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Coronavirus Occurrence in the Household Influenza Vaccine Evaluation (HIVE) Cohort of Michigan Households: Reinfection Frequency and Serologic Responses to Seasonal and Severe Acute Respiratory Syndrome Coronaviruses

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Background. We investigated frequency of reinfection with seasonal human coronaviruses (HCoVs) and serum antibody response following infection over 8 years in the Household Influenza Vaccine Evaluation (HIVE) cohort.

Methods. Households were followed annually for identification of acute respiratory illness with reverse-transcription polymerase chain reaction–confirmed HCoV infection. Serum collected before and at 2 time points postinfection were tested using a multiplex binding assay to quantify antibody to seasonal, severe acute respiratory syndrome coronavirus (SARS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike proteins and SARS-CoV-2 spike subdomains and N protein.

Results. Of 3418 participants, 40% were followed for ≥3 years. A total of 1004 HCoV infections were documented; 303 (30%) were reinfections of any HCoV type. The number of HCoV infections ranged from 1 to 13 per individual. The mean time to reinfection with the same type was estimated at 983 days for 229E, 578 days for HKU1, 615 days for OC43, and 711 days for NL63. Binding antibody levels to seasonal HCoVs were high, with little increase postinfection, and were maintained over time. Homologous, preinfection antibody levels did not significantly correlate with odds of infection, and there was little cross-response to SARS-CoV-2 proteins.

Conclusions. Reinfection with seasonal HCoVs is frequent. Binding anti-spike protein antibodies do not correlate with protection from seasonal HCoV infection.

Keywords. seasonal coronavirus; SARS-CoV-2; COVID-19; reinfection; antibody; waning; household cohort; serology; correlates of protection; immunity.

Prior to the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it was recognized that coronaviruses that infect humans (HCoVs) could be separated into the 4 seasonal, or common, coronaviruses (229E, OC43, NL53, and HKU1), which regularly cause mainly mild respiratory illnesses, and severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), which have caused epidemics of severe lower respiratory disease [1–4]. The current coronavirus disease 2019 (COVID-19) pandemic, the first recognized to be caused by an HCoV, has focused attention on the seasonal coronaviruses in comparison to SARS-CoV-2. Of particular importance are questions around possible cross-protection or enhancement of COVID-19 disease from prior seasonal virus infection and duration of infection following SARS-CoV-2 infection [5–7]. Few have examined antibody response to seasonal infection, antibody waning, or cross-response between the 4 seasonal HCoVs or with SARS-CoV-2 in large prospective cohort studies [8–10].

We have previously reported on 8 years of seasonal HCoV infection among persons in the continuing Household Influenza Vaccine Evaluation (HIVE) study being conducted in Michigan [11]. Over that period, 2010–2018, 1004 infections were detected by reverse-transcription polymerase chain reaction (RT-PCR). The infections were most frequent in children, but substantial numbers of infections were identified in adults. This, and past studies of HCoVs suggested that these agents, like most respiratory viruses, reinfect through life [12, 13]. In this report, we characterize RT-PCR–documented, symptomatic reinfections with these viruses and investigate antibody response to infection, including cross-reactivity and persistence.

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MATERIALS AND METHODS

Study Population

The complete methods of the HIVE cohort have been published previously [14]. Households with children receiving primary care from Michigan Medicine were recruited from Ann Arbor, Michigan and surrounding communities beginning in the summer of 2010. Households were retained as long as possible with replacement households enrolled and returning households reengaged in the spring or summer of each year; participant and household characteristics were recorded at this time. Adult participants provided informed consent for themselves and their children, and children ≥7 years of age provided verbal assent prior to participating. This study was reviewed and approved by the University of Michigan Medical School Institutional Review Board.

Seasonal Coronavirus Surveillance

Each study year, participants were asked to report all acute respiratory illnesses (ARIs) defined by ≥2 symptoms as soon as they occurred [14]. Participants were also actively questioned regarding their illness status via weekly calls or emails. Although year-round surveillance did not begin until the fall of 2014, complete coronavirus epidemics were likely captured in each study year because of their sharp seasonality [11]. Participants with ARI attended an illness visit within 7 days of symptom onset where study staff collected nasal and throat swabs (nasal only in children <3 years of age) combined in a single vial of viral transport media; asymptomatic infections were not assessed. Specimens were assayed for detection of respiratory viruses, including the 4 seasonal coronavirus types (229E, OC43, HKU1, and NL63). Specimens collected prior to the 2016–2017 study year were tested by singleplex RT-PCR using primers and probes developed by the CDC Division of Viral Diseases, Gastroenteritis, and Respiratory Viruses [15]. Specimens collected in the 2016–2017 and 2017–2018 years were tested using the FTD Respiratory Pathogen 33 Multiplex PCR Kit (Fast Track Diagnostics). Coinfections were defined as ARI associated with the detection of 2 or more HCoVs in the same specimen. Reinfection was defined as detection of the same or different HCoV type during an ARI with new onset of symptoms 14 or more days from the onset of a previously reported illness.

Serologic Studies

Beginning in the fall of 2011, participants aged ≥13 years were invited to provide blood specimens for serologic studies in the fall of each year prior to the respiratory virus season, and in the spring or summer following each respiratory virus season. Eligibility for serologic studies was expanded to children ≥6 months of age in the fall of 2016. We selected all individuals with PCR-confirmed common coronavirus infection between 2011 and 2018 who had paired serum collected in the fall or summer prior to their infection and in the spring or summer following their infection for serologic studies. In addition to the pair pre- and postinfection serum specimens, a subsequent postinfection specimen was selected from a later study year for each individual when available.

