Low Levels of Neutralizing Antibodies After Natural Infection With Severe Acute Respiratory Syndrome Coronavirus 2 in a Community-Based Serological Study

Thomas W. McDade,[1,2] Amelia Sancilio,[1,2] Richard D'Aquila,[4] Brian Mustanski,[3,5] Lauren A. Vaught,[6,7] Nina L. Reiser,[6,7] Matthew E. Velez,[6,7] Ryan R. Hsieh,[6,7] Daniel T. Ryan,[3,5] Rana Saber,[3,5] Elizabeth M. McNally,[6,7,8] and Alexis R. Demonbreun[6,9]

[1]Department of Anthropology, Northwestern University, Evanston, Illinois USA, [2]Institute for Policy Research, Northwestern University, Evanston, Illinois, USA, [3]Institute for Sexual and Gender Minority Health and Wellbeing, Northwestern University, Chicago, Illinois, USA, [4]Division of Infectious Diseases, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, [5]Department of Medical Social Sciences, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, [6]Center for Genetic Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, [7]Division of Cardiology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA, [8]Department of Biochemistry and Molecular Genetics, Northwestern University, Chicago, Illinois, USA, [9]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA

Background. Confidence in natural immunity after infection with severe acute respiratory syndrome coronavirus 2 is one reason for vaccine hesitancy.

Methods. We measured antibody-mediated neutralization of spike protein-ACE2 receptor binding in a large community-based sample of seropositive individuals who differed in severity of infection (N = 790).

Results. A total of 39.8% of infections were asymptomatic, 46.5% were symptomatic with no clinical care, 13.8% were symptomatic with clinical care, and 3.7% required hospitalization. Moderate/high neutralizing activity was present after 41.3% of clinically managed infections, in comparison with 7.9% of symptomatic and 1.9% of asymptomatic infections.

Conclusions. Prior coronavirus disease 2019 infection does not guarantee a high level of antibody-mediated protection against reinfection in the general population.

Keywords. COVID-19; neutralizing antibodies; SARS-CoV-2; serological testing; vaccination.

Vaccine hesitancy in the United States has slowed progress toward the goal of ending the coronavirus disease 2019 (COVID-19) pandemic through the generation of population immunity. There have been more than 62 million cases of COVID-19 [1], and for every confirmed case there are at least 2 more severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, as indicated by antibody seropositivity [2, 3]. Many people assume that natural infection generates protective immunity, and studies in the United States, United Kingdom, and Israel indicate that confidence in, or preference for, natural immunity is associated with vaccine hesitancy [4–6]. Although severe cases of COVID-19 have strained the healthcare system, the majority of infections with SARS-CoV-2 are mild or asymptomatic and do not lead to hospitalization [7]. The extent to which these infections provide protection against reinfection, comparable to the level of protection provided by vaccination, is not clear.

Prior studies investigating the level of immune protection after natural infection have drawn samples that are biased toward (1) more severe cases of COVID-19 requiring clinical management or hospitalization or (2) groups of individuals with higher or repeated occupational exposure to SARS-CoV-2 (eg, healthcare workers). Ascertaining the level of protective immunity after infection in the general population, where the level of exposure and disease severity is substantially lower, is important for evaluating the assumption that natural immunity provides adequate protection against COVID-19 in lieu of vaccination.

The objective of this report is to document the level of protective immunity in a large community-based sample of adults previously infected with SARS-CoV-2. Neutralizing antibodies play an important role in protection because they are long-lasting, and because they can bind to viral proteins and inhibit entry into host cells [8]. For SARS-CoV-2, the surface spike protein engages the human angiotensin-converting enzyme 2 (ACE2) receptor to enter host cells, and antispike neutralizing antibodies can block this interaction and prevent infection [9]. Application of this important marker of protective immunity in community settings has been limited by logistical and technical challenges associated with live virus methods and venous blood collection. We overcome these challenges by combining in-home collection of finger stick dried blood spot (DBS) samples with a surrogate virus neutralization protocol for quantifying antibody-mediated inhibition of spike-ACE2 interaction in DBS [10]. We document low levels of neutralizing activity in individuals previously exposed to SARS-CoV-2, particularly among individuals with asymptomatic and mild infections.

