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BRIEF REPORT

Absence of Severe Acute Respiratory Syndrome Coronavirus 2 Neutralizing Activity in Prepandemic Sera From Individuals With Recent Seasonal Coronavirus Infection

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(See the Editorial Commentary by Lee on pages e1212–3.)

Cross-reactive immune responses elicited by seasonal coronaviruses might affect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) susceptibility and disease outcomes. We measured neutralizing activity against SARS-CoV-2 in prepandemic sera from patients with prior polymerase chain reaction scan-confirmed seasonal coronavirus infection. Although neutralizing activity against seasonal coronaviruses was detected in nearly all sera, cross-reactive neutralizing activity against SARS-CoV-2 was undetectable.

Keywords. COVID-19; SARS-CoV-2; seasonal coronavirus; antibodies; neutralization.

Since the initial description in December 2019 of novel human coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), there has been a global effort to identify the underlying causes for the great range of disease severity observed, from mild or even asymptomatic infection to severe respiratory distress and death. One hypothesis is that cross-reactive immune responses elicited by prior infection with seasonal coronaviruses affect the course of SARS-CoV-2 infection, perhaps providing a degree of protection against severe coronavirus disease 2019 (COVID-19) disease.

The endemic seasonal human coronaviruses (HCoVs)—HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E—cause mild or subclinical respiratory infections, with severe disease being exceptionally rare [1]. Although there is low overall sequence homology between the SARS-CoV-2 Spike (S) protein and those of the endemic HCoVs, overlapping T-cell epitopes have been reported, particularly in the S2 subunit [2, 3]. It is possible that neutralizing antibodies induced by seasonal HCoV infection could cross-react with similar epitopes in SARS-CoV-2 S. Such antibodies could potentially afford some level of protection against and perhaps contribute to the wide range of outcomes of SARS-CoV-2 infection. To investigate this possibility, we analyzed sera that had been collected before the COVID-19 pandemic from patients with a recent polymerase chain reaction (PCR)-confirmed diagnosis of HCoV-OC43, HCoV-NL63, or HCoV-229E infection. Such samples should contain neutralizing antibodies against the respective seasonal HCoV, without the possibility of prior SARS-CoV-2 infection, allowing us to specifically test whether antibodies elicited by seasonal HCoV infection can neutralize SARS-CoV-2. Our results indicate a lack of SARS-CoV-2 cross-neutralization activity between the seasonal HCoVs and SARS-CoV-2.

METHODS

Identification of Patient Samples

The 37 prepandemic serum samples selected for inclusion in this study were all collected as part of routine clinical care before 2020 from patients in Edinburgh, Scotland, effectively excluding the possibility of prior SARS-CoV-2 infection. All samples were from symptomatic inpatients with PCR-confirmed diagnosis of HCoV-OC43 (n = 20), HCoV-NL63 (n = 10), or HCoV-229E (n = 7) infection, 11 to 291 days before collection of the serum sample. Ten positive control COVID-19 serum samples were collected in April-May 2020 from patients with mildly symptomatic, PCR-diagnosed SARS-CoV-2 infection, 24 to 61 days before serum collection. All samples were anonymized and ethical approval to use these patient samples was obtained through the National Health Service Lothian BioResource and the Rockefeller University Institutional Review Board.

Viruses

The seasonal coronaviruses HCoV-OC43 (strain: ATCC VR-759) and HCoV-229E (strain: ATCC VR-740) were obtained from Zeptometrix Corporation, and HCoV-NL63 (strain: Amsterdam I) was obtained from the Biodefense and Emerging Infections Research Resources Repository. Live strains of HCoV-HKU1 were not readily available and thus HCoV-HKU1 was not included this study. Viral stocks were generated by propagation on Huh7.5 cells. The replication-competent chimeric recombinant vesicular stomatitis virus encoding SARS-CoV-2 S and green fluorescent protein (GFP), rVSV/SARS-2/GFP$_{2E1}$, has been described previously and was propagated on 293T/ACE2cl.22 cells [4].

