fever is associated with higher levels of antibodies. However, neutralizing antibody responses rapidly decay and are strongly associated with declines in IgM levels. Thus, while total antibody against SARS-CoV-2 may persist, functional antibody, particularly IgM, is rapidly lost. These observations have implications for the duration of protective immunity following mild SARS-CoV-2 infection.

INTRODUCTION

In late 2019, a novel coronavirus emerged in Wuhan, China that has gone on to cause the worst global pandemic in >100 years. Within months, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) spread rapidly, reaching all regions of the globe. To date, SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19) illness, has infected nearly 104 million people and is responsible for >2.2 million deaths.1 Almost immediately, the global scientific community mobilized to begin to understand and counter the threat and countries deployed public health countermeasures to stem the rate of infection. Numerous potential long-term countermeasures, such as vaccines and therapeutics, are undergoing rapid development and clinical testing,2 with several vaccines now approved for emergency use. However, in the absence of widespread access to vaccines, much attention has been focused on whether natural infection may lead to durable protection from reinfection, potentially affecting herd immunity and personal behavior. Key to understanding this is an accurate characterization of the kinetics of total and functional antibody response following infection. Notably, while most infections are mild and do not require hospitalization, these mild infections are the least studied with regard to humoral immune responses.

Others have shown that SARS-CoV-2 infection induces varying degrees of humoral immunity. Immunoglobulin M (IgM) antibodies appear in 5–10 days but wane quickly, whereas IgG and IgA appear 14–21 days post-infection.3–12 The magnitude of IgG-binding titers is highly variable and appears to be associated with disease severity, stage of infection, and age.9,10 Neutralizing antibodies (NAbs) develop in >98% of participants3,10,12,13 and develop concurrently with IgG-binding antibodies. They are detectable within the first 2 weeks and increase in potency for at least 4 weeks post-infection.3 The development of NAbs has been confirmed by the isolation and characterization of numerous monoclonal antibodies that potently neutralize the virus.14–21 NAbs have been shown to target multiple epitopes on the Spike protein (S), with the receptor-binding domain (RBD) mediating exceptional potency.5,14,17,19 Interestingly, NAbs with little or no somatic mutation potently neutralize the virus, indicating that extensive B cell maturation is not requisite for NAb development.7,19

The durability of anti-SARS-CoV-2 humoral immunity remains ill-defined and is crucial to effectively guiding public health strategies. Studies from SARS-CoV and Middle East respiratory syndrome (MERS)-CoV suggest that humoral immunity lasts for at least 1 year,22,23 with some responses detectable after a decade
or more. While IgG-binding antibodies persist past acute infection, their neutralizing functional activity over time remains unclear. Studies on SARS-CoV-2 serological responses have been reported with follow-up up to 3 months, with some studies reporting sporadic sampling out to 7 months. Studies initially focused on the kinetics and durability of anti-S-binding IgG, observing reasonable durability at 3 months post-infection. However, viral neutralization is likely the key factor for which long-term durability must be maintained, and while NAbs have been detected as far out as 6–7 months, the kinetics of these responses remain less clear following asymptomatic or mild infection.

Here, we assessed plasma antibody responses in a cohort recovered from mild COVID-19, with follow-up for as long as 6 months. We found that IgG-binding titers to viral antigens remain durable over time, and avidity studies provide evidence of B cell maturation. Humoral responses were associated with fever, but not other clinical variables. However, neutralizing activity rapidly contracted 2–3 months following infection, despite the overall maintenance of IgG-binding antibody levels. Notably, neutralization was most strongly correlated with anti S-trimer IgM, rather than IgG or IgA, offering a possible explanation for the apparent paradox between stable IgG and waning neutralization. Our findings indicate that despite the overall maintenance and durability of humoral immune responses after infection, neutralizing activity waned rapidly as IgM titers decayed. Overall, these findings imply that mild infections may not generate lasting protection and highlight the urgent need for the rapid deployment of effective vaccines to stem the spread of SARS-CoV-2.

**RESULTS**

### Clinical characteristics

The cohort consisted of 34 individuals (Table 1). Participants presented for their initial recovered visit 14–82 days after symptom onset (median 33 days). They were 77% female and ranged in age from 24 to 74 (median 41) years. All but one participant was mildly symptomatic. The single participant who was hospitalized required supplemental oxygen but not additional support. In total, 47% reported fever, which lasted between 1 and 7 days (median 3 days). A total of 71% reported cough, which lasted between 2 and 60 days (median 6 days). The presence of fever was not associated with age or gender; however, among those who reported fever, men experienced a longer duration (4 days versus 2 days for women, p = 0.05). Women were more likely to report cough (81% versus 43% for men; adjusted odds ratio: 7.20, p = 0.04), and among those with cough, women reported a longer duration (16 days versus 3 days for men, p < 0.001). Older individuals had a trend toward increased duration of cough (1 increased day per year increase in age, p = 0.1). Fever and cough were disassociated, with 12/18 without fever reporting cough and 12/16 with fever reporting cough (p = 0.6).

Thirteen participants contributed a single time point, 12 contributed 2 time points, 8 contributed 3 time points, and 1 contributed 4 time points. When grouped by time since symptom onset, 21 participants contributed an early sample (<2 months), 22 contributed a 2-month sample, 8 contributed a 4-month sample, and 12 contributed a 6-month sample (Table 1).

