The durability of immunity against reinfection by SARS-CoV-2: a comparative evolutionary study

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Summary

Background Among the most consequential unknowns of the devastating COVID-19 pandemic are the durability of immunity and time to likely reinfection. There are limited direct data on SARS-CoV-2 long-term immune responses and reinfection. The aim of this study is to use data on the durability of immunity among evolutionarily close coronavirus relatives of SARS-CoV-2 to estimate times to reinfection by a comparative evolutionary analysis of related viruses SARS-CoV, MERS-CoV, human coronavirus (HCoV)-229E, HCoV-OC43, and HCoV-NL63.

Methods We conducted phylogenetic analyses of the *S*, *M*, and *ORF1b* genes to reconstruct a maximum-likelihood molecular phylogeny of human-infecting coronaviruses. This phylogeny enabled comparative analyses of peak-normalised nucleocapsid protein, spike protein, and whole-virus lysate IgG antibody optical density levels, in conjunction with reinfection data on endemic human-infecting coronaviruses. We performed ancestral and descendent states analyses to estimate the expected declines in antibody levels over time, the probabilities of reinfection based on antibody level, and the anticipated times to reinfection after recovery under conditions of endemic transmission for SARS-CoV-2, as well as the other human-infecting coronaviruses.

Findings We obtained antibody optical density data for six human-infecting coronaviruses, extending from 128 days to 28 years after infection between 1984 and 2020. These data provided a means to estimate profiles of the typical antibody decline and probabilities of reinfection over time under endemic conditions. Reinfection by SARS-CoV-2 under endemic conditions would likely occur between 3 months and 5·1 years after peak antibody response, with a median of 16 months. This protection is less than half the duration revealed for the endemic coronaviruses circulating among humans (5–95% quantiles 15 months to 10 years for HCoV-OC43, 31 months to 12 years for HCoV-NL63, and 16 months to 12 years for HCoV-229E). For SARS-CoV, the 5–95% quantiles were 4 months to 6 years, whereas the 95% quantiles for MERS-CoV were inconsistent by dataset.

Interpretation The timeframe for reinfection is fundamental to numerous aspects of public health decision making. As the COVID-19 pandemic continues, reinfection is likely to become increasingly common. Maintaining public health measures that curb transmission—including among individuals who were previously infected with SARS-CoV-2—coupled with persistent efforts to accelerate vaccination worldwide is critical to the prevention of COVID-19 morbidity and mortality.

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Introduction

The ongoing COVID-19 pandemic has resulted in over 4·5 million deaths worldwide. Approaches to control COVID-19 depend on the durability of immunity conferred by recovery and by vaccination. However, predicting the durability of immunity against the virus causing COVID-19, SARS-CoV-2, remains challenging amid a pandemic. During the rapid expansion of the pandemic, there have been few documented reinfections relative to the overall incidence. Short-term longitudinal studies of the levels of SARS-CoV-2 neutralising antibodies[1,2] at best provide lower bounds for the durability of immunity. By contrast, the long-term waning of antibody levels following infection has been assessed among close coronavirus relatives of SARS-CoV-2, including SARS-CoV, MERS-CoV, human coronavirus (HCoV)-OC43, HCoV-229E, and HCoV-NL63.[3–7] Extensive reinfection data over time have been collected for seasonal endemic coronaviruses (HCoV-OC43, HCoV-229E, and HCoV-NL63).[7] The zoonotic coronavirus SARS-CoV-2 is unlikely to have evolved an especially divergent interaction with the mammalian immune system compared with its close coronavirus relatives.[7] Therefore, the waning of humoral immunity against SARS-CoV-2, the observed rates of antibody decline after infection, and the probability of reinfection given antibody levels for multiple close relatives of SARS-CoV-2 can be estimated from a phylogenetic analysis of the ancestral and descendent states[8] that fills in critical gaps in our knowledge of SARS-CoV-2. This well established phylogenetic approach that weights the effect of estimates from close relatives inversely by their evolutionary divergence and the speed at which the trait

Research in context

Evidence before this study

We searched PubMed and Google Scholar for articles containing information on antibody levels after recovery from infection by the coronaviruses SARS-CoV-2, SARS-CoV, MERS-CoV, human coronavirus (HCoV)-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E, and corresponding times of reinfection. We applied no language restriction and included articles published from database inception up until June 30, 2021. Full search details are described in the Methods. We found one or more studies on each of these viral species providing data on the waning of IgG antibodies to spike protein, nucleocapsid protein, or whole-virus lysate following infection. Additionally, one study provided distributions of times to reinfection for coronaviruses. However, no studies provided estimates of the typical time to reinfection for SARS-CoV-2, SARS-CoV, or MERS-CoV.

We then searched PubMed and Google Scholar using the terms "phylogeny", "phylogenetics", "ancestral state estimation", "evolution", and "phylogenomics" in conjunction with the terms from our previous search for articles containing information on the phylogenetic relatedness of these coronaviruses, published from database inception until June 30, 2021. No language restrictions were applied to this search. We found extensive sequence data on these species and well resolved phylogenies of their relationships. However, no analysis made explicit use of the resolved phylogenetic relationships to perform rigorous estimation of durability of immunity against reinfection by SARS-CoV-2.

Added value of this study

We provide the first estimates of the expected probability of infection given IgG antibody levels to the spike protein for SARS-CoV-2, as well as for SARS-CoV, MERS-CoV, and the endemic coronaviruses HCoV-229E, HCoV-HKU1, HCoV-OC43, and HCoV-NL63, under endemic conditions. Characterising the typical waning profile over time for IgG antibody levels to the spike protein, nucleocapsid protein, and to whole viral lysate, we derive the corresponding probabilities of SARS-CoV-2 reinfection that provide a timeframe crucial to numerous aspects of public health decision making.