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Neutralization Assays

Sera were initially diluted 1:12.5, and then serially diluted 5-fold over 7 dilutions in 96-well plates. Thereafter, approximately 4×10^3 infectious units of either rVSV/SARS-2/GFP, HCoV-OC43, HCoV-NL63, or HCoV-229E were mixed with the serum dilutions and incubated at 37°C for 1 hour. Virus serum mixtures were subsequently transferred to 96-well plates containing 1×10^4 293T/ACE2cl.22 (for rVSV/SARS-2/GFP and HCoV-OC43) or HT1080/ACE2cl.14 (for HCoV-NL63 and HCoV-229E) target cells/well. Infection was allowed to proceed for 16 hours (rVSV/SARS-2) or 24 hours (HCoV-OC43, HCoV-NL63, HCoV-229E). The numbers of rVSV/SARS-2/GFP was assessed by flow cytometric detection of GFP expression as described previously [4]. For HCoV-OC43, HCoV-NL63, and HCoV-229E, cells were trypsinized and immunostained to detect nucleoprotein antigen expression in infected cells. For HCoV-OC43, Sigma MAB9013 was used, for HCoV-NL63, Eurofins M.30.HCo.B2D4 was used, for HCoV-229E: Eurofins M.30.HCo.B1E7 was used. A secondary antibody conjugate Alexa Fluor 488 Goat anti-Mouse IgG (H+L) (Thermo) was then used to and infected cells enumerated by flow cytometry.

Data Analysis

All flow cytometry data were analyzed using FlowJo software, version 10.6.1. All graphs and corresponding neutralizing titer 50 (NT_{50}) values were generated using GraphPad Prism, version 8.

RESULTS

To assess whether prior infection by seasonal coronaviruses could elicit antibodies with neutralization activity against SARS-CoV-2, we developed flow cytometry-based coronavirus neutralization assays based on the detection of nucleocapsid expression in HCoV-OC43-, HCoV-NL63-, or HCoV-229E-infected cells. Using theses neutralization assays, we confirmed that neutralizing antibodies targeting the seasonal coronaviruses were present in prepandemic samples. Indeed, all sera from individuals diagnosed with recent infection by a seasonal coronavirus neutralized that same virus (Figure 1A). Nevertheless, the neutralization titers varied between viruses. For example, although samples collected from HCoV-OC43-infected individuals typically exhibited potent neutralization of HCoV-OC43, sera collected from HCoV-229E-infected individuals had comparatively weak neutralization activity against HCoV-229E (Figure 1A). Most sera exhibited neutralizing activity against multiple seasonal coronaviruses. Indeed, some samples collected individuals with recent HCoV-229E infection neutralized HCoV-OC43 with higher titers than HCoV-229E (Figure 1A). Collectively, 73% of samples had an $NT_{50} > 500$ for HCoV-OC43 and 57% of samples $NT_{50} > 500$ for HCoV-229E, regardless of the virus detected at the time of sample collection. Neutralizing activity against HCoV-NL63 was typically of lower titer. Nevertheless, all but 1 serum sample from individuals with recently diagnosed HCoV-NL63 infection had neutralizing activity against HCoV-NL63 with NT_{50} values of >1:50 (Figure 1A). Overall, this collection of serum samples had extensive neutralizing activity against several seasonal coronaviruses including, particularly, the betacoronavirus HCoV-OC43, that is the most closely related to SARS-CoV-2 of the viruses tested. Indeed, some of the sera had potent neutralizing activity against HCoV-OC43 with NT_{50} values in excess of 10 000.

In contrast, none of the very same 37 serum samples tested had any detectable neutralization activity against rVSV/SARS-CoV-2/GFP (Figure 1B). Importantly, rVSV/SARS-CoV-2/GFP is at least as, or more, sensitive to neutralization by COVID-19 plasma analysis as SARS-CoV-2 [4]. Indeed, sera collected from 10 individuals with recently diagnosed SARS-CoV-2 infection could neutralize rVSV/SARS-CoV-2/GFP with NT_{50} values ranging from 96 to 5400 (Figure 1B). Overall, these data strongly suggest that only pandemic sera, and not prepandemic sera have neutralizing activity against SARS-CoV-2, and further suggest that preexisting serological immunity to seasonal coronaviruses is not a major driver of the diverse outcome of SARS-CoV-2 infection.