METHODS

Patient Consent Statement

All research activities were implemented under protocols approved by the institutional review board at Northwestern

University (numbers STU00212457 and STU00212472). All participants provided online informed consent.

Participants and Study Design

A community-based sample of participants in the Chicagoland area was recruited through social media, email blasts, print flyers, newspaper advertisements, and local press coverage. Between June 24 and November 11, 2020, N = 4562 adults completed a web-based survey and returned a self-collected finger stick DBS sample in the mail [3]. Participants reported whether they had experienced any of the following symptoms potentially indicative of COVID-19 infection after March 1, 2020: fever or chills; cough; shortness of breath; headache; muscle or body aches; fatigue or excessive sleepiness; diarrhea, nausea, or vomiting; loss of sense of smell or taste. Participants also indicated whether they had sought care from a healthcare provider, which was defined as in-person care in a clinic or doctor's office, emergency room, urgent care facility, or hospital, or remote care over the phone, by email, or online. Participants indicated whether a healthcare provider told you them that they had, or likely had, COVID-19. Access to diagnostic testing to confirm SARS-CoV-2 infection was very limited at the time the study was conducted, and we therefore used this variable to indicate a clinical diagnosis of COVID-19.

A pre-existing condition for severe COVID-19 was defined as the presence of chronic obstructive pulmonary disease, diabetes mellitus, cardiovascular disease, or obesity (body mass index >30 kg/m^2). Smoker was defined by the use of inhaled tobacco products. Participants also indicated sex (based on assignment at birth), primary racial/ethnic identity, and whether they were essential workers in healthcare.

Antireceptor Binding Domain Immunoglobulin G Assay

Seropositivity was determined based on the presence of antibodies against SARS-CoV-2 receptor binding domain (RBD), using an established protocol validated for use with DBS samples that shows high sensitivity and specificity, and high agreement with results from matched serum samples (R = 0.99) [11, 12]. The cutoff for seropositivity was set at the optical density value for the 0.39 µg/mL calibrator [12].

Surrogate Virus Neutralizing Assay

The competitive immunoassay to quantify neutralizing activity (%neutralization) of spike-ACE2 interaction was previously described [10]. The DBS samples were available for 790 of 820 seropositive participants and were eluted overnight along with assay controls in a nonbinding 96-well plate. Eluate was transferred to the assay plate and incubated with SARS-CoV-2 spike protein and human recombinant ACE2 conjugated with an electrochemiluminescent label (K15386U-2; Meso Scale Diagnostics). Neutralizing antibodies, if present, inhibited binding between ACE2 and spike protein, and the Meso Scale Diagnostics QuickPlex SQ 120 Imager was used to read mean

fluorescence intensity (MFI). Percentage neutralization was calculated as follows: %neutralization = $100 \times 1 -$ (sample MFI/negative control MFI). Prior validation studies indicate that results from the surrogate virus neutralization method correlate highly with results from conventional live virus (Pearson R = 0.93) and pseudovirus neutralization assays (R = 0.92) [13].

The surrogate virus neutralization assay was also applied to a sample of seronegative individuals (N = 81) who provided DBS samples 2–3 weeks after receiving the first dose of BNT162b2/Pfizer or mRNA-1273/Moderna vaccines [14]. These results provided the basis for defining low, moderate, and high levels of neutralization. Low neutralization was defined as values above the assay detection limit (13.2%) and below the median response after the first dose (38.3%). Moderate neutralization was defined as values above the median and less than the 75th percentile (73.7%). High neutralization was >73.7%.

RESULTS

A web-based, "no contact" research platform was implemented to recruit a large community-based sample of 4562 adults across the city of Chicago between June 24 and November 11, 2020 [3]. The analyses were focused on the 17.8% of participants that tested seropositive for prior infection based on the presence of IgG antibodies against the RBD of SARS-CoV-2 [3].