These serum specimens were tested in a 10-Plex electrochemiluminescence immunoassay (ECLIA) (Meso Scale Discovery, Rockville, Maryland) to measure antibody binding to the following antigens: the 4 seasonal coronavirus spike proteins (229E, OC43, HKU1, and NL63), SARS-CoV spike protein, SARS-CoV-2 spike protein, SARS-CoV-2 spike protein receptor binding domain, SARS-CoV-2 spike protein N-terminal domain, SARS-CoV-2 N protein, and a bovine serum albumin (BSA) negative control [16–19]. On the day of the assay, the plate was blocked for 60 minutes with MSD Blocker A (5% BSA). The blocking solution was washed off and test samples were applied to the wells at 4 dilutions (1:100, 1:800, 1:3200, and 1:12 800) and incubated with shaking for 2 hours. Plates were washed, and Sulfo-tag–labeled anti–immunoglobulin G antibody was applied to the wells and allowed to associate with complexed coated antigen-sample antibody. Plates were washed to remove unbound detection antibody, and a read solution containing ECL substrate was applied. In an MSD Sector instrument, a current was applied to the plate and areas of well surface where sample antibody has complexed with coated antigen and labeled reporter will emit light in the presence of the ECL substrate. An MSD Sector instrument quantitated the amount of light emitted and reported this ECL unit response, which is directly proportional to binding antibody. The area under the curve (AUC) was calculated using Prism software (GraphPad Prism, San Diego, California).

Antibody binding to the 4 common coronavirus spike proteins was also measured in singleplex ECLIA assays. The correlation between the singleplex and multiplex assays was generally high and patterns of antibody response were similar for the common coronaviruses comparing the mulitplex and singleplex assays (Supplementary Tables 1 and 2). Therefore, the results of only the multiplex analysis are presented here.

Statistical Analysis

For ease of calculation in time-to-event analyses, individuals were considered to contribute time at risk from 1 July through 30 June for each study year they were enrolled even though ARI surveillance was not carried out during the summer months in all years. Mean, median, minimum, and maximum times from 1 July of the first study year of enrollment to first infection and reinfection were estimated overall and for specific HCoV types and genera. Kaplan–Meier curves summarizing time to reinfection following each previous infection were also generated. Individuals reentered the data after each infection with time at risk of reinfection beginning at the day of onset of symptoms of their prior infection (time = 0).

Individuals who were lost to follow-up and reenrolled in a later season were censored during the period during which they were lost to follow-up.

Hazard ratios (HRs) and 95% confidence intervals (CIs) of the effect of infection in the prior year on infection in the following year were estimated in Cox proportional hazards models, stratified by year, with respective overall, type-specific, and genus-specific covariates specified as 1 if the individual was infected in the previous study year and 0 otherwise. Results of unadjusted models and models adjusted for age group (0–8, 9–17, ≥18 years) and sex are reported. Individuals contributed to a year of data if they participated in that and the prior study year. For each study year, only an individual's first HCoV infection was considered in respective any type, type-specific, and genus-specific analyses; subsequent HCoV infections during the same study year were ignored. Because individuals could contribute to multiple years and be infected multiple times, robust variance was calculated for Cox proportional hazards models.

AUC values from the ECLIA were adjusted by subtracting the BSA AUC value, and if any negative values resulted, adding a constant such that the minimum value for each target antigen was equal to 1. AUC values for each antigen were $\log_2$ transformed and the geometric mean was calculated at each time point: preinfection, postinfection, and subsequent postinfection. Changes in antibody binding from pre- to postinfection were calculated as the difference in geometric mean AUC. The number and proportion of individuals with 2-fold and 4-fold increases in AUC from pre- to postinfection were also calculated. Analyses of geometric means and infection response were only calculated among individuals with single infections; participants who had coinfections or >1 infection per year were excluded. The rate of antibody waning and half-life was estimated in linear generalized estimating equation models predicting $\log_2$ AUC by time. Pearson correlation coefficients were calculated comparing multiplex and singleplex AUC at each time point (Supplementary Tables 1 and 2).

The association between antibody binding levels and subsequent infection risk was estimated using a case test-negative design analysis. Log_2 AUC was compared between cases of single infection with a specific HCoV type and controls that were singly infected with the other 3 HCoV types in logistic generalized estimating equation regression models with exchangeable correlation structure clustered on the individual and adjusted for age, influenza vaccination, and high-risk health status. Inclusion of study year in adjusted models did not substantially change point estimates. Odds ratios (ORs) estimated from these models were interpreted as the reduction in odds of infection associated with a 2-fold increase in AUC.

RESULTS

RT-PCR–Confirmed HCoV Coinfections and Reinfections Over Time

In total, 3418 individuals participated for 1–8 study years (median, 2 [interquartile range, 1–4] years) contributing a total of 9378 person-years of observation (Figure 1). There were 1378 (40%) individuals who were under study for ≥3 years. Between 8.3% and 16.3% of the cohort had an ARI associated with seasonal HCoV infection each year. In total, 1004 ARIs associated with coronavirus infection were identified. The beta genus OC43 was most common (n = 390), followed by the alpha genus NL63 (n = 328). Less common were the beta HKU1 (n = 194), and alpha 229E (n = 152). The mean time from enrollment to first HCoV infection was 542 days. Times from enrollment to first infection for each seasonal HCoV were consistent with their