Implications of all the available evidence

Reports of eventual reinfection by SARS-CoV-2 are mounting, but they have not reached proportions within well surveilled cohorts that would enable a quantitative epidemiological study. As a pioneering estimate, our findings are consistent with the mounting reports of eventual reinfection by SARS-CoV-2, and indicate that reinfection after natural recovery from COVID-19 will become increasingly common as the pandemic progresses.

See Online for appendix 1

evolves can then provide estimates of the probabilities of reinfection. The aim of this study is to estimate these probabilities and the corresponding likely times of reinfection associated with the human-infecting coronaviruses SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, HCoV-NL63, and especially SARS-CoV-2.

Methods

Study design

We conducted phylogenetic analyses of the *S*, *M*, and *ORF1b* genes to reconstruct a maximum-likelihood molecular phylogeny of human-infecting coronaviruses. This phylogeny enabled comparative analyses of nucleocapsid protein, spike protein, and whole-virus lysate post-infection IgG antibody optical density data in response to human-infecting coronaviruses, and of the corresponding probabilities of reinfection. Ancestral and descendent states analyses provided estimates of the expected declines in antibody levels over time, as well as inferred parameters for linear logistic models relating the probabilities of reinfection to antibody level and quantifying the anticipated times to reinfection after recovery under conditions of endemic transmission.

Data acquisition

Alphacoronavirus, betacoronavirus, deltacoronavirus, and gammacoronavirus whole-genome sequences were obtained from the GenBank genome database at NCBI (appendix 1 p 1). The Markov Clustering (MCL) algorithm implemented in MCLBlastLINE[9] was used to identify homologous genes for all coronavirus genomes. We specified an inflation parameter of 1·8 that has frequently been effective in other contexts[10] and that accurately predicted homologues for all single-copy genes within these genomes. Three core genes (*S, M,* and *ORF1b*) were present as single copies in all viral genomes (appendix 1 p 2) and were chosen for further analysis. Sequences of *S, M,* and *ORF1b* were aligned with TranslatorX,[11] then concatenated.

To find data on waning antibody levels we used PubMed and Google Scholar search for terms related to human-infecting coronavirus and antibody optical density or titre. Searches were performed between Oct 1, 2020, and June 30, 2021, using each coronavirus name "SARS-CoV-2", "HCoV-NL63", "SARS-CoV", "SARS-CoV-1", "MERS-CoV", "HCoV-229E", "HCoV-OC43", and "HCoV-HKU1" in combination with "antibodies", "antibody response", "coronavirus", "ELISA", "IgG", "immunity", "immune response", "longitudinal monitoring", "N protein", "Nucleocapsid", "neutralising antibodies", "optical density", "S protein", "Spike protein", "reinfection", "serological", and "titer". There were no language restrictions imposed on this search.

ELISA-based optical density measures of IgG antibody levels over time consequent to infection by each of the six human-infecting coronaviruses were extracted from published, peer-reviewed research papers. Studies were deemed sufficient for inclusion when they reported ELISA optical density data for anti-N IgG, anti-S IgG, or anti-whole virus lysate IgG antibody levels that extended more than 3 months after the peak of the respective IgG antibody response to infection.

Phylogenetic analyses

We analysed the concatenated alignment of the *S, M,* and *ORF1b* genes to reconstruct a maximum-likelihood molecular phylogeny of the four coronavirus genera, specifying a general time-reversible model of nucleotide substitution incorporating discretised gamma-distributed rate variation across sites and a proportion of invariable sites (GTR + I + Γ_4 model). To assess the effect of the likelihood search algorithm on our inference, we used two maximum-likelihood methods, IQ-TREE v2.0.6[12] and RAxML v7.2.8.[13] with 1000 non-parametric bootstrap replicates to assess node support. We time-calibrated maximum-likelihood phylogenies using least-squares dating (LSD2)[14] in IQ-TREE v2.0.6,[12] then two additional methods to assess consistency: Relative Times (RelTime)[15] in MEGA X v10.1.9,[16] and TreeTime v0.7.6. For the RelTime analysis, we provided the RAxML-derived and IQ-TREE-derived maximum-likelihood phylogeny with estimated branch lengths as the input phylogeny with the deltacoronavirus clade designated as the outgroup as indicated by Chan and colleagues.[17] Divergence times were calibrated using the earliest time each virus was sampled. The TreeTime analysis was performed by providing the same information that was provided to RelTime, including branch lengths and the root specification. Tips that did not follow a loose clock were not ignored in the analysis. To assess the effect of our choice of outgroup lineage, we repeated the TreeTime analysis with an unrooted input phylogeny, and used the option to estimate a root, with other parameter settings unchanged from those already specified. Because some areas of the SARS-CoV-2 genome have been suggested to recombine,[18] phylogenetic analyses were repeated using non-recombining blocks of sequence[18] that were realigned and analysed using the methods already identified. This additional analysis enabled us to ascertain whether a history of recombination among or within the *S, M,* and *ORF1b* genes had any discernible effect on our estimates. For all analyses, divergence times were scaled proportionally to the most recent common ancestor.