### IgG is durable and associated with fever

We evaluated the development of IgG antibody responses longitudinally after infection. We tested IgG-binding endpoint titers to recombinant S trimer, to the RBD subdomain on S1 (the site of angiotensin I-converting enzyme-2 [ACE2] receptor attachment), and to nucleocapsid protein (NP) (Figure S1). Of 34 participants, 31 (91%) developed anti-S trimer IgG antibodies. At entry point into the study, we observed a wide range of endpoint IgG titers to trimer, from 1:232 to 1:2,686,3 (Figure 1A). Similarly, 32 of 34 participants (94%) developed anti-RBD IgG, and endpoint titers ranged from 1:135 to 1:28,488 (Figure 1A). A total of 32 of 34 participants (94%) developed anti-NP titers, with titers that ranged from 1:50 to 1:111,192 (Figure 1A).

#### Table 1. Cohort features: demographic characteristics and disease features of the cohort and the samples included in this study

| Characteristics          | Gender    | Number (Percentage) |
|--------------------------|-----------|---------------------|
| Female, n (%)            | 26 (77%)  |                     |
| Male, n (%)              | 8 (23%)   |                     |
| Age, y (range)           | 41 (24–74)|                     |
| Fever, n (%)             | 16 (47%)  |                     |
| Days with fever (range)  | 3 (1–7)   |                     |
| Cough, n (%)             | 25 (71%)  |                     |
| Days with cough (range)  | 6 (2–20)  |                     |

#### Time points

| Time points | No. | Symptom days |
|-------------|-----|--------------|
| Early       | 21  | 24 (14–41)   |
| 2 months    | 22  | 63 (43–82)   |
| 4 months    | 8   | 97 (86–139)  |
| 6 months    | 12  | 154 (140–181)|

Within the cohort, we observed a strong correlation between the level of anti-S IgG (delta per day cough: 0.009, p = 0.04) and anti-RBD IgG (delta: 0.48, p = 0.002), and anti-NP IgG (delta: 0.82, p < 0.001) (Figures 1B-1E). However, several participants were discordant for antibodies across the three antigens. Two participants had detectable NP antibodies despite undetectable IgG binding to S trimer, whereas one participant lacked measurable binding to NP despite anti-S titers >1:500. A single participant did not seroconvert against any antigen. At the early time point, NP titers were higher than anti-trimer titers (delta: 0.34, p = 0.02) or anti-RBD titers (delta: 0.43, p = 0.004); anti-trimer and anti-RBD titers were not different from each other. At all other time points, titers against the three antigens were similar.

In longitudinal models, fever was associated with higher anti-S trimer IgG (delta: 0.46, p < 0.001), anti-RBD IgG (delta: 0.48, p = 0.002), and anti-NP IgG (delta: 0.82, p < 0.001) (Figures 1B-1E). Among those with fever, the duration of fever was positively associated with the level of anti-trimer IgG (delta per day fever: 0.15, p < 0.001) and anti-NP IgG (delta per day fever: 0.07, p = 0.001), but not anti-RBD IgG. Although there was no association with presence of cough and antibody levels, among those who reported cough, the duration of cough was associated with the level of anti-S IgG (delta per day cough: 0.009, p = 0.04) and...
anti-RBD IgG (delta per day cough: 0.01, p < 0.001), but not anti-NP IgG. Male gender was associated with higher anti-NP IgG only (delta: 0.34, p = 0.04). Thus, participants who reported more severe symptoms, or longer persistence of symptoms, tended to develop higher levels of IgG-binding antibodies.

We additionally considered the decay rate of antibody response against the three antigens. There was no significant decay of anti-S trimer IgG over time (delta per month: −0.02, p = 0.5); however, anti-RBD antibody showed a trend toward slight decay over time (delta per month: −0.04, p = 0.07), and anti-NP significantly decayed over time (delta per month: −0.15, p < 0.001). Fever did not modify the rate of decay for anti-S trimer antibodies; however, those with fever had a faster rate of decay for anti-RBD antibodies (interaction term: p = 0.05; delta per month with fever: −0.06, p = 0.01, delta per month without fever: 0.01, p = 0.6) and anti-NP antibodies (interaction term: p < 0.001; delta per month in fever: −0.20, p < 0.001; delta per month without fever: −0.05, p = 0.1). There was evidence of effect modification in the rate of anti-S trimer decay by gender, where the levels decayed in males but not females (interaction term: p = 0.07; delta per month in males: −0.15, p = 0.06; delta per month in females: 0.01, p = 0.8), but not the other antigens.

Viral neutralization rapidly decays over time
Neutralizing antibodies are thought to be a critical component of the antiviral antibody response and are a correlate of protection for numerous licensed vaccines. Neutralization is the capacity of antibodies to bind the virus and prevent entry into target cells and are differentiated from non-neutralizing antibodies that bind the viral antigens but do not prevent infection. While the correlates of protective antibody immunity against SARS-CoV-2 are not clearly understood, it is likely that neutralizing antibodies will be a critical component of protective immunity after infection and vaccination. We measured the ability of plasma antibodies to neutralize SARS-CoV-2 in a lentiviral pseudovirus assay, in which mature S is pseudotyped onto a viral backbone with a reporter gene and entry measured in HEK293T/hACE2 cells (summarized in Figure S2).

Figure 1. Comparison of IgG responses to SARS-CoV-2 antigens in recovered participants after COVID-19 with versus without fever
Plasma IgG titers were determined for S, RBD, and NP at all available time points. Lines represent linear regression best-fit lines. Each point represents the average value from 2 replicate assays, each containing 2 technical replicates. Black lines: all participants, blue dots/lines: participants without fever, red dots/lines: participants with fever. Dotted lines: 95% confidence band. (A) Participants with fever developed higher IgG titers against all 3 antigens (anti-S delta: 0.46, p < 0.001, anti-RBD IgG delta: 0.48, p = 0.002, and anti-NP IgG delta: 0.82, p < 0.001). (B) Correlations between anti-S, anti-RBD, and anti-NP titers including data from all time points. NP titers were higher at the early time point, relative to S trimer (delta: 0.34, p = 0.02) or RBD (delta: 0.43, p = 0.004). (C–E) Decay rates for IgG over time by fever status. Anti-S trimer IgG did not decay over time (delta per month: −0.02, p = 0.5). Anti-RBD IgG slowly decayed over time (delta per month: −0.04, p = 0.07), whereas anti-NP IgG significantly decayed over time (delta per month: −0.15, p < 0.001). In addition, those with fever had a faster rate of decay for anti-RBD and anti-NP IgG despite having higher titers at the early time points, such that by 6 months, antibody titers were similar between those with and without fever for all 3 antigens.