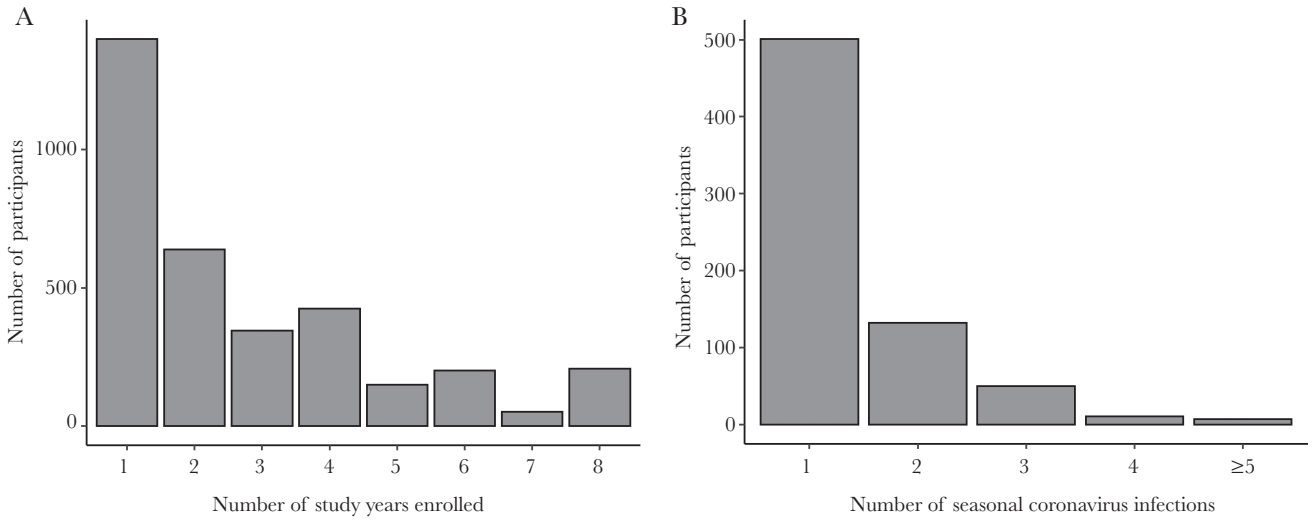

Figure 1. Distributions of follow-up time (*A*) and observed acute respiratory illnesses (*B*) associated with reverse-transcription polymerase chain reaction (RT-PCR)–confirmed seasonal coronavirus infection, Household Influenza Vaccine Evaluation (HIVE) cohort, 2010–2018. The number of observed acute respiratory illnesses associated with RT-PCR–confirmed seasonal coronavirus infection per individual ranged from 1 to 13.

relative incidence—that is, shorter times for the more common OC43 and NL63, and longer for the less common HKU1 and 229E (Supplementary Table 3).

Of the 1004 HCoV-associated ARIs, there were 53 (5.3%) instances of coinfection with 2 HCoV types detected from the same specimen and 3 (0.3%) in which 3 different types were detected. Combinations of all 4 HCoV types were observed in these coinfections, but alpha and beta genus coinfections were most common (Figure 2A and 2B).

HCoV reinfections were common in the cohort (Figure 3; Supplementary Figure 1). Overall, the 1004 ARI with HCoV occurred among 701 individuals; 303 (30%) of these represented documented reinfection. The number of ARI episodes with HCoV infection ranged from 1 to 13 per individual (Figure 1). Of the overall 303, 81 any type reinfections were identified in the same study year (1 July–30 June), a relatively short period given their seasonality. Of the 81 reinfections during the same study year, 12 were of the same HCoV type potentially representing prolonged shedding (range of days between illnesses, 14–152). Considering any type reinfections, the mean time to reinfection was 505 days. The mean time to same type reinfection was estimated at 983 days for 229E, 578 days for HKU1, 615 days for OC43, and 711 days for NL63 (Supplementary Table 3). Distributions of HCoV type pairings in consecutive any type reinfections were similar to those for coinfections, with consecutive alpha and beta genus infections most common (Figure 2C and 2D).

Overall, the hazard of infection with any coronavirus was more than twice as high among subjects with documented infection of any type in the immediately prior study year relative to those who were not (HR, 2.2 [95% CI, 1.7–2.7]). Similarly, the hazard of reinfection with a beta genus HCoV was more than twice as high among subjects infected with a beta genus HCoV in the previous study year; this effect was consistent for both beta genus viruses, HKU1 and OC43 (Table 1). There was no statistically significant effect of prior season alpha genus HCoV infection on hazard of alpha genus reinfection in the following season. To investigate the possibility that the increased risk of reinfection might be due to confounding by unmeasured risk factors for infection, we performed a sensitivity analysis restricting to only those individuals with any seasonal HCoV infection in the prior year. HRs consistently shifted lower in this analysis (Table 1); the increased hazard of beta coronavirus reinfection was attenuated and no longer statistically significant, while the reduced hazard of alpha coronavirus reinfection was strengthened and statistically significant.

Serologic Studies

We selected all individuals with common coronavirus infection between 2011 and 2018 who had paired serum collected in the in the fall or summer prior to their infection and in the spring or summer following their infection for serologic studies (201 of the 1004 total infections). Of these 201, 42 were from individuals infected with 229E, 41 with HKU1, 40 with NL63, and 78 with OC43. Of these, 167 had a subsequent postinfection specimen collected in a later study