The sample includes women and men across race/ethnic identities between the ages of 18 and 81 (Table 1). Only 64 of 790 participants were essential healthcare workers. Comparisons were made across 3 groups, based on self-reported severity of infection and engagement with clinical care. The "clinical" group included individuals who reported experiencing symptoms of infection, who received care from a healthcare provider (phone/online or in a clinical setting), and who received a diagnosis of COVID-19. The "symptomatic" group reported experiencing 1 or more symptoms of COVID-19 but did not seek clinical care. The "asymptomatic" group reported no symptoms and did not seek clinical care. There was no difference in the number of days between symptom report and blood collection across the symptomatic and clinical groups (Table 1). All 3 study groups were comparable in the number of days since March 1, 2020 and the timing of blood collection.

Symptomatic infections, without clinical treatment or diagnosis, were most frequent at 46.5% of all infections. A total of 39.8% of infections were asymptomatic. Only 13.8% of infections were symptomatic with clinical care, and of these only 3.7% required hospitalization (0.5% of all infections). The clinical group reported a median of 5 symptoms of infection, in comparison with 2 for the symptomatic group. Median %neutralization of spike-ACE2 binding was significantly higher in the clinical group than the symptomatic group (29.8 vs 8.0%; Wilcoxon rank-sum z = 8.54, $P < .001$) (Figure 1A). Although the difference was relatively small, neutralization was

Table 1. Descriptive Statistics for the Three Study Groups[a]

Variable	Total	Asymptomatic	Symptomatic	Clinical
N	790	314	367	109
Age, years (SD)	38.6 (13.2)	37.8 (13.0)	38.0 (13.0)	43.0 (13.9)
Female	55.6	51.0	57.8	57.8
Race/ethnicity				
White	44.2	47.8	41.7	42.2
Hispanic/Latinx	21.3	13.4	24.8	32.1
Asian	23.5	29.0	23.2	9.2
Black	8.5	8.0	7.4	13.8
Other	2.5	1.9	3.0	2.8
Healthcare worker	8.1	7.3	9.0	7.3
Pre-existing condition	25.7	20.7	26.7	36.7
Smoker	8	8.0	7.1	11.0
Positive COVID-19 test	3.9	0.0	0.3	27.5
Number of symptoms (IQR)	1 (0–3)	0 (0–0)	2 (1–4)	5 (3–7)
Days between symptoms and blood collection (SD)	--	--	134.0 (61.6)	132.2 (63.5)
%neutralization (IQR)	8.0 (0–17.9)	4.8 (0–13.6)	8.0 (0.4–16.9)	29.8 (12.7–53.7)
Days between March 1, 2020 and blood collection (SD)	195.7 (36.4)	196.0 (35.2)	194.4 (37.3)	199.3 (37.3)
Level of neutralization (%)				
High	2.3	0.0	1.9	10.1
Moderate	7.9	1.9	6.0	31.2
Low	26.1	24.2	25.6	33.0
Undetectable	63.8	73.9	66.5	25.7

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; SD, standard deviation.

[a]The groups differed significantly in mean age (one-way analysis of variance, $F = 7.1$, $P < .001$), race/ethnicity (Pearson $\chi^2 = 37.5$, $P < .001$), presence of a pre-existing condition ($\chi^2 = 11.2$, $P < .01$), positive diagnostic COVID-19 test ($\chi^2 = 186.8$, $P < .001$), median number of symptoms (non-parametric k-sample $\chi^2 = 458.9$, $P < .001$), and median %neutralization (non-parametric k-test $\chi^2 = 68.1$, $P < .001$).

significantly lower in the asymptomatic than the symptomatic group (4.8 vs 8.0%; $z = 3.90$, $P < .001$). In comparison, vaccination results in median neutralization of 38.3% after 1 dose, and 97.9% after 2 doses, of mRNA vaccine.