There was no evidence of effect modification by age or cough. Despite having higher initial titers, the faster rate of decay of RBD and NP titers in participants with fever led to titers similar to those without fever by 6 months after symptom onset. Overall, anti-S trimer antibodies appear to be durable out to at least 6 months, regardless of clinical features.
Considering the early and 2-month time point from symptom onset, 29 of 33 participants (88%) demonstrated a neutralizing response, which ranged from 1:80 to nearly 1:3,000 with a median NAb 50% inhibitory dose (ID50) titer of 1:370. At the 4- and 6-month time points, 23 of 33 participants (70%) exhibited a neutralizing response, which ranged from 1:50 to 1:1,135, with a median NAb titer of 1:250, whereas 6 participants had lost detectable neutralization. Declines were evident even in individuals with initial high neutralizing capacity. For example, one participant’s NAbs declined from ID50 titers of 1:2,732 to 1:354 over the course of 3 months and another declined from 1:2,087 to 1:64 over the course of 4 months.

In our longitudinal model, fever was associated with higher NAb (delta: 0.36, p = 0.01) (Figure 2A). Unlike anti-S trimer and RBD IgG, NAb activity rapidly declined over time (delta per month: −0.10, p = 0.001) (Figures 2B and 2C), with the exception of a single participant who showed increasing NAb activity over time (Figure 2D). The rate of decay was modified by gender and age where the rate was faster among men (interaction term: p < 0.001; delta per month in men: −0.30, p < 0.001; delta per month in women: −0.05, p = 0.03) and older individuals (interaction term: p = 0.06, delta per month in <40 years: −0.07, p = 0.05; delta per month in ≥40 years: −0.14, p = 0.02). Thus, although the majority of participants developed NAbs in response to infection, neutralization ID50 titers were relatively modest and waned quickly over time, calling into question whether mild COVID-19 elicits durable immunity capable of mediating protection from reinfection.

**Avidity of IgG to S and RBD increases over time**

To understand B cell maturation over time, we measured the relative avidity of IgG responses to S trimer and RBD in a chaotrope-modified ELISA, which provides a measure of the strength of antibody binding and serves as a surrogate for ongoing B cell maturation.31 We assessed avidity throughout the follow-up period, observing a wide range of avidity indices, as low as <0.2 and as high as >0.9, which were similar between S trimer and RBD. In longitudinal models, similar to antibody titers, fever was associated with higher anti-S trimer avidity (delta: 0.09, p = 0.04) (Figure 3A) and a trend toward higher RBD avidity (delta: 0.06, p = 0.1) (Figure 3B). Among those with fever, the duration of fever had a trend toward association with S trimer avidity (delta per day fever: 0.04, p = 0.08), but not RBD avidity. Although there was no overall association with the presence of cough, among those with cough, the duration trended toward association with RBD avidity (delta per day: 0.002, p = 0.07), but not S trimer avidity. Avidity was not associated with age or gender. When assessed over time, we observed a strong association between avidity and day since symptom onset for both S trimer (delta per month: 0.06, p < 0.001) and RBD (delta per month: 0.06, p < 0.001) (Figures 3A and 3B). The avidity of S trimer increased faster among those with cough (interaction term: p = 0.08, with cough delta per month: 0.07, p < 0.001; without cough delta per month: 0.04, p < 0.001), whereas the avidity of RBD increased faster among those without fever (interaction term: p = 0.08; with fever delta per month: 0.05, p < 0.001; without fever delta per month: 0.08, p < 0.001).

We next assessed whether avidity predicted neutralization. When we considered only the early time point, there was a
non-significant positive association between both S trimer (delta = 0.55, p = 0.4) and RBD avidity (delta = 1.24, p = 0.2) and neutralization. In longitudinal models that adjusted for days from symptom onset, S trimer avidity did not predict neutralization (delta: -0.02, p = 1.0) (Figure 3C); however, there was a non-significant positive association between RBD avidity and neutralizing effect (delta: 0.76, p = 0.15) (Figure 3D). These findings imply that although avidity may be a determinant of the neutralizing effect at early time points, increasing the avidity of serum IgG via somatic hypermutation may not lead to increased neutralization, suggesting selection, maturation, and maintenance of predominantly non-neutralizing B cell clones.

**Anti-S trimer IgM and IgA are present in most subjects**

To further explore the apparent paradox between relatively stable anti-S trimer IgG and rapid loss of neutralization, we measured anti-S trimer IgM and IgA in our subjects. Overall, 29 of 34 (85%) subjects were ever positive for IgM, with endpoint titers ranging from 1:23 to 1:2,180. Four of the 5 negative subjects entered the study late (52–82 days from symptom onset), while the single subject who entered early (17 days) was also IgG negative to all of the antigens tested. In longitudinal models, fever was associated with increased levels of IgM (delta: 0.28, p = 0.02) (Figure 4A). IgM decayed over time (delta per month: -0.11, p < 0.001) (Figure 4B), with the rate of decay faster among men (interaction: p = 0.03; men delta per month: -0.21, p < 0.001, women: -0.09, p < 0.001).