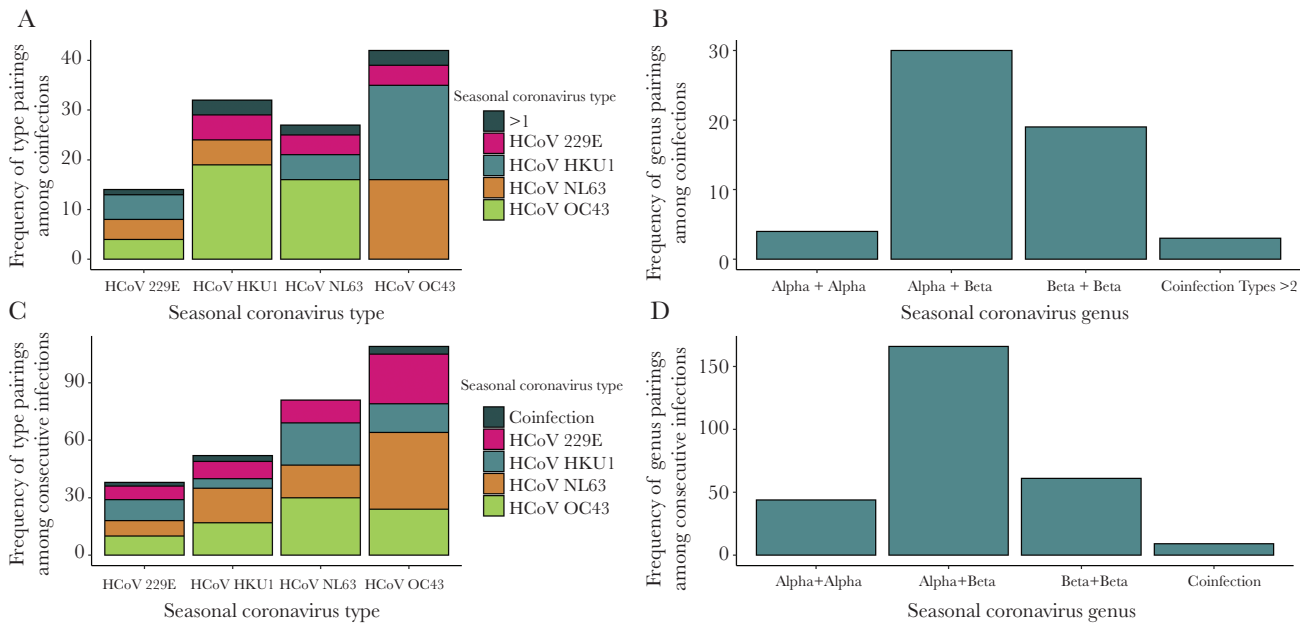

Figure 2. Frequency of seasonal human coronavirus (HCoV) pairings in coinfections by type (*A*) and genus (*B*), and frequency of seasonal HCoV pairings in consecutive reinfections by type (*C*) and genus (*D*).

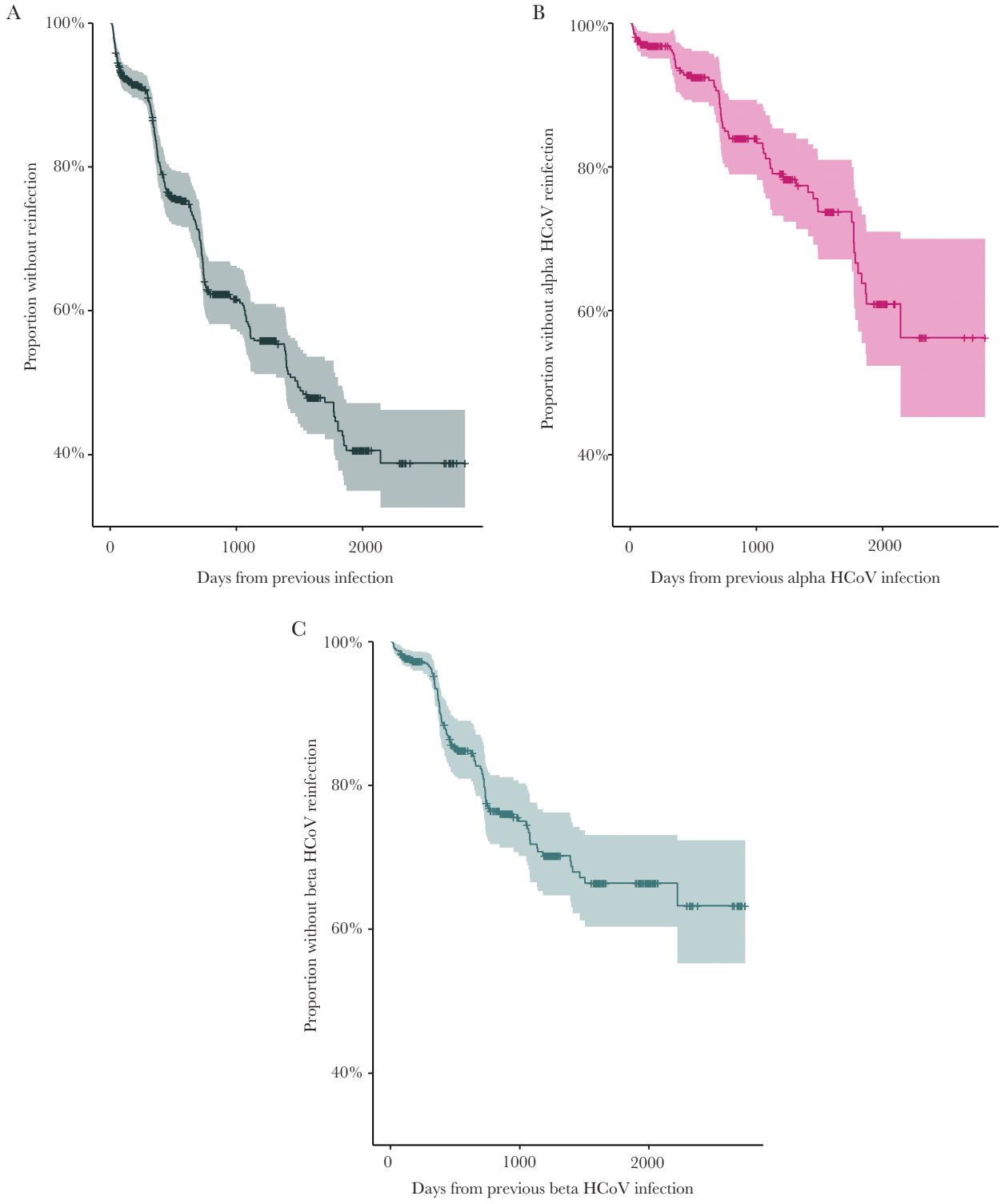

Figure 3. Kaplan–Meier curves of time to reinfection following a prior seasonal human coronavirus (HCoV) infection for any seasonal HCoV reinfection (*A*), and for same-genus alpha (*B*) and beta (*C*) coronavirus reinfections.

year. The ages of individuals included in serologic studies ranged from 3 to 67, mainly from younger adults (median age, 38 years). Preinfection specimens were collected between 2 and 216 days prior to infection (median, 60 days); postinfection specimens were collected between 19 and 244 days after infection (median, 110 days); and subsequent specimens were collected between 79 and 517 days after infection (median, 198 days).