Waning antibody profiles and baselines

To construct profiles of antibody waning through time, we first extracted antibody levels after peak infection for SARS-CoV-2, SARS-CoV, HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HCoV-229E from published studies identified in PubMed and Google Scholar searches. We normalised all optical density quantiles to ensure that the post-infection peak optical density was 1·0 for each virus. This normalisation accounts for arbitrary scaling associated with assay-specific optical density measurements from ELISAs, ensuring a consistent scale relative to peak when analysing optical density data between studies. The normalised optical density data were analysed using Mathematica v12.0.0.6206964 to calculate the average rate of antibody decline at 0·05 intervals between 0 and 1. A typical antibody waning profile was then calculated by decreasing antibody levels each day from 1·0 (the peak) by the average rate of decline attributed to the 0·05 bracket enclosing the previous daily value, to the point whereby the decline rates for lesser brackets were no longer available (because of the absence of long-term antibody waning data for that virus) or until the empirical baseline was reached. For endemic coronaviruses, the baseline coronavirus IgG antibody level to nucleocapsid (N) protein was directly quantified as the lowest level observed in an extensive longitudinal study by Edridge and colleagues.[7] In the absence of long-term longitudinal studies of endemic infections by MERS-CoV, SARS-CoV, and SARS-CoV-2, the baseline IgG antibody levels for these viruses could not be estimated by such empirical observation. Instead, we estimated the baseline antibody levels for MERS-CoV, SARS-CoV, and SARS-CoV-2 using phylogenetic ancestral and descendent states analysis via Rphylopars v0.2.12.[8] This approach applies a Brownian model of trait evolution along a phylogenetic tree to estimate unobserved trait values for a taxon or taxa, providing best linear unbiased predictions that are mathematically equivalent to universal kriging (Gaussian process regression).[8] Using Mathematica, we next computed the best least-squares fit of an augmented exponential function

$$\omega + (1 - \omega) \times e^{-\lambda}$$

to each typical antibody waning profile, in which ω was the observed (in the case of endemic coronaviruses) or phylogenetically informed baseline antibody level, and λ was the corresponding exponential decline to the baseline specific to the virus and antibody under consideration. With this phylogenetically informed function, we projected the time-course for each typical antibody waning profile beyond the extant dataset to the duration of the longest full typical antibody waning profile inferred (HCoV-229E, 4393 days after peak infection).

To place this projection of antibody waning into a probabilistic framework for infection, we performed a linear logistic regression of daily probability of infection against antibody level based on the data from Edridge and colleagues,[7] yielding an infection function

$$[1 + e^{-(a + b \times g)}]^{-1}$$

with parameters *a* (intercept) and *b* (slope) for each endemic coronavirus, dependent on *g*, the peak-normalised antibody level. Using Rphylopars v0.2.12, we then performed ancestral and descendent states analysis to estimate the *a* and *b* parameters for the zoonotic viruses, specifying as coevolving and correlated traits our quantifications of λ and their phylogenetically informed baseline antibody levels. In Mathematica, we used the typical plus projected antibody waning time courses for each virus and the logistic infection function inferred for each virus to calculate the probability of infection on each day, and finally calculated how many days it took for the probability of infection by a given day to accumulate to 0·05, 0·5, and 0·95. These quantiles correspond to the times by which 5%, 50%, and 95% of individuals would be expected to become reinfected under endemic conditions. Comprehensive custom Mathematica notebooks illustrating our approach and used to perform the analyses are available on Zenodo. To assess the effect of method of phylogenetic inference on our phylogenetic trait estimation of the baseline antibody level ω and the linear logistic infection function parameters *a* and *b*, we repeated phylogenetic ancestral and descendent states analysis via Rphylopars[8] using resulting molecular phylogenies from IQ-TREE and RAxML and the relative phylogenetic chronograms estimated using RelTime and TreeTime, as well as the phylogenies produced using the non-recombinant alignment. The resulting parameter estimates for linear logistic infection function parameters *a* and *b* and the baseline antibody level ω were compared with the results conditioned on the relative phylogenetic chronogram estimated in IQ-TREE.

For the **Mathematica notebooks** please see https://doi.org/10.5281/zenodo.5146327

See **Online** for appendix 2

To assess the effect of using alternate sources of IgG antibody data on our analyses, we performed five additional analyses, designated 2–6. (2) We substituted an alternate anti-N IgG optical density dataset for SARS-CoV,[19] and performed an analysis that was otherwise identical to the original analysis (analysis 1). (3–4) We inferred a linear model in Mathematica relating anti-S IgG antibody levels to anti-N IgG antibody levels based on a SARS-CoV-2 cohort,[20] and applied it to anti-N IgG antibody data for the endemic coronaviruses to specify putative anti-S IgG antibody waning. We paired these putative anti-S IgG antibody level data for the endemic coronaviruses with direct anti-S IgG antibody level data for SARS-CoV-2 along with two distinct datasets on anti-S IgG antibody level in response to infection by MERS-CoV,[3,21] constituting two analyses (3 and 4) without SARS-CoV, each yielding a distinct result. (5) We inferred a linear model relating anti-virus IgG antibodies to anti-N IgG antibody levels based on a SARS-CoV cohort in which both were measured[19] and applied it to anti-N IgG antibody data for the endemic coronaviruses to specify putative anti-virus IgG antibody waning for the endemic coronaviruses. We paired these putative anti-virus IgG antibody levels for the endemic coronaviruses with directly measured anti-virus IgG antibodies for SARS-CoV-2 and performed an analysis without MERS-CoV. (6) We conducted an analysis that was otherwise identical to analysis 5, except that we used alternate anti-virus IgG antibody levels in response to infection by SARS-CoV.[5]