Overall, 31 of 34 (91%) subjects were positive for IgA at any time point, with titers ranging from 1:25 to 1:1,283. The three subjects who were negative for IgA were among those who were negative for IgM, and one was also negative for all IgGs. In longitudinal models, fever was associated with higher IgA lower IgA (delta per year: -0.01, p = 0.02), and men had higher IgA levels (delta: 0.36, p = 0.02). IgA had a slow decline over time (delta per month: -0.08, p < 0.001) (Figure 4C), and the rate of decay did not vary significantly by any factor.

**Association between anti-S trimer IgM, IgA, and IgG and neutralization**

To understand whether neutralization was principally driven by anti-S trimer IgM, IgA, or IgG, we assessed the relationship between inverse titer and neutralization in longitudinal models that adjusted for time from symptom onset. There was a strong association between IgM and neutralization (delta per log increase: 0.80, p < 0.001) (Figure 4D), with less significant positive associations for IgA (delta per log increase: 0.38, p = 0.05) (Figure 4E) and IgG (delta per log increase: 0.39, p = 0.004) (Figure 4F). Despite being correlated (IgM and IgA: R² = 0.38, p = 0.002; IgM and IgG: R² = 0.31, p = 0.02; IgA and IgG: R² = 0.20, p = 0.1), when all three antibody titers were included in the model together, they each were independently associated with neutralization, although IgM continued to have the strongest effect (IgM delta per log increase: 0.71, p < 0.001; IgA delta per log increase: 0.28, p < 0.001; IgG delta per log increase: 0.19, p = 0.02). These findings suggest that neutralization may be predominantly driven by anti-S trimer IgM, with more limited contributions from IgA or IgG. This observation may explain the apparent paradox of relatively stable IgG levels but rapid loss of neutralization concomitant with the decay of IgM titers.

**DISCUSSION**

The consequences of naturally acquired SARS-CoV-2 immunity remain ill-defined, particularly following mild cases,
representing most infections. A number of recent studies describe the development of antibodies in COVID-19-recovered participants, with a focus on severe or hospitalized cases. Relatively less emphasis has been placed on the evaluation of humoral immunity to mild or asymptomatic disease. There are varying estimates for what proportion of infections involve either few or no symptoms, but recent studies indicate that as many as 50%–90% of infections are asymptomatic or undiagnosed. Thus, defining the features and durability of humoral immunity following mild COVID-19 is of importance, as it may profoundly affect both public health policy and counseling of individuals regarding the risk of reinfection and disease following recovery. Here, we assessed the development, kinetics, and durability of antibody responses to SARS-CoV-2 in the setting of mild COVID-19.

We observed that following mild COVID-19, nearly all of the participants in our cohort developed antibodies and that anti-S trimer and anti-RBD IgG persisted relatively unchanged through at least 6 months. Initially, NP IgG titers were higher in magnitude than anti-S antibodies, but NP antibodies decayed more quickly and reached similar levels to those of S antigens by 6 months of follow-up. Others have reported that the development of humoral immunity is associated with disease severity. The presence of fever was associated with higher IgG titers to S trimer, RBD, and NP, as well as higher IgM and IgA to S trimer, suggesting that even in mild disease, an immune response that causes systemic symptoms may initially result in higher initial antibody response. Notably, despite initially higher titers, the rate of IgG decay was more marked in those with fever, such that antibody titers were similar in magnitude in those with and without fever by 4–6 months post-initial onset of symptoms. We additionally found that some antibody titers were higher in men, which is consistent with a recent report. Thus, regardless of clinical features, IgG levels to S trimer, RBD, and NP appear to reach a stable setpoint that was sustained through at least 6 months. Anti-S trimer IgA had a similar slow rate of decline, whereas IgM more rapidly declined across the follow-up of our study.

While the correlates of protection from SARS-CoV-2 infection are not yet fully established, it is likely that antibody-mediated neutralization of the virus is a key determinant. Here, we found neutralizing antibodies in 88% of participants but that the NAb activity waned quickly in most participants and became undetectable during follow-up in 30% of the cohort. As with binding antibodies, ID50 NAb titers initially were higher in those who reported fever but also decayed at a greater rate than in those without fever. The titer of plasma NAbs required to protect from SARS-CoV-2 infection is not yet established in humans, and thus we cannot predict whether the NAb levels developed after mild infection would be protective. However, the rapid contraction of NAb activity over time raises the specter that even if the initial NAb levels are sufficient to provide protection, they may rapidly decay below the protective threshold and leave
the individual susceptible to infection. Neutralization capacity was most strongly associated with anti-S trimer IgM, although both IgA and IgG appeared to contribute to the total response, which is consistent with a recent report.\textsuperscript{26} This observation offers a compelling explanation for the apparent paradox between stable IgG levels and declining neutralization capacity. Furthermore, these findings are consistent with other recent reports demonstrating an important role for non-IgG immunoglobulins in neutralizing SARS-CoV-2 and have potentially important implications for the design of therapeutics for COVID-19 based on recovered plasma or monoclonal antibodies.\textsuperscript{15,36,37} In addition, the rapid loss of anti-S trimer IgM over time may leave recovered individuals susceptible to reinfection.