Table 1. Hazard Ratios of Coronavirus Infection by Prior Year Infection Status, Overall and by Type and Genus

Cohort and Infection	No. of Infections/Person-Years Not Infected in Previous Year (%)	No. of Infections/Person-Years Infected in Previous Year (%)	HR[a] (95% CI)	Adjusted[a,b] HR (95% CI)
Full cohort				
All HCoV infections	429/5170 (8.3)	113/639 (17.7)	2.3 (1.8–2.9)	2.2 (1.7–2.7)
229E infections	118/5695 (2.1)	1/114 (0.9)	0.7 (.1–5.3)	0.7 (.1–5.0)
HKU1 infections	118/5690 (2.1)	9/119 (7.6)	5.6 (2.5–12.3)	4.8 (2.2–10.4)
NL63 infections	170/5605 (3.0)	4/204 (2.0)	0.8 (.3–2.2)	0.7 (.3–1.8)
OC43 infections	179/5517 (3.2)	18/292 (6.2)	2.6 (1.6–4.1)	2.3 (1.4–3.6)
Alpha genus infections	275/5494 (5.0)	14/315 (4.4)	0.9 (.5–1.7)	0.8 (.5–1.5)
Beta genus infections	256/5421 (4.7)	50/388 (12.9)	2.9 (2.1–4.1)	2.7 (1.9–3.7)
Subset with infection in previous year				
229E infections	18/525 (3.4)	1/114 (0.8)	0.5 (.1–3.7)	0.5 (.1–3.4)
HKU1 infections	23/520 (4.4)	9/119 (7.6)	2.7 (1.2–6.1)	2.4 (1.1–5.2)
NL63 infections	29/435 (6.7)	4/204 (2.0)	0.3 (.1–.8)	0.2 (.1–.6)
OC43 infections	25/347 (7.2)	18/292 (6.2)	1.1 (.6–2.0)	1.0 (.6–1.8)
Alpha genus infections	38/324 (11.7)	14/315 (4.4)	0.4 (.2–.7)	0.3 (.2–.6)
Beta genus infections	22/251 (8.8)	50/388 (12.9)	1.5 (.8–2.5)	1.4 (.8–2.4)

Abbreviations: CI, confidence interval; HCoV, human coronavirus; HR, hazard ratio.

[a]Estimated in study year–stratified Cox proportional hazards model predicting time to infection by prior year infection status.

[b]Model adjusted for age group (0–8, 9–17, ≥18 years) and sex.

Antibody Against Seasonal Coronaviruses

Individuals included in the serologic portion of this analysis were infected despite high binding antibody levels against the coronavirus with which they were infected. In general, preinfection antibody levels were high against all 4 seasonal HCoVs, with geometric mean AUC ranging from 155 751 against NL63 to 2 225 663 against OC43 (Table 2; Supplementary Figure 2). These geometric mean AUCs were approximately 25- to 360-fold higher than those binding the SARS-CoV-2 spike protein. Geometric mean AUCs were remarkably similar overall at preinfection, postinfection, and subsequent time points (Supplementary Figure 2).

Only modest increases in antibody levels to homologous HCoV types were observed following single infection (Table 2; Figure 4). Homologous geometric mean AUC values increased from pre- to postinfection by 1 450 426 for individuals infected with 229E, by 278 799 with HKU1, by 44 706 with NL63, and by 618 993 with OC43. Antibody levels did not substantially change from pre- to postinfection for other nonhomologous common coronaviruses, although sporadic individuals had cross-reactive responses following infection. Antibody levels postinfection were durable; statistically significant waning of homologous antibody was not observed (Figure 4; Supplementary Table 4).

There was no significant effect of antibody level on odds of infection detected in test-negative analyses comparing those testing positive for 1 coronavirus type to those testing positive for other types. A doubling in homologous AUC was estimated to reduce odds of infection by 7% (OR, 0.93 [95% CI, .76–1.14]) for 229E, 18% (OR, 0.82 [95% CI, .61–1.08]) for NL63, and by 6% (OR, 0.94 [95% CI, .68–1.31]) for OC43; a doubling in AUC was estimated to increase odds of infection by 9% (OR, 1.09 [95% CI, .83–1.45]) for HKU1.

Cross-Reactive Antibody to Epidemic Coronaviruses

Antibody levels were also measured against SARS-CoV spike protein, SARS-CoV-2 spike protein, SARS-CoV-2 spike protein receptor binding domain, SARS-CoV-2 spike protein N-terminal domain, and SARS-CoV-2 N protein (Table 3). With the exception of the SARS-CoV-2 N protein, antibody levels were low against these targets and did not substantially change from pre- to postinfection with each of the 4 seasonal coronaviruses. While much lower than antibody levels to the seasonal coronaviruses, the preinfection geometric mean AUC for the SARS-CoV-2 N protein was higher than for other SARS-CoV targets. Modest, but not statistically significant, increases in geometric mean AUC for SARS-CoV-2 N protein were observed for those infected with 229E where nearly 20% had ≥4-fold rise in $\log_2$ AUC.

DISCUSSION

During an influenza pandemic, past experience is useful in many areas of planning, but the novel COVID-19 pandemic has left us with few precedents for a number of critical

Table 2. Geometric Mean Area Under the Curve Levels of Antibodies Binding Seasonal Coronavirus Spike Protein at Preinfection and at 2 Postinfection Time Points for Individuals Infected With 229E, HKU1, NL63, or OC43; and the Proportion of Individuals Who Demonstrated a ≥2-Fold or ≥4-Fold Rise in Binding Antibody Following Infection

	Antigen			
Infection	229E	HKU1	NL63	OC43
229E (n = 42)				
Preinfection GM-AUC	1 131 162.57	784 535.98	15 5751.62	2 225 663.81
Postinfection GM-AUC	**2 581 588.76**	882 948.38	177 840.92	2 532 165.45
Subsequent GM-AUC	2 091 271.52	821 827.33	168 956.51	2 179 466.83
≥2-fold rise from pre- to postinfection, No. (%)	22 (52.4)	8 (19.0)	5 (11.9)	5 (11.9)
≥4-fold rise from pre- to postinfection, No. (%)	10 (23.8)	3 (7.1)	2 (4.8)	3 (7.1)
HKU1 (n = 41)				
Preinfection GM-AUC	1 175 392.03	837 183.13	211 015.36	2 572 590.25
Postinfection GM-AUC	1 315 880.69	**1 115 981.78**	202 850.69	2 474 157.99
Subsequent GM-AUC	1 459 890.11	104 3611.88	230 423.33	2 513 233.77
≥2-fold rise from pre- to postinfection, No. (%)	5 (12.2)	9 (22.0)	1 (2.4)	2 (4.9)
≥4-fold rise from pre- to postinfection, No. (%)	3 (7.3)	1 (2.4)	0 (0.0)	1 (2.4)
NL63 (n = 40)				
Preinfection GM-AUC	1 449 510.47	764 070.12	143 224.93	1 964 950.38
Postinfection GM-AUC	1 139 895.79	750 090.91	**187 930.95**	2 035 442.24
Subsequent GM-AUC	1 209 333.46	685 540.48	182 482.86	2 251 171.93
≥2-fold rise from pre- to postinfection, No. (%)	0 (0.0)	4 (10.0)	7 (17.5)	6 (15.0)
≥4-fold rise from pre- to postinfection, No. (%)	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)
OC43 (n = 78)				
Preinfection GM-AUC	123 6257.52	774 334.37	162 126.04	2 183 705.59
Postinfection GM-AUC	1 344 293.21	811 925.45	173 449.60	**2 802 698.81**
Subsequent GM-AUC	1 339 786.71	830 427.15	177 626.98	2 740 582.39
≥2-fold rise from pre- to postinfection, No. (%)	9 (11.5)	9 (11.5)	6 (7.7)	14 (17.9)
≥4-fold rise from pre- to postinfection, No. (%)	3 (3.8)	3 (3.8)	2 (2.6)	0 (0.0)