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

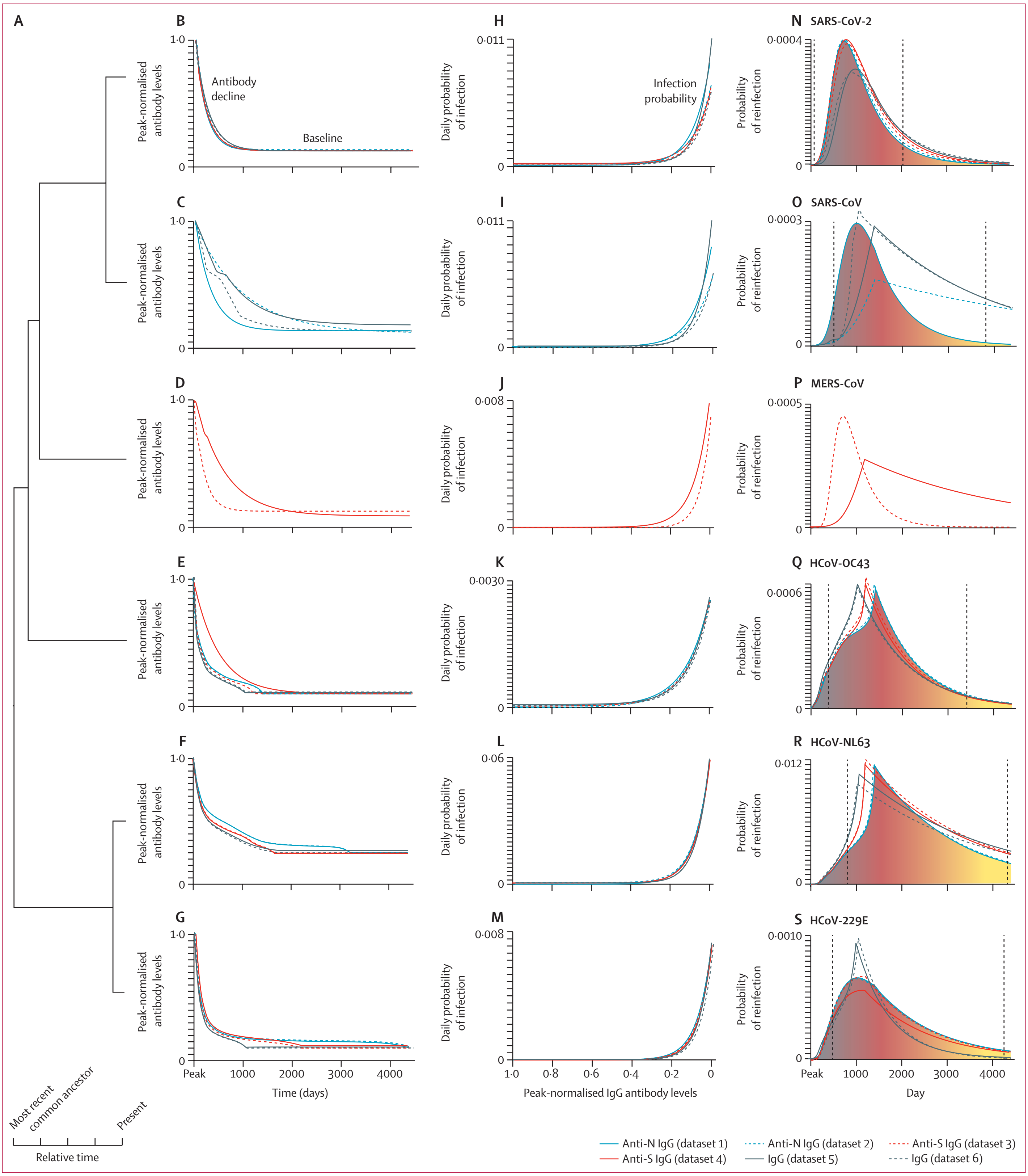
Results

Between Feb 12 and June 15, 2020, we accessed 58 alphacoronavirus, 105 betacoronavirus, 11 deltacoronavirus, and three gammacoronavirus genome sequences for analysis (appendix 1 p 1). Our phylogenetic analyses generated a topology of the evolutionary relationships for the seven human-infecting coronaviruses, SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-NL63, HCoV-229E, and HCoV-HKU1 (figure 1A; appendix 2 p 3). We excluded the human-infecting endemic coronavirus HCoV-HKU1 from figure 1A and from subsequent analyses because there were only two data points for infections by this virus, within just one individual from Edridge and colleagues.[7] Our phylogenetic analysis shows that SARS-CoV and SARS-CoV-2 are closely related, MERS-CoV is the sibling lineage to this SARS-CoV clade, with other endemic coronaviruses representing more distant outgroups (figure 1A). Our estimates of these phylogenetic relationships were congruent across multiple methods of inference with strong (100% bootstrap) support for all nodes (appendix 2 p 4), consistent with previous hypotheses of evolutionary relationships among coronaviruses (figure 1A; appendix 2 pp 5–6).[23]

Our literature search for antibody data subsequent to infection identified seven studies that met the criteria of having sufficient ELISA optical density data on anti-N IgG, anti-S IgG, or anti-whole virus lysate IgG antibody levels for comparative analysis.[3,5,7,19–21,22] These studies yielded six comparative datasets that provided insight into the durability of immunity as well as into the

Figure 1: **Evolutionary divergences, peak-normalised coronavirus anti-spike protein IgG antibody levels, daily probabilities of infection given antibody level, and probabilities of reinfection for human-infecting coronaviruses SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-NL63, and HCoV-229E**

(A) Phylogenetic chronogram of the evolutionary divergence of human-infecting coronaviruses relative to the most recent common ancestor. Bootstrap support was 100% for all nodes on this phylogeny. Peak-normalised antibody levels with fitted exponential waning (B–G) to a phylogenetically informed (B–D) or empirically determined baseline (E–G), in days from peak antibody level at 3 months. Daily probabilities of infection given peak-normalised S IgG antibody levels (H–M) from phylogenetically informed estimates (H–J) or from a maximum-likelihood fit of a linear-logistic model of probability of infection given antibody level (K–M). (N–S) Daily probability (curve with relative gradient from grey [low], to red [moderate], to yellow [high] for each virus) of reinfection over time, and central 90% interval of the reinfection day (black dashed vertical lines). Curves each correspond to parameters estimated from datasets 1–6.[3,5,7,19–21,22] HCoV=human coronavirus.