There is significant heterogeneity regarding the interpretation of SARS-CoV-2 antibodies detected in recovered individuals among recent studies. Multiple studies reported significant declines in humoral immunity over the first 3–5 months post-infection.\textsuperscript{9,10,33,38–40} Other studies concluded that humoral immunity, including neutralization, is stable out to 7 months.\textsuperscript{12,26,41} Our findings that IgG responses to S reach a durable setpoint and persist to at least 6 months agree with the latter studies, as does our finding that IgG avidity increases over time. Similarly, the role for IgM in neutralization and its association with declines in neutralization agree with a recent report.\textsuperscript{27} Despite the contraction in neutralizing activity seen across our cohort as a whole, NAb titers were detected in 70% of participants out to 6 months, similar to recent reports that concluded that NAbs are durable over time.\textsuperscript{12,28,42} However, as we observed here, these studies also reported marked decreases in neutralization potency over time.\textsuperscript{12,26} Thus, the key issue is not whether NAbs persist, but whether the levels that are maintained are sufficient to protect from reinfection and provide sterile immunity or diminish secondary disease. Our findings indicate that after recovery from mild COVID-19, NAbs wane quickly, and that this contraction may permit SARS-CoV-2 reinfection. However, other arms of adaptive immunity likely contribute to the protection and modulation of disease severity, and thus the effect of waning humoral immunity is likely to be nuanced.

This study provides a detailed assessment of the kinetics of humoral immunity after mild COVID-19 infection. While it is encouraging that IgG responses appear to be durable for at least 6 months, our findings suggest caution in interpreting this durability as immunity from reinfection, as other IgGs appear to have a significant role in neutralizing the virus. Rather, infection-induced humoral immunity may only provide a limited duration of protection, particularly for those with asymptomatic or mild disease.

Limitations of study

The serological readouts described in this study were generated with \textit{in vitro} assays, including the binding and neutralization assays. Binding was measured against several viral antigens that were produced in HEK293 cells and purified with affinity tags, and therefore may have subtle differences from native antigens that could affect binding. The S trimers also have several modifications that differentiate it from the native S, including deletion of the protease cleavage site, stabilizing proline mutations, and an exogenous T4 fibrin trimerization motif. Thus, the antibody recognition of native viral antigens may have subtle differences from what we observe here. Similarly, viral neutralization was measured not against native SARS-CoV-2 virus, but in a pseudovirus system that places the native S protein on the surface of an HIV-1 virion lacking its endogenous envelope and measures single round viral entry. Viral infection is measured in HEK293 cells engineered to overexpress human ACE2. Thus, the neutralization assay, while standard among studies, represents an artificial surrogate that may have differences in activity compared to native SARS-CoV-2 virus and target cells. The assays described above are used uniformly across the field for diagnostic and research purposes, and thus do not represent difficulty for cross-study comparisons. However, the direct link between the surrogate neutralization assays to native viral neutralization and \textit{in vivo} protection is not yet well established, and caution is urged on interpreting the potential effect of the rapid decline in neutralizing antibodies seen here.

Finally, the cohort described here has limitations worth noting. The cohort biases toward females, which make up 77% of the cohort. The gender-specific responses reported here and previously (described above), while statistically supported, should be interpreted with caution. Furthermore, the cohort did not have uniform sampling over time and the intervals vary among subjects, due to differences in intake from time since infection and the fact that follow-up sampling on regular intervals was not done. Thus, the temporal analysis presented here could have failed to identify important changes in serological responses that occurred in the intervening gaps between sampling events.

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2021.100253.
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AUTHOR CONTRIBUTIONS

Conceptualization, W.E.H. and D.N.S; methodology, W.E.H., L.F., O.T., N.D., and D.N.S; investigation, O.T., N.D., D.V.A., A.R., J.H., W.S., A.Y., V.V., W.Y., M.H., J.W., A.O., S.H., S.W.A.S., and A.G.; resources, W.V.V.; writing – original draft, W.E.H. and D.N.S.; writing – review & editing, W.E.H. and D.N.S.; supervision, W.E.H., L.F., and D.N.S.; funding acquisition, W.E.H., L.F., and D.N.S.

DECLARATION OF INTERESTS

The authors declare no competing interests.
26. Wajnberg, A., Amanat, F., Firpo, A., Altman, D.R., Bailey, M.J., Mansour, M., McMahon, M., Meade, P., Mendu, D.R., Muellers, K., et al. (2020). Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science 370, 1227–1230.

27. Prévost, J., Gasser, R., Beaudoin-Bussières, G., Richard, J., Duerr, R., Laumaea, A., Anand, S.P., Goyette, G., Benlarbi, M., Ding, S., et al. (2020). Cross-Sectional Evaluation of Humoral Responses against SARS-CoV-2 Spike. Cell Rep. Med. 1, 100126.

28. Gaebler, C., Wang, Z., Lorenzi, J.C.C., Muecksch, F., Finkin, S., Tokuyama, M., Cho, A., Jankovic, M., Schaefer-Babajew, D., Oliveira, T.Y., et al. (2021). Evolution of antibody immunity to SARS-CoV-2. Nature 591, 639–644.

29. Rodda, L.B., Netland, J., Shehata, L., Pruner, K.B., Morawski, P.A., Thouvenel, C.D., Takehara, K.K., Eggenberger, J., Herrmann, E.A., Waterman, H.R., et al. (2021). Major role of IgM in the neutralizing activity of convalescent plasma against SARS-CoV-2. Cell Rep. 34, 108790.

30. Liu, A., Wang, W., Zhao, X., Zhou, X., Yang, D., Lu, M., and Lv, Y. (2020). Disappearance of antibodies to SARS-CoV-2 in a -COVID-19 patient after recovery. Clin. Microbiol. Infect. 26, 1703–1709.

31. Wang, J.-Y., Song, W.-T., Li, Y., Chen, W.-J., Yang, D., Zhong, G.-C., Liu, A., Wang, W., Zhao, X., Zhou, X., Yang, D., Lu, M., and Lv, Y. (2020). Mucosal versus systemic antibody responses to SARS-CoV-2 antigens in COVID-19 patients. medRxiv. https://www.medrxiv.org/content/10.1101/2020.08.01.20166553v2.

32. Chen, Y., Tong, X., Li, Y., Gu, B., Yan, J., Liu, Y., Shen, H., Huang, R., and Wu, C. (2020). A comprehensive, longitudinal analysis of humoral responses specific to four recombinant antigens of SARS-CoV-2 in severe and non-severe COVID-19 patients. PLoS Pathog. 16, e1008796.