Statistically significant differences (paired *t* test $P < .05$) between preinfection and postinfection GM-AUC are shown in bold.

Abbreviation: GM-AUC, geometric mean area under the curve.

subjects, particularly duration of immunity postinfection or vaccination [20]. Prior experience with epidemic HCoVs offers little help; SARS-CoV was eliminated and MERS-CoV has not spread widely [21, 22]. As a result, we have looked to the 4 seasonal HCoV viruses causing typically mild disease to gain insights on how SARS-CoV-2 might behave going forward. We have previously demonstrated that these HCoVs are truly seasonal, transmitting mainly in the months between November and May and peaking in December–March, and only time will tell if the SARS-CoV-2 occurrence will begin to follow the same pattern as immunity increases in the population [11].

While it was clear from studies conducted years ago that reinfection with seasonal HCoVs, like other common respiratory viruses, occurred throughout life, their frequency had not been a matter of great interest [12, 13]. This has become more urgent currently given the importance of knowing how long immunity might last after SARS-CoV-2 infection and vaccination. Given the age structure of our cohort, and high levels of preexisting antibody, nearly all infections observed in this study are likely reinfections. However, nearly a third of all identified infections were confirmed reinfections during enrollment with an average duration of 505 days between infections. In primary analyses, we observed that the risk of infection with beta genus coronaviruses was higher if an individual had a beta coronavirus infection in the immediately prior study year. This effect was attenuated in sensitivity analyses conditioning only on those with a prior infection, suggesting that this result is likely due to confounding by unmeasured shared risk factors for infection rather than a specific biological effect. Regardless, this finding underscores the frequency of reinfection in this cohort.

While there have been several recent studies on antibody to the seasonal HCoVs in those infected with SARS-CoV-2, there have been few looking at the antibody response of RT-PCR–confirmed seasonal infection [23, 24]. We used a multiplex binding assay that included SARS-CoV-2, SARS-CoV, and seasonal coronavirus prefusion-stabilized spike protein constructs made using techniques developed for studying vaccine response