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N SARS-CoV-2
O SARS-CoV
P MERS-CoV
Q HCoV-OC43
R HCoV-NL63
S HCoV-229E
Peak-normalised antibody levels
Antibody decline
Baseline
Daily probability of infection
Infection probability
Probability of reinfection
1·0
0
0·011
0·008
0·0030
0·06
0·0004
0·0003
0·0005
0·0006
0·012
0·0010
Peak
1000
2000
3000
4000
Time (days)
1·0
0·8
0·6
0·4
0·2
Peak-normalised IgG antibody levels
Day
Most recent common ancestor
Present
Relative time
Anti-N IgG (dataset 1)
Anti-N IgG (dataset 2)
Anti-S IgG (dataset 3)
Anti-S IgG (dataset 4)
IgG (dataset 5)
IgG (dataset 6)

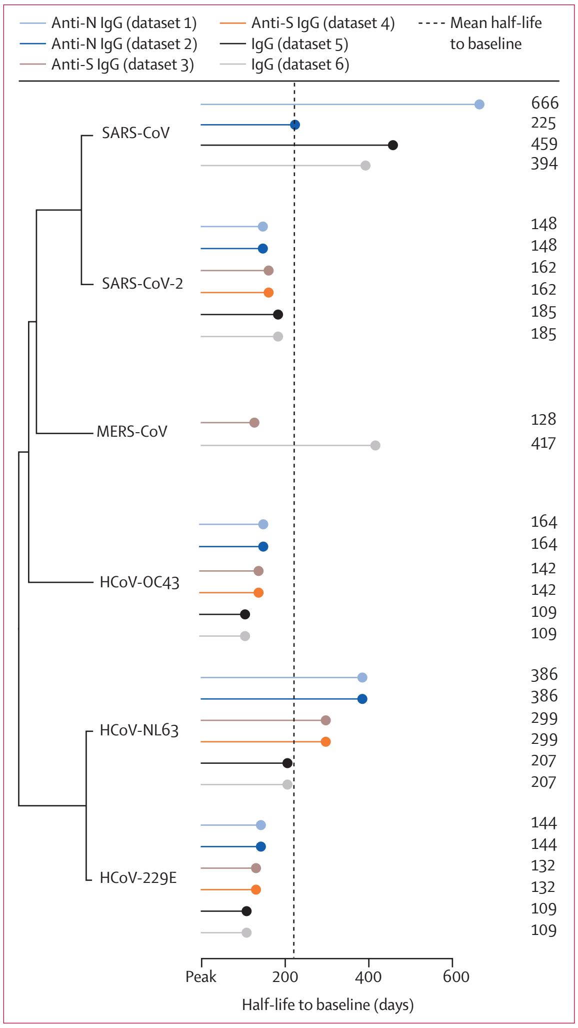

Figure 2: **Evolutionary divergences of human-infecting coronaviruses and estimated half-lives of antibody decline to baseline 3 months after infection by human-infecting coronaviruses.**
Estimated half-life to baseline for SARS-CoV-2 and other human-infecting coronaviruses are colour coded by dataset. The estimated half-lives resulting from analyses of datasets 1–6 are plotted in comparison to the mean half-life to baseline across all coronaviruses (dashed vertical line). HCoV=human coronavirus.

robustness of our findings to data selection (figure 1B–S). Dataset 1 comprised anti-N IgG antibody data over 240 days post-onset of symptoms from 20 individuals who had SARS-CoV infection-associated pneumonia;[22] from a population sample of 1797 individuals extending over 125 days after diagnosis of infection by SARS-CoV-2,[20] and from ten men aged 27–75 years who were assayed for antibody response to infection by HCoV-OC43, HCoV-NL63, and HCoV-229E over 28 years spanning the periods 1984–97 and 2003–20 (appendix 1 p 3).[7] Dataset 2 included alternate SARS-CoV data on 30 individuals (13 male and 17 female; mean age 37 years [SD 11]) monitored over 2 years after onset of symptoms.[19] Datasets 3 and 4 included putative endemic coronavirus anti-S IgG antibody waning data from our linear model relating anti-N and anti-S IgG (appendix 1 p 4) and MERS-CoV data from two sources (dataset 3 containing nine individuals [five male and four female; aged 27–54 years] with symptoms ranging from asymptomatic to severe, monitored up to 18 months,[3] and dataset 4 containing 11 individuals [five with severe disease and six with mild disease]) monitored over 1 year after symptom onset.[21] Datasets 5 and 6 included putative endemic anti-virus IgG antibody waning data from our linear model relating anti-N and anti-virus IgG (appendix 1 p 5) and alternate SARS-CoV data. Dataset 5 included 30 individuals monitored for over 2 years[19] and dataset 6 included 176 individuals monitored for more than 3 years after onset of symptoms[5] (appendix 1 p 3).

Comparison of the waning antibody levels in response to infection revealed that infections by all coronaviruses were followed by similar rates of antibody decline (figure 1B–G) and half-lives to baseline (figure 2). The rates of decline of antibody levels following infection by SARS-CoV-2 (148–185 days half-life to baseline; figures 1B, 2) and endemic coronaviruses HCoV-OC43 (109–164 days; figures 1E, 2) and HCov-229E (109–144 days; figures 1G, 2) were similar. The decline following infection by HCoV-NL63 was notably longer, with estimates of half-life to baseline being 207–386 days. Estimates for SARS-CoV also indicate a longer half-life to baseline; however, the degree of that longer half-life is variable between anti-N IgG datasets.[19,22] Estimates for half-life to baseline following MERS-CoV infection are inconsistent between anti-S IgG antibody datasets,[3,21] leading to considerable uncertainty regarding the typical rate following infection by this virus across the range of declines exhibited by other human-infecting coronaviruses. All of these results were consistent regardless of whether a chronogram or a molecular evolutionary tree was used, and regardless of which method of phylogenetic inference was used (appendix 2 pp 7–8).