DISCUSSION

These data demonstrate that neutralization activity against seasonal coronaviruses is nearly ubiquitous in sera collected from individuals with PCR-confirmed prepandemic seasonal coronavirus infection. Indeed, most sera had neutralizing activity against multiple seasonal coronaviruses and some sera had greater neutralization potency against different coronaviruses than the one detected at the time of sample collection. This may be due to inherent differences in neutralization sensitivity among the seasonal coronaviruses, and is most likely the result of prior, undocumented infection with different seasonal coronaviruses. That we observed more potent antibody responses to HCoV-OC43 regardless of the PCR results may suggest that recent infection with this virus is more common. Indeed, previous observations suggest that reinfection with HCoV-OC43 and HCoV-229E occurs at a greater frequency than HCoV-NL63 [5–8] and that infection with HCoV-OC43 is common in this geographic locale.

Although we cannot exclude the possibility that that seasonal coronavirus elicits cross-reactive antibodies, the divergence between seasonal coronaviruses S proteins would suggest that the degree of cross reactivity is limited (HCoV-OC43 S shares 22.6% and 24.5% identical amino acids with HCoV-229E and HCoV-NL63 S, respectively, whereas HCoV-NL63 and HCoV-229E S share 55% identical amino acids). Consistent with this notion, none of the samples tested had any neutralization activity against SARS-CoV-2, whose spike protein shares 24% to 29% amino acid identity with the seasonal coronaviruses. Moreover, many of the monoclonal antibodies cloned from