33. Pollan, M., Pérez-Gómez, B., Pastor-Barruso, R., Oteo, J., Hernán, M.A., Pérez-Olmeda, M., Sanmartín, J.L., Fernández-García, A., Cruz, I., Fernández de Larrea, N., et al.; ENE-COVID Study Group (2020). Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet 396, 535–544.

34. Garcia-Beltran, W.F., Lam, E.C., Astudillo, M.G., Yang, D., Miller, T.E., Feldman, J., Hauser, B.M., Caradonna, T.M., Clayton, K.L., Nitico, A.D., et al. (2021). COVID-19 neutralizing antibodies predict disease severity and survival. Cell 184, 476–488.e11.

35. Klingler, J., Weiss, S., Itri, V., Liu, X., Oguntuyo, K.Y., Stevens, C., Ikegame, S., Hung, C.T., Enyindah-Asonye, G., Amanat, F., et al. (2021). Role of IgM and IgA Antibodies in the Neutralization of Severe Acute Respiratory Syndrome Coronavirus 2. J. Infect. Dis. 223, 957–970.

36. Sterlin, D., Mathian, A., Miyara, M., Mohr, A., Anna, F., Claire, L., Quentric, P., Fadlallah, J., Devilliers, H., Ghiliani, P., et al. (2021). IgA dominates the early neutralizing antibody response to SARS-CoV-2. Sci. Transl. Med. 13, eabd2223.

37. Fisher, B.S., Dambrauskas, N., Trakhimets, O., Andrade, D.V., Smedley, J., Sodora, D.L., and Sather, D.N. (2020). Oral Immunization with HIV-1 Envelope SOSIP trimers elicits systemic immune responses and cross-reactive anti-V1V2 antibodies in non-human primates. PLoS ONE 15, e0233577.

38. Liu, A., Wang, W., Zhao, X., Zhou, X., Yang, D., Lu, M., and Lv, Y. (2020). Disappearance of antibodies to SARS-CoV-2 in a -COVID-19 patient after recovery. Clin. Microbiol. Infect. 26, 1703–1709.

39. Beaudoin-Bussières, G., Laumaea, A., Anand, S.P., Prévost, J., Gasser, R., Goyette, G., Medjahed, H., Perreault, J., Tremblay, T., Lewin, A., and et al. (2020). Decline of Humoral Responses against SARS-CoV-2 Spike in Convalescent Individuals. MBio 11, e02590-20.
# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**
  Pacific Blue anti-human CD3 (SP34-2) | BD Biosciences | Cat# 558124; RRID: AB_397044 |
  APC anti-human CD19 (HIB19) | BioLegend | Cat# 982406; RRID: AB_2650645 |
  BV570 anti-human CD20 (2H7) | BioLegend | Cat# 302332; RRID: AB_2563805 |
  BV711 anti-human CD21 (B-ly4) | BD Biosciences | Cat# 563163; RRID: AB_2738040 |
  BV785 anti-human CD27 (O323) | BioLegend | Cat# 302832; RRID: AB_2562674 |
  PE-Cy7 anti-human CD38 (HIT2) | BD Biosciences | Cat# 560677; RRID: AB_1727473 |
  BV605 anti-human IgM (G20-127) | BD Biosciences | Cat# 562977; RRID: AB_2737928 |
  Alexa Fluor 488 anti-human IgD (IA6-2) | BioLegend | Cat# 348216; RRID: AB_11150595 |
  SARS-CoV-2 (2019-nCoV) Spike S1 Antibody | Sino Biological | Cat# 40150-R007 RRID: AB_2827979 |
| **Recombinant proteins**
  Recombinant SARS-CoV-2 spike trimer protein | This study | N/A |
  Recombinant SARS-CoV-2 NtNP protein | This study | N/A |
  Recombinant SARS-CoV-2 spike RBD domain protein | This study | N/A |
| **Biological samples**
  Human plasma from SARS-COV-2 infected donors | This study | N/A |
  Peripheral blood mononuclear cells (PBMCs) from SARS-COV-2 infected donors or uninfected donors | This study | N/A |
| **Chemicals, peptides, and recombinant proteins**
  GE Healthcare Ficoll-Paque PLUS Media | Fisher Scientific | Cat# 45001-749 |
  Human BD Fc block | BD Biosciences | Cat# 564220; RRID: AB_2869554 |
  PEI MAX | Polysciences | Cat# 24765-100 |
  Phosphate buffered saline (PBS) | VWR | Cat# 45000-446 |
  Bovine Serum Albumin (BSA) | VWR | Cat# 97061-420 |
  Fetal bovine serum (FBS) | Gemini Bio | Cat# 900-208 |
  Advanced RPMI 1640 Medium | ThermoFisher | Cat# 97061-420 |
  Dimethyl sulfoxide | VWR | Cat# 80058-040 |
  FreeStyle 293 Expression Medium | ThermoFisher | Cat# 12338018 |
  HisPur Ni-NTA Resin | ThermoFisher | Cat# A32557 |
  Tween® 20 (Polyorbate) | VWR | Cat# 97063-872 |
  TMB Peroxidase Substrate | SeraCare Life Sciences Inc | Cat# 5120-0083 |
  Steady Glo | Promega | Cat# E2920 |
  DMEM [+ 4.5 g/L glucose, L-glutamine, sodium pyruvate | VWR | Cat# 45000-306 |
  Penicillin-Streptomycin | VWR | Cat# 45000-652 |
| **Critical commercial assays**
  Live/dead Violet Fixable Dead Stain | ThermoFisher | Cat#L34955 |
  Streptavidin APC Fire 750 | BioLegend | Cat# 405250 |
  Streptavidin-PercpCy5.5 | BioLegend | Cat# 405214 |

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, D. Noah Sather (noah.sather@seattlechildrens.org)

Materials availability
The plasmid for SARS-CoV-2 spike RBD domain can be requested from the lead contact.