Our ancestral and descendent states analysis of the logistic regression parameters for the time-dependent probabilities of reinfection revealed the relationships between the antibody waning profile (figure 1B–G) and the probabilities of reinfection given antibody levels across human-infecting coronaviruses (figures 1H–M, 3A–F). SARS-CoV-2 exhibited the comparatively lowest probabilities of remaining reinfection-free through time (figure 3A). This low probability of remaining reinfection-free for SARS-CoV-2 arises jointly from the moderately fast rate of antibody decline (figure 1B) and a higher probability of infection given a specified antibody level (figure 1H). All of these results were again consistent regardless of whether a chronogram or a molecular evolutionary tree was used, and regardless of which method of phylogenetic inference was used (appendix 2 pp 9–10). The estimated median time to reinfection following peak antibody response for SARS-CoV-2 is 16 months (figure 3A), with alternate compositions of the antibody waning datasets producing estimates ranging from 16 to 21 months

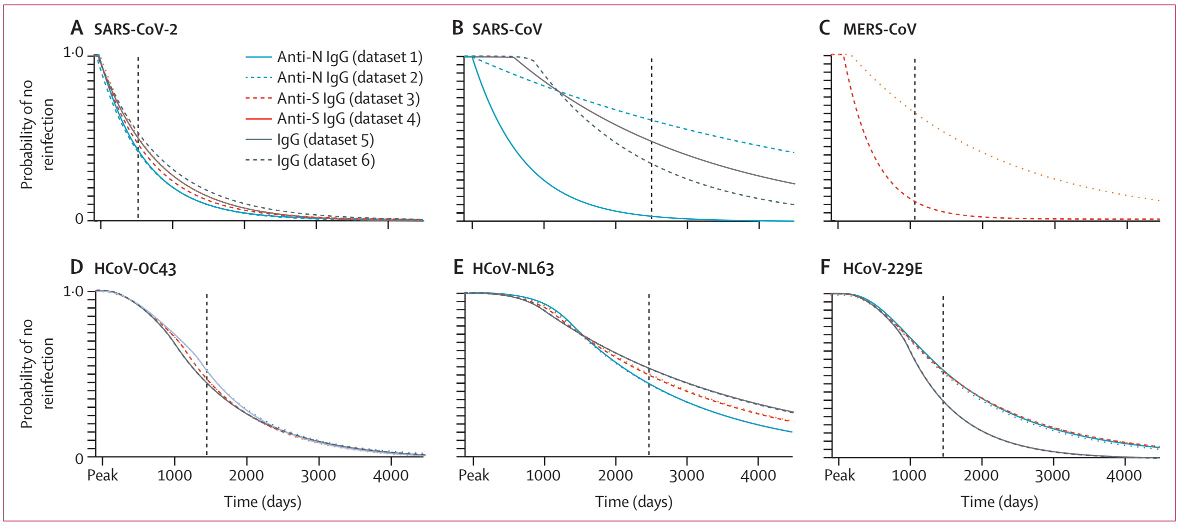

***Figure 3:* Probability of remaining free of reinfection over time and median times to reinfection for human-infecting coronaviruses SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-OC43, HCoV-NL63, and HCoV-229E.**
Probability of remaining free of reinfection (curves) and median times to reinfection (black dashed vertical line) resulting from analyses of datasets 1–6, in days from peak antibody level at 3 months. HCoV=human coronavirus.

(appendix 2 p 11). The consistency of median reinfection time estimates across antibody datasets reflects a strong correlation between post-peak infection levels of anti-N IgG and anti-S IgG antibodies (r^2=0·998; p<0·0001) and levels of anti-N and anti-virus IgG antibodies (r^2=0·904; p=0·0006; appendix 1 p 4–5; appendix 2 p 12).

Collectively, there is substantial heterogeneity in antibody decline and reinfection probabilities through time among human-infecting coronaviruses. Nevertheless, all viral lineages exhibited substantial overlap in their probabilities of reinfection over time (figure 1 N–S), revealing the evolutionary conservation of the immunological relationship between coronaviruses and humans. For SARS-CoV-2, our primary analysis yielded 5–95% quantiles of 3 months to 5·1 years after peak antibody response (figure 1N). Quantiles for other viruses spanned a typically later and distinctly wider range with 4 months to 6 years for SARS-CoV, 15 months to 10 years for HCoV-OC43, 31 months to 12 years for HCoV-NL63, and 16 months to 12 years for HCoV-229E (figure 1O, Q–S). The 5–95% quantiles for SARS-CoV-2, HCoV-OC43, HCoV-NL63, and HCoV-229E were very similar across datasets 1–6. For SARS-CoV and MERS-CoV, there was greater sensitivity to the dataset employed (figure 1N; appendix 2 p 11).

Discussion

In this study we have inferred phylogenetic relationships among the human-infecting coronaviruses, demonstrating that a phylogenetic analysis of the ancestral and descendent states can inform our understanding of virus-specific waning of antibodies post-infection, the probability of infection at a given antibody level, and the distribution of likely times to reinfection. Our analyses show that both the waning antibody profiles and the probabilities of infection at a given antibody level are heterogeneous among human-infecting coronaviruses. Quantifying both of these parameters by ancestral and descendent states analysis enabled us to infer a timescale to likely reinfection for each coronavirus. Reinfection by SARS-CoV-2 under endemic conditions would likely occur between 3 and 63 months after peak antibody response, with a median of 16 months. This protection is of less than half the duration revealed for the endemic coronaviruses circulating among humans.

Our estimated times to reinfection are consistent with the low numbers of validated cases of reinfection. However, our results caution that reinfection will become increasingly common as pandemic disease transitions into endemic disease. Our estimated timing of the waning of immunity can facilitate quantitative analyses of all policy decision making about individuals who have recovered from COVID-19 and who might be viewed as temporarily immune to reinfection. In particular, our estimate argues strongly against the claim that a long-standing resolution of the epidemic could arise due to herd immunity from natural infection or that mitigation of the long-term risks of morbidity and mortality can be achieved without vaccination. Relying on herd immunity without widespread vaccination jeopardises millions of lives, entailing high rates of reinfection, morbidity, and death. In areas with low vaccination, our data-driven analysis reinforces the need for continued safety practices such as social distancing, proper indoor ventilation, and mask wearing to avoid reinfection as pandemic conditions continue. These estimates of the likely time course of SARS-CoV-2 reinfection also have implications for travel restrictions, decisions regarding how students obtain their education, as well as the opening and closing of economic sectors in response to predictive models of the epidemic.[24] Epidemiological modelling, which has served a crucial role in public health policy and disease management in the time of COVID-19, has been restricted in time scale and vague in long-term implications because of the absence of any previous rigorous base-case estimate of the time of waning of immunity for SARS-CoV-2.[24,25] Further modelling in light of our results is warranted.