In the clinical known COVID-19 group, 41.3% of individuals had moderate or high neutralizing activity after infection (Figure 1B). Only 7.9% of symptomatic and 1.9% of asymptomatic cases show evidence of neutralization at this level. Furthermore, neutralizing activity was indistinguishable from zero for 66.5% of symptomatic and 73.9% of asymptomatic individuals, in comparison with 36.7% of the clinical group.

DISCUSSION

As the number of SARS-CoV-2 infections continues to grow in the United States, it is possible that a substantial proportion of the population will decline COVID-19 vaccination based on the assumption that natural immunity provides sufficient immune protection against reinfection. Our findings suggest caution in making this assumption: we document low levels of surrogate virus neutralization after natural infection for the vast majority of adults with prior exposure to SARS-CoV-2.

Our sample is large and community-based, and it is not enriched with healthcare workers or more severe hospitalized cases
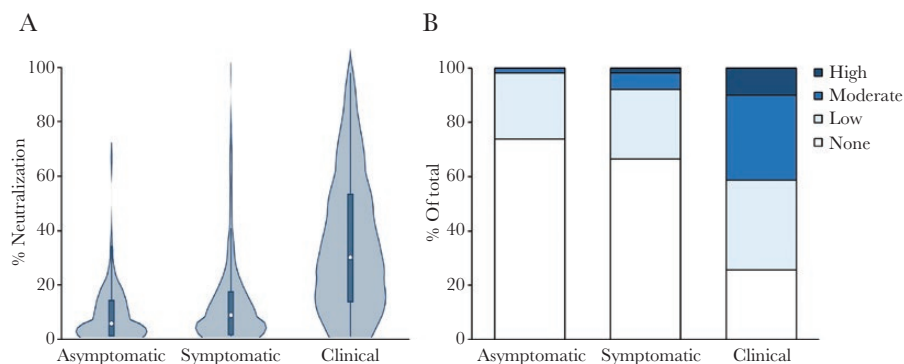
Figure 1. Antibody-mediated neutralization of spike-ACE2 interaction by severity of severe acute respiratory syndrome coronavirus 2 infection. (A) Violin plot shows median %neutralization and interquartile range, with kernel density, for each group. (B) Proportion of cases with low, moderate, and high levels of neutralizing activity.

of COVID-19. As such, it may present a better reflection of the "on the ground" experience of most Americans, the majority of whom will not require inpatient care. Our findings indicate that natural infection results in a level of neutralizing antibody protection that is, at best, comparable to the antibody response after 1 dose of mRNA vaccine. For people with mild or asymptomatic infections, the average response is lower. This pattern of results is consistent with smaller studies showing (1) heterogeneity in antibody levels after natural infection and (2) weaker responses after mild/asymptomatic infections [15–17]. It is also consistent with prior reports of stronger neutralization and reduced risk of infection after vaccination, in comparison with natural infection [18, 19].

Recent studies have reported robust antibody responses to the first dose of currently available mRNA COVID-19 vaccines after natural infection [20–22], supporting the proposition that a second vaccine dose may not be needed for previously infected individuals. However, these studies are relatively small, and the samples exclusively comprised healthcare workers who have a unique profile of SARS-CoV-2 exposure. Our findings caution against overgeneralizing results from studies of healthcare workers, and more severe clinical cases of COVID-19, to the general population when evaluating the immune response to natural infection and vaccination. This point may be particularly important as the omicron variant reaches more unvaccinated people globally and tends to cause milder infections in comparison with prior variants [23].

A limitation of our study is that it does not include measures of cellular immunity, which can reduce the severity of COVID-19 if a breakthrough or reinfection occurs [24]. In addition, although our method for quantifying surrogate neutralization is relatively low cost and high throughput, it is not directly comparable to results from studies using live virus or pseudovirus methods for assessing neutralization. Finally, even though the 3 study groups were relatively balanced, it is possible that factors other than severity of SARS-CoV-2 infection contributed to differences in neutralization.