Data and code availability
All data are included in the manuscript. This study did not generate new or custom code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement
The Human Subjects Protocols for this study were approved by the Seattle Children’s Institutional Review Board and written informed consent was obtained from all participants. Adults with COVID-19 from the Seattle Children’s workforce and community members, diagnosed by detection of SARS-CoV-2 nucleic acids in nasal specimens were offered enrollment into the Seattle Children’s SARS2 Recovered Cohort. Additionally, our workforce and their families were offered enrollment into the Seattle Children’s SARS2 Prospective Cohort, an observational study of new onset of SARS-CoV-2 infection detected by weekly self-collected nasal specimens for testing by PCR. A summary of demographic parameters and timing of clinical samples collection are included in Table 1.

HEK293 hACE2 cells
HEK293 hACE2 expressing cells were obtained from Dr. Jesse Bloom, the use of which is described elsewhere. These cells are human embryonic kidney cells that have been engineered to overexpress human ACE2 on the surface, which serves as the receptor for SARS-CoV-2 viral entry. The cells are grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 I.U./ml Penicillin, 100 μg/ml Streptomycin, and 4 mM L-glutamine at 37C and 5% CO2.
HEK293T-17 cells
HEK293T-17 cells were obtained from ATCC. The cells are human embryonic kidney cells that are transfected with viral plasmids to produce pseudovirus. The cells are grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 I.U./ml Penicillin, 100 µg/ml Streptomycin, and 4 mM L-glutamine at 37°C and 5% CO2.

Freestyle 293F cells
Freestyle 293F cells are a HEK293 cell line adapted for serum free suspension culture and were obtained from ThermoFisher. The cells are used for transient transfection of DNA to produce spike proteins. The cells are grown in Freestyle 293 Expression Medium at 37°C and 5% CO2 with shaking.

METHOD DETAILS

Collection of samples from human cohort
Longitudinal blood samples were obtained beginning at a minimum of 14 days from symptom onset and at 2 months, 4 months, and 6 months following SARS-CoV-2 infection. The early time point was defined as < 6 weeks from symptom onset; the 2 month time point was defined as 6-11.9 weeks from symptom onset; the 4 month time point was defined as 12-19.9 weeks from symptom onset, and the 6 month time point was defined as 20-27.9 weeks from symptom onset.

A case report form was completed for each participant that documented the day of symptom onset, day of PCR positive test, and presence and duration of the following symptoms: fever (temperature > 100.4 F), sore throat, sneezing, cough, vomiting, diarrhea, loss of smell/taste, headache, muscle/body aches, shortness of breath, chest tightness, and fatigue/tiredness, as well as a free response for other symptoms.

Blood/plasma processing
Whole blood tubes were centrifuged at 400 g for 10 minutes. Plasma was harvested and aliquoted into 2ml microcentrifuge tubes and stored at −80°C. PBS was added into blood tube to bring blood to its original volume. PBMCs were isolated using standard Ficoll gradient centrifugation. Briefly, 15ml of Ficoll-Paque gradient was pipetted into 50 ml centrifuge tubes. Diluted blood was carefully layered over the Ficoll-Paque gradient (25 to 35 ml/tube). The tubes were centrifuged for 20 min at 800-1000 g. The cell interface layer was harvested carefully, and the cells were washed twice in PBS (10 min at 400 g) and resuspended in complete RPMI 1640 medium before counting. Cells were cryopreserved in freezing medium (10% DMSO, 90% fetal bovine serum) and stored in liquid nitrogen (5×106 or 10×106 per vial).

Protein antigen production
The SARS-CoV-2 trimer construct was generously provided by Dr. Jason McLellan (University of Texas at Austin) and Dr. Barney Graham (National Institutes of Health). Codon-optimized sequence encoding full-length SARS-CoV-2 spike protein was obtained from Sino Biological (cat. no. VG40589-UT) and used to generate the RBD-encoding construct in the pcDNA3.4 backbone (Thermo Fisher Scientific, Waltham, MA, USA). The N-terminal portion of the Nucleoprotein (UniProt:NCAP_SARS2) contains encoding amino acids 47–173 and was produced in E. coli by NiNTA affinity purification, followed by SEC on a Superdex 75 16/600. Recombinant N-Nt was a generous gift from Wes Van Voorhis, Lynn Barrett, Roger Shek, Justin Craig, Logan Tillery, Julie Early, and Peter Myler of Seattle Structural Genomics Center of Infectious Diseases (SSGCID.org), National Institute of Allergy and Infectious Diseases contract numbers HHSN272201700059C. Samples of N-Nt protein have been deposited in the BEI Resources repository and can be acquired from https://www.beiresources.org/.