Our estimates should be understood as a prediction of probabilistic immunity through time, underscoring a concept in which there is no fixed durability of immunity or absolute protection from infection. This approach contrasts with other approaches that classify reinfection risk for an individual based on a specific threshold antibody level.[26] Such a binary distinction forces an artificial categorisation of risk that could provide unintentionally misleading scientific and public health messaging. The probabilistic framework for reinfection enables the adoption of quantitative modelling that accounts for individual low-probability events such as short-term reinfections. For an individual, such a reinfection is extremely unlikely. However, during a pandemic with hundreds of thousands of individuals being infected, occurrence of these rare events at quantifiable frequencies is highly probable and might have substantial public health implications.

Our study has several limitations. First, our study was limited by the absence of longitudinal data gathered on anti-S IgG and anti-virus IgG antibody response to endemic coronavirus infection, which obligated us to rely for some of our analyses on imputation based on the high correlations among antibodies to some targets (anti-N and anti-S, and anti-virus and anti-S). Moreover, the antibody declines and infection probabilities determined by long-term studies of SARS-CoV, MERS-CoV, HCoV-229E, HCoV-OC43, and HCoV-NL63 that we used in our analyses are averaged among an unfortunately small number of infected individuals; any one individual might have longer or shorter durations of immunity. For an individual, reinfection risks depend on immune status, infection severity, cross-immunity, age, and other immunological factors such as T-cell and B-cell memory or lack of antibody neutralising capacity.[27–29] The probabilistic framework of our analysis does not capture these aspects, their interactions, and other aspects of SARS-CoV-2 infection that merit special attention. For example, asymptomatic infection by SARS-CoV-2 can induce a weaker immune response than symptomatic infection,[2] which in turn would result in lower production of antibodies, and consequently shorter-term resistance against reinfection over time. This observation is of particular importance as reinfection can lead to lower infection severity than primary infection.[30] For predictive modelling of epidemiology that is dependent on the consequences of natural infections, it might be important to recognise lower waning times of immunity depending on symptomaticity.[25]

An additional limitation is that protective immunity consists of both humoral (antibody based) immunity and cell-mediated immunity conferred by cooperation between B and $CD4^+$ and $CD8^+$ T cells.[28] The identification of B-cell and T-cell populations—including their quantity, subsets, effector or memory phenotype, or persistence—could be more directly causal of immunity or better indicators of the durability of immunity than antibody level alone. Although antibody levels have been shown to correlate with protection from SARS-CoV-2 in humans in specific high attack rate settings[27] and for severe disease,[31] emerging studies have shown the action of memory B cells and memory and effector T cells and their cytokines after infection with the various coronaviruses.[32,33] It would be worthwhile to collect longitudinal data on these immunological traits for the various endemic human-infecting coronaviruses and for historical zoonotic human-infecting coronaviruses, so that their potentially higher explanatory power regarding immunity could be incorporated into a correlated-trait ancestral and descendent states analysis.[8] Regardless of the nature of the components of the immune response that are most immediately causal of immunity, the inferential basis of our analysis relies only on the correlation between antibody level and reinfection in endemic human coronaviruses. Given the close evolutionary relationships of human-infecting coronaviruses, it is probable that immunological correlates are similar among the human-infecting endemic and zoonotic coronaviruses.

Undue public confidence in the long-term durability of immunity following natural infection by SARS-CoV-2 has been shown to contribute to vaccine hesitancy,[34] perhaps because of a false equivalence with the long-term immunity after natural recovery from evolutionarily divergent viruses causing diseases such as measles, mumps, and rubella. By contrast, numerous respiratory viruses such as influenza, human rhinoviruses, or coronaviruses can overcome the immunity conferred by previous infections by evolving new variants in the protein domains most frequently surveilled and targeted by the human immune system. Just over a year into the COVID-19 pandemic, novel SARS-CoV-2 variants that can vary in severity of infection and evoke differential immune system responses and that can thwart the durability of immunity started arising.[35] Such novel variants probably play a similar evolutionary role in the persistence of lower-severity, endemic human coronaviruses.[4] Mitigation of the potential evolution of immune-evading SARS-CoV-2 variants in the near-future might depend crucially on a rapid global deployment of vaccination, which can induce higher immunogenicity than natural infection.[36]

The hallmark of the modern world is going to be the evolution of new threats to human health. Evolutionary biology, which provided the theoretical foundations for these analyses, is traditionally considered a historical discipline. However, our findings underscore its important role in informing decision making. Our results provide a crucial stepping stone toward robust knowledge of our prospects of resistance to SARS-CoV-2 reinfection. These prospects can guide myriad public health decisions until a long-term cohort study comprehensively and definitively quantifying SARS-CoV-2 reinfection risks becomes feasible. When more data become available on antibody declines following vaccination, our approach could be extended to assess which vaccines provide longer immunity than natural infection and stronger protection against emerging variants. Moreover, evolutionary immunological inference can be deployed on future emerging diseases, rapidly informing critical gaps in knowledge necessary for effective pandemic response.

