Evolution of Early SARS-CoV-2 and Cross-Coronavirus Immunity

Carolin Loos,a,b Caroline Atyeo,a,c Stephanie Fischinger,a,d John Burke,a Matthew D. Slein,a Hendrik Streeck,e Douglas Lauffenburger,b Edward T. Ryan,f,g,h Richelle C. Charles,f,g Galit Altera

aRagon Institute of MGH, MIT, and Harvard, Cambridge, Massachusetts, USA
bMassachusetts Institute of Technology, Cambridge, Massachusetts, USA
cHarvard Virology Program, Boston, Massachusetts, USA
dVirology and Immunology Program, University of Duisburg-Essen, Essen, Germany
eDepartment of Virology, University Hospital Bonn, Bonn, Germany
fDivision of Infectious Disease, Massachusetts General Hospital, Boston, Massachusetts, USA
ghHarvard Medical School, Boston, Massachusetts, USA
igHarvard T.H. Chan School of Public Health, Boston, Massachusetts, USA

Carolin Loos, Caroline Atyeo, and Stephanie Fischinger contributed equally; author order was determined in order of decreasing seniority.

ABSTRACT The novel coronavirus, SARS-coronavirus (CoV)-2 (SARS-CoV-2), has caused over 17 million infections in just a few months, with disease manifestations ranging from largely asymptomatic infection to critically severe disease. The remarkable spread and unpredictable disease outcomes continue to challenge management of this infection. Among the hypotheses to explain the heterogeneity of symptoms is the possibility that exposure to other coronaviruses (CoVs), or overall higher capability to develop immunity against respiratory pathogens, may influence the evolution of immunity to SARS-CoV-2. Thus, we profiled the immune response across multiple coronavirus receptor binding domains (RBDs), respiratory viruses, and SARS-CoV-2, to determine whether heterologous immunity to other CoV-RBDs or other infections influenced the evolution of the SARS-CoV-2 humoral immune response. Overall changes in subclass, isotype, and Fc-receptor binding were profiled broadly across a cohort of 43 individuals against different coronaviruses—RBDs of SARS-CoV-2 and the more common HKU1 and NL63 viruses. We found rapid functional evolution of responses to SARS-CoV-2 over time, along with broad but relatively more time-invariant responses to the more common CoVs. Moreover, there was little evidence of correlation between SARS-CoV-2 responses and HKU1, NL63, and respiratory infection (influenza and respiratory syncytial virus) responses. These findings suggest that common viral infections including common CoV immunity, targeting the receptor binding domain involved in viral infection, do not appear to influence the rapid functional evolution of SARS-CoV-2 immunity, and thus should not impact diagnostics or shape vaccine-induced immunity.

IMPORTANCE A critical step to ending the spread of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the ability to detect, diagnose, and understand why some individuals develop mild and others develop severe disease. For example, defining the early evolutionary patterns of humoral immunity to SARS-CoV-2, and whether prevalent coronaviruses or other common infections influence the evolution of immunity, remains poorly understood but could inform diagnostic and vaccine development. Here, we deeply profiled the evolution of SARS-CoV-2 immunity, and how it is influenced by other confections. Our data suggest an early and rapid rise in functional humoral immunity in the first 2 weeks of infection across antigen-specific targets, which is negligibly influenced by cross-reactivity to addi-
tional common coronaviruses or common respiratory infections. These data suggest that preexisting receptor binding domain-specific immunity does not influence or bias the evolution of immunity to SARS-CoV-2 and should have negligible influence on shaping diagnostic or vaccine-induced immunity.