Enzyme linked immunosorbent assay
Plasma IgG titers to SARS-CoV-2 trimer, RBD, and NP were determined using direct immobilization ELISA. Plasma was heat-inactivated for 1 hour at 56°C prior to the assay and centrifuged at 17,000 x g for 10 min. Immulon 2HB 96-well plates (Thermo Scientific, 3455) were coated with fifty nanograms per well of SARS-CoV-2 trimer, RBD, or NP in 0.1M NaHCO3, pH 9.5 for 1 hour at 37°C. Plates were washed between each ELISA step with PBS containing 0.2% Tween-20. Coated plates were blocked with PBS, 10% non-fat milk, and 0.3% Tween-20. Following blocking, plasma samples were serially diluted over a range of 1:50 to 1:36,450 in PBS, 10% non-fat milk, 0.03% Tween-20 and incubated for 1 hour at 37°C. Bound antibodies were detected using goat anti-human IgG Fc-
Neutralizing antibody activity against SARS-CoV-2 pseudovirus was measured using 293T/hACE2 cells. 293T/hACE2 cells were plated at a density of 1 × 10^4 per well of a 96-well plate (Falcon, 353075) 24 hours prior to the assay, and polybrene reagent was added to the cells at 2 μg/ml thirty minutes prior to inoculation with pseudovirus. Heat-inactivated plasma was serially diluted over a range of 1:50 to 1:109,350 and pre-incubated with pseudovirus for 60 minutes at 37°C. The following morning, media containing the transfection mixture was replaced with fresh cell media and the cells were moved to a 32°C, 5% CO2 incubator overnight. Following the incubation, polybrene-containing medium was aspirated from the 293T/hACE2 cells and the virus+plasma mixture was added to the cells. After 65 hours at 37°C, 100 μl of Steady Glo reagent (Promega, E2520) was mixed into each well. Cells were allowed to lyse for 5 minutes at room temperature in the dark and luciferase activity was measured as RLU using a Fluoroskan Ascent FL luminometer. Percent neutralization was calculated using the following formula: 1 - (RLU plasma/RLU_{psv}) x 100.

Pseudovirus production and validation
Pseudovirus plasmids and 293T/hACE2 cells were generously provided by Dr. Jesse Bloom, Fred Hutchinson Cancer Research Center. Pseudovirus was produced using 293T-17 cells (ATCC), as described previously with minor modifications. 293T cells were seeded in a T225 flask in DMEM (Corning, 10-013-CM) supplemented with FBS, L-glutamine, Penicillin, Streptomycin. At 24 hours post-seeding, cells were transfected with the pseudovirus plasmid mix and PEI, and incubated in a 37°C, 5% CO2 incubator overnight. The following morning, media containing the transfection mixture was replaced with fresh cell media and the cells were moved to a 32°C, 5% CO2 incubator. Media containing pseudovirus was harvested at 72 hours post-transfection, centrifuged at 300 x g, and stored in aliquots at –80°C for future use. Pseudovirus was titrated over 293T/hACE2 cells to determine a dilution of pseudovirus yielding approximately 1000-fold relative light units (RLU) over cell background RLU, and this dilution was used for all future assays.

Neutralization assay
Neutralizing antibody activity against SARS-CoV-2 pseudovirus was measured using 293T/hACE2 cells. 293T/hACE2 cells were plated at a density of 1 × 10^4 per well of a 96-well plate (Falcon, 353075) 24 hours prior to the assay, and polybrene reagent was added to the cells at 2 μg/ml thirty minutes prior to inoculation with pseudovirus. Heat-inactivated plasma was serially diluted over a range of 1:50 to 1:109,350 and pre-incubated with pseudovirus for 60 minutes at 37°C. Following the incubation, polybrene-containing medium was aspirated from the 293T/hACE2 cells and the virus+plasma mixture was added to the cells. After 65 hours at 37°C, 100 μl of Steady Glo reagent (Promega, E2520) was mixed into each well. Cells were allowed to lyse for 5 minutes at room temperature in the dark and luciferase activity was measured as RLU using a Fluoroskan Ascent FL luminometer. Percent neutralization was calculated using the following formula: 1 - (RLU_{plasma}/RLU_{psv}) x 100.

Avidity measurements by chaotrope-modified ELISA
Plasma IgG avidity to SARS-CoV-2 trimer and RBD were measured using the direct ELISA format described for determining plasma IgG titers, with the following exceptions. Plasma samples were titrated from a range of 1:50 to 1:36,450 in quadruplicate wells, as opposed to duplicate. Following a 1 hour incubation at 37°C, half of the sample wells were treated with PBS and the other half with 1M NH₄SCN, for 20 minutes at room temperature. Detection was then performed with goat anti-human IgG Fc-HRP as described above. Avidity was determined by dividing the area under the curve (AUC) of the NH₄SCN-treated samples by the AUC of the untreated (PBS) samples.

Quantification and Statistical Analysis
In order to assess the impact of demographic factors on clinical symptoms, we built multivariate logistic models that included age and gender to predict either fever or cough. We next developed multivariate linear regression models that included age and gender to predict number of days of fever or cough restricted to those individuals among whom the symptom was present.

For serologic evaluation, inverse antibody titers and ID50s were log transformed and then modeled as linear variables. S trimer and RBD avidity were modeled as linear variables. All samples were considered by absolute number of days from symptom onset rather than time blocks. Linear regression was used to model serologic outcomes at a single time point (e.g., the early time point), whereas generalized estimating equations models with robust standard errors were used for longitudinal analysis including rates of decay. Models were adjusted for days since symptom onset, age, gender, fever, and cough. Secondary analysis considered effect of number of days of fever or cough among those who reported each symptom. Effect modification in rate of decay was evaluated by generating an interaction term between time from symptom onset and gender, age, fever, or cough. Participants who had no detectable total antibody titer against S trimer or RBD antigen were excluded from avidity analysis of the respective antigen. Participants who had neither anti S trimer or RBD total antibody were excluded from ID50 analysis; the one participant responded to RBD but not S was included in the ID50 analysis. For those who had detectable anti-S trimer or anti-RBD antibody but did not have a measurable ID50 (i.e., the lowest dilution of 1:50 yielded a neutralizing effect < 50%) the ID50 was set to 1:49. Linear regression was used to assess the relationship between anti-S trimer IgM, IgG, and IgA and ID50, with adjustment for days from symptom onset first individually, then modeled together. Individuals who were negative for either IgM or IgA were included in the model with an antibody titer of 1:19.