Contributors

JPT conceived the project and designed the study. JPT, NHR, AD, JS, ZW, and HBH did the literature review and assessed suitable datasets. AD, JPT, and HBH accessed and processed anti-N IgG, anti-S IgG, and anti-virus IgG antibody data for each virus. HBH accessed and processed all virus sequence data. JPT developed and coded analytical approaches and piloted their application to data. HBH performed analyses with contributions from AD, SM, ZW, and SK. AD, HBH, and JPT designed and implemented data visualisations. JPT and AD wrote the manuscript, APG and SK edited the manuscript, and all authors reviewed the manuscript before submission. All authors approved the manuscript and JPT and AD had the final decision to submit for publication. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. Data were verified by JPT, HBH, NHR, and AD.

Declaration of interests

We declare no competing interests.

Data sharing

Data and code are publicly available. Supplemental tables, alignments, phylogenies, and code used to generate these analyses are available on Zenodo at DOI:10.5281/zenodo.5146327.

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References

1 Wajnberg A, Mansour M, Leven E, et al. Humoral immune response and prolonged PCR positivity in a cohort of 1343 SARS-CoV 2 patients in the New York City region. *medRxiv* 2020; published online May 5. https://doi.org/10.1101/2020.04.30.20085613.

2 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.

3 Alshukairi AN, Khalid I, Ahmed WA, et al. Antibody response and disease severity in healthcare worker MERS survivors. *Emerg Infect Dis* 2016; **22:** 1113–15.

4 Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol Infect* 1990; **105:** 435–46.

5 Wu L-P, Wang N-C, Chang Y-H, et al. Duration of antibody responses after severe acute respiratory syndrome. *Emerg Infect Dis* 2007; **13:** 1562–64.

6 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.

7 Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. *Nat Med* 2020; **26:** 1691–93.

8 Goolsby EW, Bruggeman J, Ané C. Rphylopars: fast multivariate phylogenetic comparative methods for missing data and within-species variation. *Methods Ecol Evol* 2017; **8:** 22–27.

9 van Dongen S, Abreu-Goodger C. Using MCL to extract clusters from networks. *Methods Mol Biol* 2012; **804:** 281–95.

10 Brohée S, van Helden J. Evaluation of clustering algorithms for protein-protein interaction networks. *BMC Bioinformatics* 2006; **7:** 488.

11 Abascal F, Zardoya R, Telford MJ. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. *Nucleic Acids Res* 2010; **38** (suppl 2): W7-13.

12 Minh BQ, Schmidt HA, Chernomor O, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol* 2020; **37:** 1530–34.

13 Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014; **30:** 1312–13.

14 To T-H, Jung M, Lycett S, Gascuel O. Fast dating using least-squares criteria and algorithms. *Syst Biol* 2016; **65:** 82–97.

15 Tamura K, Battistuzzi FU, Billing-Ross P, Murillo O, Filipski A, Kumar S. Estimating divergence times in large molecular phylogenies. *Proc Natl Acad Sci USA* 2012; **109:** 19333–38.

16 Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 2018; **35:** 1547–49.

17 Chan JF-W, To KK-W, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. *Trends Microbiol* 2013; **21:** 544–55.

18 Boni MF, Lemey P, Jiang X, et al. Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nat Microbiol* 2020; **5:** 1408–17.

19 Li T, Xie J, He Y, et al. Long-term persistence of robust antibody and cytotoxic T cell responses in recovered patients infected with SARS coronavirus. *PLoS One* 2006; **1:** e24.

20 Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020; **383:** 1724–34.

21 Choe PG, Perera RAPM, Park WB, et al. MERS-CoV antibody responses 1 year after symptom onset, South Korea, 2015. *Emerg Infect Dis* 2017; **23:** 1079–84.

22 Woo PCY, Lau SKP, Wong BHL, et al. Longitudinal profile of immunoglobulin G (IgG), IgM, and IgA antibodies against the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in patients with pneumonia due to the SARS coronavirus. *Clin Diagn Lab Immunol* 2004; **11:** 665–68.

23 Fu X, Fang B, Liu Y, et al. Newly emerged porcine enteric alphacoronavirus in southern China: identification, origin and evolutionary history analysis. *Infect Genet Evol* 2018; **62:** 179–87.

24 Kissler SM, Tedijanto C, Goldstein E, Grad YH, Lipsitch M. Projecting the transmission dynamics of SARS-CoV-2 through the postpandemic period. *Science* 2020; **368:** 860–68.

25 Holmdahl I, Buckee C. Wrong but useful—What COVID-19 epidemiologic models can and cannot tell us. *N Engl J Med* 2020; **383:** 303–05.

26 Sui Y, Bekele Y, Berzofsky JA. Potential SARS-CoV-2 immune correlates of protection in infection and vaccine immunization. *Pathogens* 2021; **10:** 138.

27 Addetia A, Crawford KHD, Dingens A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol* 2020; **58:** 21.

28 Cox RJ, Brokstad KA. Not just antibodies: B cells and T cells mediate immunity to COVID-19. *Nat Rev Immunol* 2020; **20:** 581–82.

29 Gaebler C, Wang Z, Lorenzi JCC, et al. Evolution of antibody immunity to SARS-CoV-2. *Nature* 2021; **591:** 639–44.

30 Hanrath AT, Payne BAI, Duncan CJA. Prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection. *J Infect* 2021; **82:** e29–30.

31 Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27:** 1205–11.

32 Mateus J, Grifoni A, Tarke A, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science* 2020; **370:** 89–94.

33 Iwasaki A, Yang Y. The potential danger of suboptimal antibody responses in COVID-19. *Nat Rev Immunol* 2020; **20:** 339–41.

34 Gerussi V, Peghin M, Palese A, et al. Vaccine hesitancy among Italian patients recovered from COVID-19 infection towards influenza and SARS-CoV-2 vaccination. *Vaccines (Basel)* 2021; **9:** 172.

35 Wheatley AK, Juno JA, Wang JJ, et al. Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat Commun* 2021; **12:** 1162.

36 Callaway E, Ledford H. How to redesign COVID vaccines so they protect against variants. *Nature* 2021; **590:** 15–16.