**KEYWORDS** Fc-receptor binding, SARS-CoV-2, antibody response, cross-reactivity

The SARS-CoV-2 pandemic continues to burden the health care system and has had a major impact on the global economy and social dynamics. While coronaviruses (CoVs) have entered into the human population repeatedly over the past century (1), severe outbreaks with this family of viruses are rare, with reported outbreaks in 2002 caused by the severe acute respiratory syndrome coronavirus (SARS-CoV-1) (2) and in 2012 caused by the Middle East respiratory syndrome coronavirus (MERS-CoV), resulting in 10% and 40% mortality, respectively (3). While SARS-CoV-2 infection appears to be less lethal than SARS-CoV-1 and MERS-CoV, the absence of detailed data on the rate of asymptomatic or mild infections, which do not prompt medical attention, has complicated a true comparison of mortality rates. Well-documented transmission by pre- or asymptomatically infected humans has contributed to the remarkable speed and often uncontrollable spread of SARS-CoV-2. In addition to these lethal CoVs, other CoVs, such as HKU1 and NL63, cause milder influenza virus-like disease (1, 4). Despite their broad prevalence, repeated infections occur with these CoVs over life, hypothesized to occur due to the poor durability of the immune response to these viruses (5). However, despite the lack of seasonal protection against common CoVs, speculations have arisen related to the potential role of cross-CoV immunity in shaping the response to SARS-CoV-2.

SARS-CoV-1 and SARS-CoV-2 share 74% identity, even in the receptor binding domain (RBD), the most variable part of the coronavirus genome (2, 6). In contrast, other coronaviruses such as the NL63 RBD exhibit only 20% sequence identity and HKU1 harbors 2% sequence identity with the SARS-CoV-2 RBD (7). Thus, this limited sequence identity suggests little potential cross-reactivity between the sequences. However, given the increasing possible influence of preexisting cross-reactive antibodies on potential protection or disease enhancement (8), here we aimed to deeply profile and determine whether previous common CoV immunity shapes the evolution of the response to SARS-CoV-2. Both levels of subclasses/isotypes and Fc-receptor binding profiles were interrogated across the RBDs of several CoVs, capturing overall levels and recent inflammatory status of the humoral immune responses. The study highlights the rapid evolution of a robust and highly functional humoral immune response to SARS-CoV-2. However, common CoV RBD-specific immunity appears to have limited to no impact on shaping the SARS-CoV-2 response.

**RESULTS**

Dissecting the early evolution of SARS-CoV-2 humoral immunity. In order to decipher the humoral immune response to SARS-CoV-2 and cross-reactivity to other common coronaviruses, quantitative data for a cross-sectional sample set of 43 individuals captured at variable time points after symptom onset and hospitalization were generated and analyzed. The heatmap (Fig. 1A) displays the immune responses to SARS-CoV-2 spike (S), nucleocapsid (N), and the spike receptor binding domain (RBD) across subjects, with lower antibody reactivity in non-SARS-CoV-2-infected individuals. To gain a deeper multidimensional analysis of the data, principal-component analysis (PCA) showed expected distinct antibody profiles among RNA⁻ and RNA⁺ individuals (Fig. 1B), where individuals were considered to be RNA⁻ if they had a negative nasopharyngeal (NP) swab PCR test. Within the RNA⁺ individuals, individuals who passed away due to coronavirus disease (COVID-19) were generally older (Fig. 1C). While limited differences were observed between female and male participants, samples from RNA⁺ individuals early following symptom onset (up to 6 days) clustered with
individuals, whereas antibody responses clearly evolved in samples drawn more than 6 days from symptom onset (Fig. 1D).

To explore the kinetics of the evolution of the humoral immune response to SARS-CoV-2, we stratified individuals by time from symptom onset. The evolutions of the S-, N-, and RBD-specific immune responses were compared. Comparable induction of IgG1 responses was observed across all three antigens, emerging in nearly all individuals by day 14 following symptom onset, as has been previously observed (9) (Fig. 2). Similar kinetics were observed for IgG3, an early highly functional antibody subclass (10), particularly for N-specific immunity; these N-specific IgG3 responses appeared to track with background cross-reactive IgG3 responses. More erratic and
inconsistent IgG2 and IgG4 responses were observed across the population, albeit more robustly to N, as expected given that these antibody subclasses are less functional and largely selected in the context of nonviral disease (11). Conversely, despite some baseline cross-reactivity in RNA− individuals, robust IgA1 and IgM evolution was observed across antigens (12). Interestingly, IgA1 and IgM responses seem to have emerged synchronously and slightly earlier than IgG1 responses, capturing all infected individuals by day 10, highlighting the unusual class-switching and potential utility of these isotypes in early detection.

