Twelve-Month Follow-up of Early COVID-19 Cases in the United States: Cellular and Humoral Immune Longevity

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We quantify antibody and memory B-cell responses to severe acute respiratory syndrome coronavirus 2 at 6 and 12 months postinfection among 7 unvaccinated US coronavirus disease 2019 cases. All had detectable S-specific memory B cells and immunoglobulin G at both time points, with geometric mean titers of 117.2 BAU/mL and 84.0 BAU/mL at 6 and 12 months, respectively.

Keywords. COVID-19; immunity; memory B cells; SARS-CoV-2 infection.

The longevity of cellular and humoral immune responses after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a critical element informing the trajectory of the coronavirus disease 2019 (COVID-19) global pandemic. SARS-CoV-2 seroconversion occurs in >90% of individuals after infection [1], with neutralizing antibodies and spike (S)-specific immunoglobulin G (IgG) antibodies present up to 10 months after infection [2]. SARS-CoV-2 memory B-cell responses have been reported up to 8 months after infection [3], and protection from reinfection is high in the first 6–9 months after infection [4, 5].

We characterize antibody and memory B-cell responses to SARS-CoV-2 infection at 12 months postonset among early US COVID-19 cases [6, 7]. Additionally, we describe immunologic trends in an individual with possible reinfection 10.5 months after initial infection. As one of the earliest detailed descriptions

of SARS-CoV-2-specific immune responses at 12 months, this investigation provides further insight into the natural history of SARS-CoV-2.

METHODS

Among the first 14 detected COVID-19 cases in the United States who previously underwent specimen collection and interviews during acute illness or at 6 months post–illness onset [6, 7], 7 were included in this follow-up investigation at 12 months (±2 weeks). These 7 participants underwent a semistructured interview about their health, health care use, COVID-19 tests, possible exposures, and vaccination status since the investigation 6 months prior [7]. Blood was collected for antibody and memory B-cell assessment.

Plasma specimens from the 12-month investigation were assessed for neutralizing antibodies and S-specific IgG, immunoglobulin M (IgM), and immunoglobulin A (IgA) by in-house enzyme-linked immunosorbent assays (ELISAs), as previously described [6, 7]. Plasma specimens from all available acute and convalescent time points were also analyzed at 1:100 and 1:5000 dilutions for IgG and IgM to SARS-CoV-2 nucleocapsid (N), receptor binding domain (RBD), and S protein (V-PLEX SARS-CoV-2 Panel 2 Kit, Meso Scale Discovery [MSD]). Peripheral blood mononuclear cells (PBMCs) collected at the 6- and 12-month time points were used to assess S-specific IgG and IgA memory B-cell responses. PBMC stimulation was performed as previously described [8, 9]. One residual SARS-CoV-2 RNA-positive nasal specimen that had been collected at a community testing site was submitted to the Centers for Disease Control and Prevention (CDC) for confirmatory virologic testing. Reverse transcription polymerase chain reaction (RT-PCR), virus culture, and whole-genome sequencing were performed as previously described [6]. Additional laboratory methods are available in the Supplementary Data.

RESULTS

Interviews were conducted, and blood was collected from 7 early US COVID-19 cases at 12 months postonset during January 2021. As reported during acute illness [6], 2 (B, G) reported underlying comorbidities and 2 (B, F) required supplemental oxygen without mechanical ventilation (Supplementary Table 1). At the time of the 12-month investigation, none of the 7 participants had received a COVID-19 vaccination, and none reported close contact with a known COVID-19 case since their initial illness.

One participant (K) reported a positive SARS-CoV-2 test in December 2020, ~10.5 months after onset of initial illness. As

previously reported [6], the initial illness in January 2020 consisted of 9 days of fever and cough without supplemental oxygen; SARS-CoV-2 RNA was detected in the upper respiratory tract up to day 19 postonset. SARS-CoV-2 virus culture and sequencing were successful from the earliest collected nasopharyngeal specimen (GENBANK MT039887). In December 2020, participant K underwent local SARS-CoV-2 testing because of a mild sore throat for 1 day and perceived recent exposure (though no confirmed COVID-19 contact). The SARS-CoV-2-positive nasal swab was RT-PCR-positive at the CDC, with a cycle threshold of 29.7; attempted virus isolation and sequencing were unsuccessful. Despite further interview, the most likely exposure event and timing of infection were unclear. There was no indication of transmission to household members (n = 4).

At 12 months postonset compared with 6 months postonset, all 7 participants still had detectable S-specific IgG by the same in-house assay [7], none had detectable S-specific IgA, and 4 (B, F, G, J) still had detectable neutralizing antibodies (Supplementary Figure 1, Supplementary Table 1). Additional antibody data across the time course of infection were collected using a standardized multiplex assessment of S-, RBD-, and N-specific IgG (Figure 1) and IgM (Supplementary Figure 2). All 7 participants had detectable S-specific IgG at 6 and 12 months, with geometric mean titers (GMTs; range) of 117.2 (16.7–253.9) BAU/mL and 84.0 (16.6–187.0) BAU/mL, respectively, reflecting a 28% decrease. RBD-GMTs (range) were 93.1 (24.6–291.1) BAU/mL and 62.3 (14.8–183.2) BAU/mL at 6 and 12 months, respectively, representing a 33% decrease. N-specific IgG was detectable in only 4/7 participants at 12 months (B, J, K, N), among whom the N-GMT (range) decreased 63% from 114.6 (59.0–276.9) BAU/mL at 6 months to 42.5 (20.4–80.9) BAU/mL at 12 months.