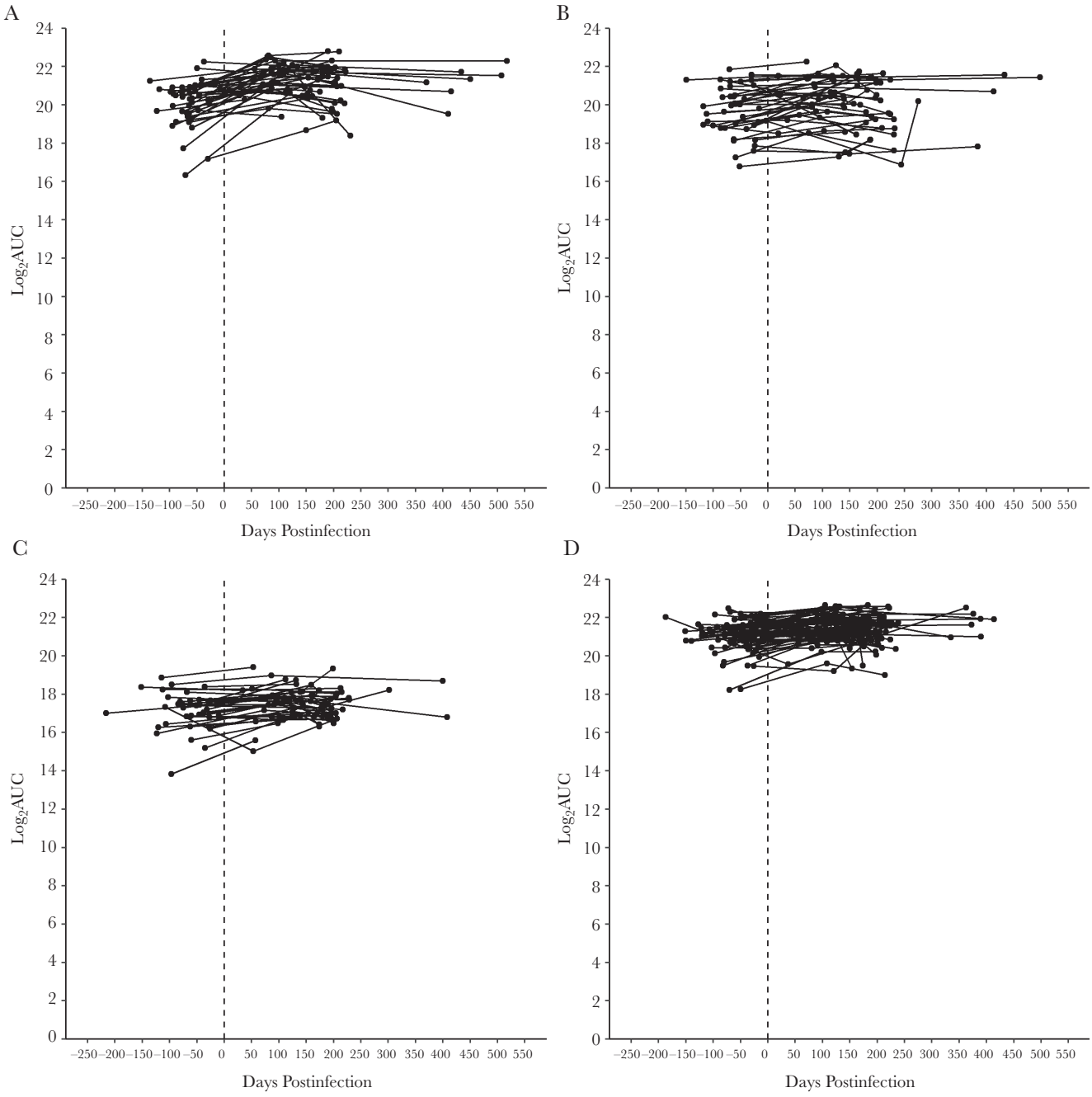

Figure 4. Infection-homologous $\log_2$ area under the curve (AUC) values preinfection and at 2 postinfection time points for individuals infected with human coronaviruses 229E (*A*), HKU1 (*B*), NL63 (*C*), and OC43 (*D*).

[19, 25–27]. We found high levels of antibody binding the spike protein of the seasonal viruses and very low levels binding SARS-CoV-2 antigens in this pre–COVID-19 population. Individuals in this study were infected with seasonal HCoV despite these high levels of binding antibody, and had very modest increases in antibody following infection, suggesting a ceiling effect. Consistent with this observed infection despite high antibody levels, there was no significant evidence that homologous binding antibody correlated with protection. This analysis was limited by lack of antibody measurements in a completely uninfected control group and by a relatively narrow distribution of preinfection antibody binding levels in this population. We also did not measure neutralizing antibody, which is likely to be a better correlate of protection. While binding assays for SARS-CoV-2 have correlated well with neutralizing antibodies in recent studies of response to infection or vaccination with the

Table 3. Geometric Mean Area Under the Curve Levels of Antibodies Binding Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and SARS-CoV-2 Antigens at Preinfection and at 2 Postinfection Time Points for Individuals Infected With 229E, HKU1, NL63, or OC43; and the Proportion of Individuals Who Demonstrated a ≥2-Fold or ≥4-Fold Rise in Binding Antibody Following Infection

	Antigen				
Infection	SARS-CoV Spike	SARS-CoV-2 Spike	SARS-CoV-2 Spike-NTD	SARS-CoV-2 Spike-RBD	SARS-CoV-2 N Protein
229E (n = 42)					
Preinfection GM-AUC	5562.88	6134.71	2552.83	7301.94	23 461.49
Postinfection GM-AUC	4331.25	7226.25	**1880.80**	6143.84	42 016.96
Subsequent GM-AUC	5657.59	6236.92	2413.06	8303.43	29 981.52
≥2-fold rise from pre- to postinfection, No. (%)	3 (7.1)	7 (16.7)	6 (14.3)	4 (9.5)	13 (31.0)
≥4-fold rise from pre- to postinfection, No. (%)	3 (7.1)	3 (7.1)	2 (4.8)	1 (2.4)	8 (19.0)
HKU1 (n = 41)					
Preinfection GM-AUC	5609.93	4303.93	2212.49	5489.75	23 466.27
Postinfection GM-AUC	4868.87	4447.64	2347.31	7045.39	19 975.46
Subsequent GM-AUC	6530.69	4668.69	2979.96	6795.63	22 969.35
≥2-fold rise from pre- to postinfection, No. (%)	2 (4.9)	4 (9.8)	9 (22.0)	11 (26.8)	7 (17.1)
≥4-fold rise from pre- to postinfection, No. (%)	0 (0.0)	1 (2.4)	4 (9.8)	7 (17.1)	1 (2.4)
NL63 (n = 40)					
Preinfection GM-AUC	4270.94	6733.11	1975.73	5610.12	18 421.41
Postinfection GM-AUC	3959.94	6056.06	2665.88	5926.12	20 615.65
Subsequent GM-AUC	4088.78	6005.97	2036.92	7221.81	24 803.20
≥2-fold rise from pre- to postinfection, No. (%)	3 (7.5)	2 (5.0)	10 (25.0)	7 (17.5)	9 (22.5)
≥4-fold rise from pre- to postinfection, No. (%)	1 (2.5)	1 (2.5)	4 (10.0)	2 (5.0)	1 (2.5)
OC43 (n = 78)					
Preinfection GM-AUC	6374.95	6479.42	3260.21	6444.89	25 972.48
Postinfection GM-AUC	**5052.53**	5831.20	2119.52	6190.94	19 376.76
Subsequent GM-AUC	4602.15	6287.42	1928.70	5624.17	18 284.01
≥2-fold rise from pre- to postinfection, No. (%)	3 (3.8)	8 (10.3)	5 (6.4)	11 (14.1)	10 (12.8)
≥4-fold rise from pre- to postinfection, No. (%)	1 (1.3)	1 (1.3)	2 (2.6)	7 (9.0)	4 (5.1)

Statistically significant differences (paired *t* test, $P < .05$) between preinfection and postinfection GM-AUC are shown in bold.

Abbreviations: GM-AUC, geometric mean area under the curve; NTD, N-terminal domain; RBD, receptor binding domain; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

novel virus [26, 28–31], it is possible that is not the case with the repeat infection that occurs with the seasonal viruses.

We also found that antibody was remarkably persistent over time with little indication of waning. This stability of antibody is in contrast to several studies that have observed rapidly waning immunity following SARS-CoV-2 infection [8, 28, 32, 33], although other studies do suggest a more persistent antibody response [29, 30, 34]. As with correlates of protection, it may be the case that persistence of neutralizing antibody differs from that of binding antibodies.

There were few responses in SARS-CoV-2 antibodies following seasonal HCoV infection, indicating that the cross-response is infrequent. An exception was higher preinfection antibody to the SARS-CoV-2 N protein, known to be more conserved among the HCoVs [35]. Measuring antibody directed to the SARS-CoV-2 N protein has been suggested as a way to distinguish individuals who are infected vs vaccinated, as both will exhibit spike-directed antibody. The antigen we used was the full N protein; using epitopes restricted to SARS-CoV-2 may be more specific for this strategy.