CONCLUSIONS

In conclusion, natural infection did not generate detectable levels of neutralizing antibodies in 63.8% of exposures in our large, community-based study. Moderate to high levels of neutralizing antibodies were present in only 10.1% of previously infected individuals. These results suggest that prior infection with SARS-CoV-2 does not guarantee a high level of antibody-mediated protection against reinfection. This information may be important for public health messaging to the large and growing proportion of the global population that has been previously infected with SARS-CoV-2 and remains unvaccinated, or only partially vaccinated.

Acknowledgments

References

1. Centers for Disease Control and Prevention. COVID Data Tracker. Available at: https://covid.cdc.gov/covid-data-tracker/#datatracker-home. Accessed 13 January 2022.
2. Jones JM, Stone M, Sulaeman H, et al. Estimated US infection-and vaccine-induced SARS-CoV-2 seroprevalence based on blood donations, July 2020-May 2021. JAMA **2021**; 326:1400–9.
3. Demonbreun AR, McDade TW, Pesce L, et al. Patterns and persistence of SARS-CoV-2 IgG antibodies in Chicago to monitor COVID-19 exposure. JCI Insight **2021**; 6:e146148.
4. Shekhar R, Sheikh AB, Upadhyay S, et al. COVID-19 vaccine acceptance among health care workers in the United States. Vaccines **2021**; 9:119.
5. Paul E, Steptoe A, Fancourt D. Attitudes towards vaccines and intention to vaccinate against COVID-19: implications for public health communications. Lancet Reg Health Eur **2021**; 1:100012.
6. Dror AA, Eisenbach N, Taiber S, et al. Vaccine hesitancy: the next challenge in the fight against COVID-19. Eur J Epidemiol **2020**; 35:775–9.
7. Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection: a narrative review. Ann Intern Med **2020**; 173:362–7.
8. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature **2020**; 590:630–4.
9. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell **2020**; 181:271–80.e8.
10. Sancilio A, D'Aquila R, McNally E, et al. A surrogate virus neutralization test to quantify antibody-mediated inhibition of SARS-CoV-2 in finger stick dried blood spot samples. Sci Rep **2021**; 11:15321.
11. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med **2020**; 26:1033–6.
12. McDade TW, McNally EM, Zelikovich AS, et al. High seroprevalence for SARS-CoV-2 among household members of essential workers detected using a dried blood spot assay. PLoS One **2020**; 15:e0237833.
13. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat Biotechnol **2020**; 38:1073–8.
14. Demonbreun AR, Sancilio A, Velez ME, et al. Comparison of IgG and neutralizing antibody responses after one or two doses of COVID-19 mRNA vaccine in previously infected and uninfected persons. EClinicalMedicine **2021**; 38:101018.
15. Karuna S, Li SS, Grant S, et al. Neutralizing antibody responses over time in demographically and clinically diverse individuals recovered from SARS-CoV-2 infection in the United States and Peru: a cohort study. PLoS Med **2021**; 18:e1003868.
16. De Giorgi V, West KA, Henning AN, et al. Naturally acquired SARS-CoV-2 immunity persists for up to 11 months following infection. J Infect Dis **2021**; 224:1294–304.
17. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature **2021**; 595:426–31.
18. Cavanaugh AM, Spicer KB, Thoroughman D, Glick C, Winter K. Reduced risk of reinfection with SARS-CoV-2 after COVID-19 vaccination—Kentucky, May–June 2021. Morb Mortal Wkly Rep **2021**; 70:1081.
19. Stamatatos L, Czartoski J, Wan Y-H, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science **2021**; 372:1413–8.
20. Saadat S, Tehrani ZR, Logue J, et al. Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. JAMA **2021**; 325:1467–9.
21. Manisty C, Otter AD, Treibel TA, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. Lancet **2021**; 397:1057–8.
22. Krammer F, Srivastava K, Alshammary H, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med **2021**; 384:1372–4.
23. Kupferschmidt K, Vogel G. Omicron threat remains fuzzy as cases explode. Science **2021**; 375:9–10.
24. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals. Cell Rep Med **2021**; 2:100355.