Participant K, who was reinfected 1 month before the 12-month investigation, had an S-specific IgG titer of 42.7 BAU/mL. At the 6-month investigation, participant K had an S-specific IgG titer of 55.8 BAU/mL, which was low compared with most other participants (n = 6; GMT = 132.7 BAU/mL). Participant K had undetectable S-specific IgA at 6 months and 12 months postonset by in-house IgA ELISA. During Participant K's acute illness, neutralizing antibodies were detectable but low [6] and were undetectable at 6 months [7] and 12 months (Supplementary Figure 2).

All participants had evidence of S-specific IgG memory B cells at 6 and 12 months (Figure 2). Participant K had the lowest IgG B-cell responses of all participants, with no indication of IgG-specific boosting at 12 months (1 month after reinfection). Notably, participant K did not have detectable S-specific IgA memory B cells at 6 months but did have a low response at 12 months. All participants except N had detectable S-specific IgA memory B-cell responses at 12 months.

DISCUSSION

We characterize antibody and memory B-cell responses among the earliest reported cases of SARS-CoV-2 infection in the United States. All participants had detectable S-specific IgG and S-specific memory B cells at 12 months postonset. Additionally, we report a possible SARS-CoV-2 reinfection 10.5 months after initial infection in an immunocompetent individual with documentation of low humoral and cellular immunity at 6 months after initial infection.

SARS-CoV-2-specific antibodies have been detected up to 10 months after infection [2]. In our study, all 7 participants retained detectable S-specific IgG in the blood at 12 months postonset, and only 4/7 participants had detectable N-specific IgG. N-specific waning was greater than S-specific waning, as previously reported [10], though lack of detection could be related to lower assay sensitivity. As time since infection passes, N-specific assays may become a less sensitive marker of prior infection. S-specific IgG correlates for 80% and 50% vaccine effectiveness (VE) against symptomatic COVID-19 for ChAdOx1 nCoV-19 (AZD1222) [11] and 90% VE against symptomatic
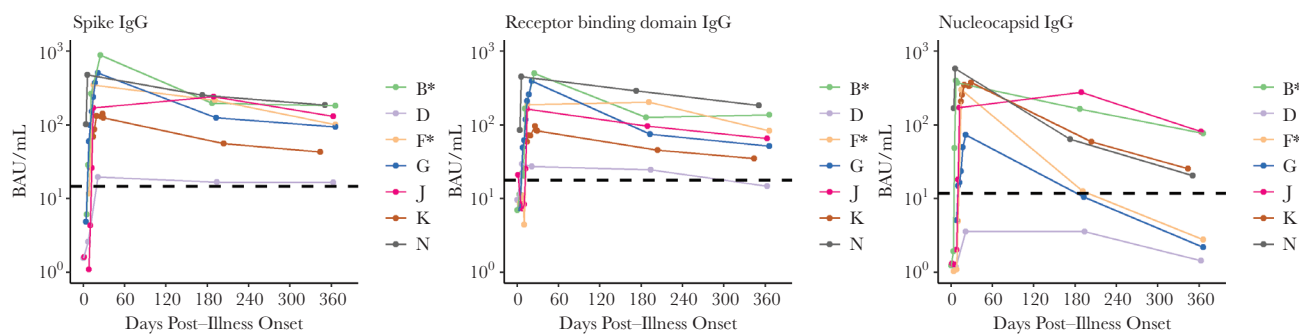