This study has demonstrated that seasonal HCoV reinfection frequently occurs over a relatively short time period and is possibly affected by the prior infecting virus type. However, we have not determined precisely how frequently this occurs in a broad population nor is it evident whether this will apply to SARS-CoV-2 reinfection or infection after vaccination. Still, it does suggest that duration of immunity will probably be limited by either waning immunity or viral antigenic drift, at least in some of the population, and supports careful determination of the need for booster vaccinations as time from original immunization increases. Careful monitoring of the ways in which repeated vaccination and reinfection shape the development of

immunity is also warranted. This has implications for the use of immune assays as it is possible that the close relation between binding and neutralizing antibodies for SARS-CoV-2 relates to the novelty of the virus. The need for periodic revaccination should not be viewed with alarm, since it has been the practice for many years with influenza. Such booster immunizations, through updated reformulation, could also address possible antigenic changes, now becoming a major concern as novel variants continue to be identified globally.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Natl Acad Sci U S A **1967**; 57:933–40.
2. Monto AS. Medical reviews. Coronaviruses. Yale J Biol Med **1974**; 47:234–51.
3. Drosten C, Günther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med **2003**; 348:1967–76.
4. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med **2012**; 367:1814–20.
5. Huang AT, Garcia-Carreras B, Hitchings MDT, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. Nat Commun **2020**; 11:4704.
6. Beretta A, Cranage M, Zipeto D. Is cross-reactive immunity triggering COVID-19 immunopathogenesis? Front Immunol **2020**; 11:567710.
7. Aran D, Beachler DC, Lanes S, Overhage JM. Prior presumed coronavirus infection reduces COVID-19 risk: a cohort study. J Infect **2020**; 81:923–30.
8. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med **2020**; 26:1691–3.
9. Ringlander J, Nilsson S, Westin J, Lindh M, Martner A, Hellstrand K. Low incidence of reinfection with endemic coronaviruses diagnosed by real-time PCR [manuscript published online 10 October 2020]. J Infect Dis **2021**; 26:2013–2014.
10. Galanti M, Shaman J. Direct observation of repeated infections with endemic coronaviruses. J Infect Dis **2020**; 223:409–15.
11. Monto AS, DeJonge PM, Callear AP, et al. Coronavirus occurrence and transmission over 8 years in the HIVE cohort of households in Michigan. J Infect Dis **2020**; 222:9–16.
12. Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. J Infect Dis **1974**; 129: 271–6.
13. Hamre D, Beem M. Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance. Am J Epidemiol **1972**; 96:94–106.
14. Monto AS, Malosh RE, Evans R, et al; HIVE Study Research Staff. Data resource profile: Household Influenza Vaccine Evaluation (HIVE) Study. Int J Epidemiol **2019**; 48:1040–1040g.
15. Sakthivel SK, Whitaker B, Lu X, et al. Comparison of Fast-Track Diagnostics respiratory pathogens multiplex real-time RT-PCR assay with in-house singleplex assays for comprehensive detection of human respiratory viruses. J Virol Methods **2012**; 185:259–66.
16. Johnson M, Wagstaffe HR, Gilmour KC, et al. Evaluation of a novel multiplexed assay for determining IgG levels and functional activity to SARS-CoV-2. J Clin Virol **2020**; 130:104572.
17. Majdoubi A, Michalski C, O'Connell SE, et al. Antibody reactivity to SARS-CoV-2 is common in unexposed adults and infants under 6 months. medRxiv [Preprint]. Posted online 6 November **2020**. doi:10.1101/2020.10.05.20206664.

18. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science **2020**; 367:1260–3.
19. Hsieh CL, Goldsmith JA, Schaub JM, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science **2020**; 369:1501–5.
20. Monto AS, Fukuda K. Lessons from influenza pandemics of the last 100 years. Clin Infect Dis **2020**; 70:951–7.
21. Lipsitch M, Cohen T, Cooper B, et al. Transmission dynamics and control of severe acute respiratory syndrome. Science **2003**; 300:1966–70.
22. Hui DS, Azhar EI, Kim YJ, Memish ZA, Oh MD, Zumla A. Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. Lancet Infect Dis **2018**; 18:e217–27.
23. Anderson EM, Goodwin EC, Verma A, et al. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. medRxiv [Preprint]. Posted online 10 November **2020**. doi: 10.1101/2020.11.06.20227215.
24. Poston D, Weisblum Y, Wise H, et al. Absence of SARS-CoV-2 neutralizing activity in pre-pandemic sera from individuals with recent seasonal coronavirus infection [manuscript published online ahead of print 3 December 2020]. Clin Infect Dis **2020**. doi:10.1093/cid/ciaa1803.
25. Jackson LA, Anderson EJ, Rouphael NG, et al; mRNA-1273 Study Group. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med **2020**; 383:1920–31.
26. Anderson EJ, Rouphael NG, Widge AT, et al; mRNA-1273 Study Group. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med **2020**; 383:2427–38.
27. Widge AT, Rouphael NG, Jackson LA, et al; mRNA-1273 Study Group. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. N Engl J Med **2021**; 384:80–2.
28. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. Nat Microbiol **2020**; 5:1598–607.
29. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. Science **2020**; 370:1227–30.
30. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Sci Immunol **2020**; 5:eabe0367.
31. To KK-W, Tsang OT-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis **2020**; 20:565–74.
32. Kellam P, Barclay W. The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. J Gen Virol **2020**; 101:791–7.
33. Vabret N. Antibody responses to SARS-CoV-2 short-lived. Nat Rev Immunol **2020**; 20:519.
34. Lumley SF, Wei J, O'Donnell D, et al. The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers [manuscript published online 6 January 2021]. Clin Infect Dis **2021**. doi:10.1093/cid/ciab004.
35. Dutta NK, Mazumdar K, Gordy JT. The nucleocapsid protein of SARS–CoV-2: a target for vaccine development. J Virol **2020**; 94:e00647-20.