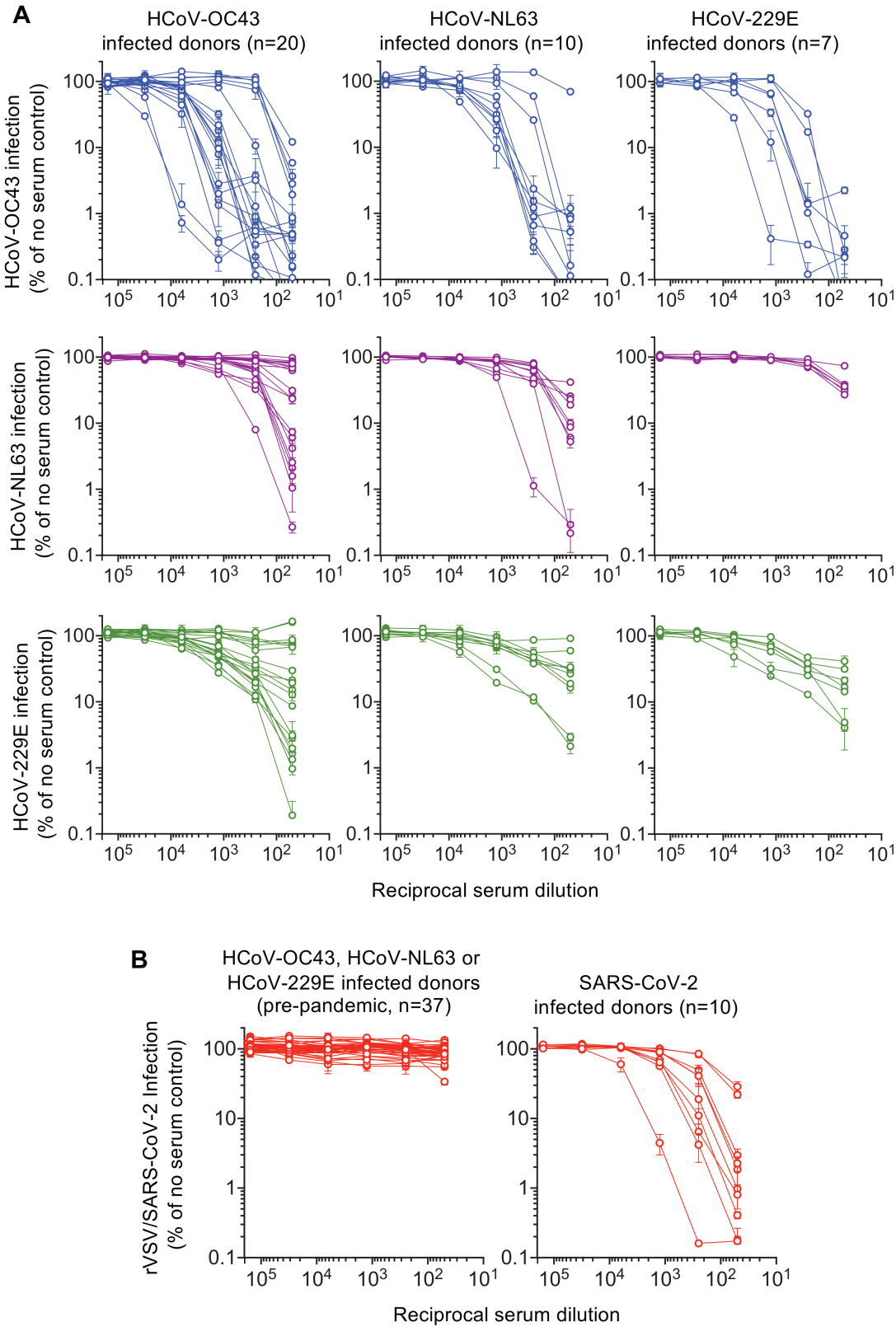

Figure 1. Coronavirus neutralizing activity in sera from individuals with recent coronavirus infection. (A) Infection by HCoV-OC43 (blue), HCoV-NL63 (purple), and HCoV-229E (green) in the presence of the indicated dilutions of pre-COVID-19 pandemic sera, from individuals recently diagnosed by PCR with HCoV-OC43, HCoV-NL63, or HCoV-229E infection, as indicated. Infected cells were enumerated by flow cytometry and the number of infected cells is plotted as a percentage of the number of infected cells (~30%) obtained in the absence of serum. (B) Infection by rVSV/SARS-CoV-2 in the presence of the indicated dilutions of pre-COVID-19 pandemic sera from individuals recently diagnosed by PCR with HCoV-OC43, HCoV-NL63, or HCoV-229E infection (left), or COVID-19 convalescent sera (right). Infected cells were enumerated by flow cytometry and the number of infected cells is plotted as a percentage of the number of infected cells (~30%) obtained in the absence of serum. Abbreviations: COVID-19, coronavirus disease 2019; HCoV, human coronavirus; PCR, polymerase chain reaction; rVSV, recombinant vesicular stomatitis virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2–infected individuals contain very low levels of somatic hypermutation [9]. This finding suggests potent neutralizing antibodies targeting SARS-CoV-2 arise de novo rather than from recall B-cell responses. However, some instances of cross-reactive antibodies with high levels of somatic hypermutation have been reported, indicating that in some cases memory B cells evoked by prior seasonal HCoV infection may be recalled during infection with a SARS-like coronavirus [10].

Although other groups have reported the existence of SARS-CoV-2 cross-reactive neutralizing antibodies in sera from individuals who were not infected with SARS-CoV-2, the neutralization activity observed appears low [11, 12]. Unlike other reports, the prepandemic sera used in our study that have undetectable neutralization activity against SARS-CoV-2 can neutralize seasonal HCoVs, in some cases quite potently. Although it is possible that there are rare instances of individuals possessing antibodies from prior seasonal HCoV infection may be able to also target SARS-CoV-2 S, our data argue against a broad role for preexisting protective humoral immunity against SARS-CoV-2.

Notes

Author contributions. H. W., S. J., Y. W., T. H., and P. D. B. conceived and designed the study. D. P. performed the neutralization assays. D. P., T. H., and P. D. B. wrote the manuscript with input from all authors.

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