Figure 1. S-, RBD-, and N-specific IgG responses up to 12 months after COVID-19 illness onset among early US COVID-19 cases. Asterisks indicate that the patient received supplemental oxygen during their acute COVID-19 illness. Dashed lines indicate the threshold of detection for each assay. Spike-specific IgG titers from the acute time points (<45 days) and 6-month time points were previously published using a different assay [7]; specimens were retested using the V-PLEX SARS-CoV-2 Panel 2 Kit (Meso Scale Discovery) assay for standardized comparisons in this analysis. Abbreviations: COVID-19, coronavirus disease 2019; IgG, immunoglobulin G; N, nucleocapsid; RBD, receptor binding domain; S, spike.
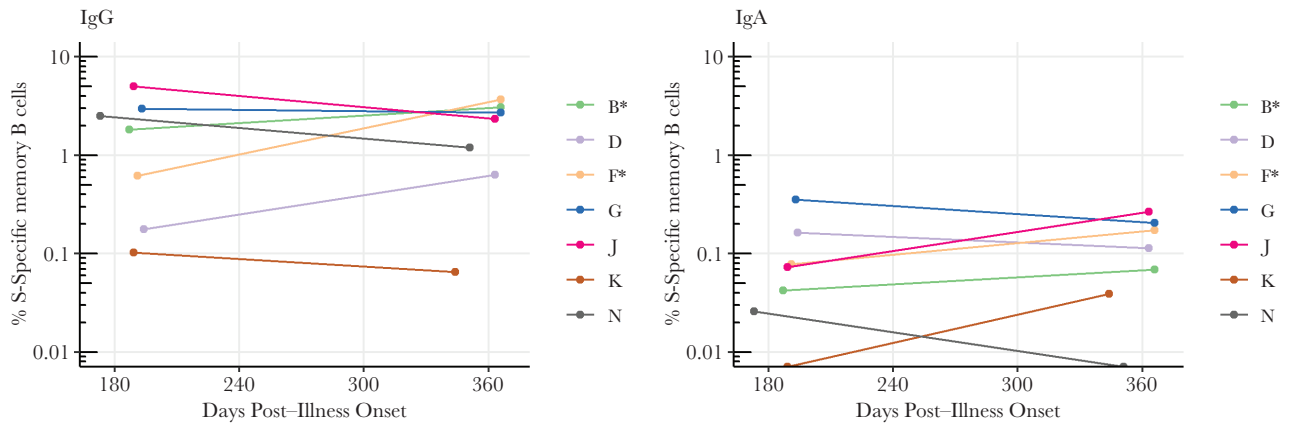
Figure 2. Percentage of S-specific IgG and IgA memory B cells at 6 and 12 months post–illness onset among early US COVID-19 cases. The percentage of S-specific memory B cells among all memory B cells is depicted. Asterisks indicate that the patient received supplemental oxygen during their acute COVID-19 illness. Participant K had no detectable IgA memory B cells at 189 days post–illness onset, and participant N had no detectable IgA memory B cells at 351 days. Abbreviations: COVID-19, coronavirus disease 2019; IgA, immunoglobulin A; IgG, immunoglobulin G; S, spike.

COVID-19 for mRNA-1273 (Moderna) [12, 13] were recently reported. VE against severe disease is likely higher at these antibody levels [14]. Among our 7 participants, none had S-specific IgG at levels above the 80% VE correlate (264 BAU/mL) for ChAdOx1 nCoV-19 or 90% VE correlate (298 BAU/mL) to mRNA-1273 at either 6 or 12 months postonset. However, 6 of 7 participants, including participant K, had levels above the 50% VE correlate (29 BAU/mL) for ChAdOx1 nCoV-19 at 6 and 12 months postonset [11]. The implications of these comparisons for infection-induced protection from reinfection are not yet fully understood.

As residual serum antibodies wane over time, memory B cells become an increasingly critical component of the immune response, facilitating rapid production of new antibodies upon reinfection to attenuate virus replication and possibly illness severity. We detected S-specific IgG memory B cells in all participants and IgA memory B cells in 6 of 7 participants at 12 months postonset, exhibiting similar levels to those reported after influenza virus infection [9]. In previous reports, SARS-CoV-2-specific memory B cells were stable up to 6 months postinfection [3]. Understanding the complexities of B- and T-cell responses to SARS-CoV-2 will be crucial to identifying immunologic correlates of viral clearance and protection against reinfection.

The mild (or possibly asymptomatic) reinfection case (participant K) exhibited low antibody and B-cell responses at 6 months postonset (~4 months before reinfection); specifically, participant K had no detectable neutralizing antibodies, had no detectable S-specific IgA- or IgA-producing memory B cells, and had low levels of S-specific IgG- and IgG-producing memory B cells compared with other participants. At 12 months (~1 month after reinfection), participant K did not have detectable neutralizing antibodies and did not exhibit detectable serum IgG or IgA boosting; however, S-specific

IgA-producing memory B cells, which were not detectable at 6 months, were detectable at 12 months. These findings may reflect a limited mucosal immune response following reinfection or simply the heterogenous nature of infection-acquired immune responses.

Generalizations from this investigation are limited by the small sample size. Infections occurred early in the pandemic, and data reflect immune responses to early SARS-CoV-2 strains. Routine screening for SARS-CoV-2 reinfection among participants was not systematically available, and reinfections could have been missed. Cellular analyses were performed on previously frozen PBMCs, which may have been less sensitive than using freshly isolated PBMCs. Variant-specific immunologic assays were not available in this study. Additionally, measures of T-cell responses and mucosal antibodies were not included but may play an important role in immune memory and protection against SARS-CoV-2 reinfection.

We demonstrate detection of S-specific IgG and memory B cells at 1 year after SARS-CoV-2 infection and describe humoral and cellular responses before and 1 month after a single reinfection case. Additional studies are needed to further characterize the correlation of B and T cells with protection against reinfection and to better assess the duration of protection conferred by infection alone or in conjunction with vaccination.

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