Beyond isotype/subclass detection, Fcγ-receptor (FcγR) binding represents a marker of induction of highly functional and proinflammatory antibodies (13). Rapid evolution of broad FcγR binding antibodies was observed across antigens, with early and highly specific detection of FcγRIIA binding antibodies by day 10 following symptom onset, with no background reactivity. Similar profiles were observed across the Fc-receptors, despite some low-level background reactivity. An early rise in N-specific FcγRIII immunity was observed for FcγRIII binding antibodies. Along these lines, a similar early increase in N-specific IgA1, IgM, IgG2, IgG3, and FcγRIII binding was detected compared to RBD and S. Although less clear for IgG1 and FcγRIIA, this early rise in N-specific immunity may be related to the earlier and more abundant expression of nucleocapsid transcripts during viral infection (14).

**Probing the influence of cross-reactivity to RBDs of other CoVs on SARS-CoV-2 humoral evolution.** The presence of low-level IgM and IgA1 SARS-CoV-2 binding among RNA− individuals pointed to either potential cross-reactivity to other common CoVs or preexistence of immunity among these early and mucosal responses (15). Given the relatively high seroprevalence of common CoVs, questions have been raised related to the potential influence of these responses on the overall trajectory and quality of the humoral immune response to SARS-CoV-2. Despite the mild sequence identity among the common CoVs, we next compared the overall humoral profiles across common CoV-RBDs (HKU1 and NL63) and other respiratory viruses (influenza virus and respiratory syncytial virus [RSV]). Thus, we analyzed the differences in antibody titers across this spectrum of antigens between SARS-CoV-2-positive and -negative individuals (Fig. 3A). While there was a clear enrichment of responses to SARS-CoV-2 antigens among SARS-CoV-2-infected individuals, no strong differences
Given the more profound differentiation of SARS-CoV-2-infected individuals by IgA1 and IgM immunity (Fig. 3A), we next aimed to dissect the dynamics of the changes in the response to SARS-CoV-2. Low-level cross-reactivity was observed for IgM, IgA1, and FcγRIIB for the SARS-CoV2 N-specific response (Fig. 3B). Conversely, an expansion of IgA1 and IgM N-specific humoral immunity during the early days of infection (0 to 6 days from symptom onset) was observed, followed by RBD- and then S-specific humoral profiles. This cross-reactivity to N may be explained by the fact that N is highly conserved between coronaviruses (16–18) such that preexisting antibodies specific to the N of coronaviruses may be able to bind to the N of SARS-CoV-2. Yet, the responses increased substantially across all antigens (Fig. 3B). Whether these early responses and trajectories were linked to the potential presence of N-cross-reactivity, where class-switched antibodies may represent a surrogate for a helper T cell response, remains uncertain.
To parse the potential influence of cross-CoV-reactivity on shaping the trajectory of the SARS-CoV-2-specific response, the overall profile of reactivity was probed against HKU1, NL63, influenza virus, and RSV (Fig. 3C). A highly synchronized IgG, IgA1, and IgM response was noted across individuals, linked to robust evolution of Fcγ/H9253 receptor binding profile. Detectable IgG1 and IgA1 responses were noted to both common CoVs, linked to robust FcγRIIA and FcγRIIIA binding antibodies. Similar IgG1 and IgA1 responses were observed to influenza virus, associated with more functional FcγR binding profiles. Conversely, a broader antibody subclass/isotype and FcγR binding profile was seen for RSV. However, importantly, notable differences were not found in the overall response profile to any of these pathogens across non-SARS-CoV-2-infected, early SARS-CoV-2-infected, or later SARS-CoV-2-infected individuals, which should expand in synchrony to SARS-CoV-2 immunity if cross-reactive. Due to the lack of substantive profile changes across these pathogens with SARS-CoV-2 infection, these data argue for limited cross-reactivity or influence across the responses.

We next studied the relationship between SARS-CoV-2 immunity across antigens and across other pathogens (Fig. 4). Strong correlations were observed across the SARS-CoV-2 antigens, highlighting the coordinated induction of highly functional immunity, across isotype/subclass/Fc-receptor binding, to the RBD, S, and N antigens. While some positive relationships were observed among the IgG2, IgG4, and IgM response to the common CoVs and SARS-CoV-2 RBD-specific immunity, most relationships between the SARS-CoV-2 response and common CoVs, influenza virus, and RSV were largely driven by individuals exhibiting low to undetectable titers. Overall, these data suggest that preexisting CoV RBD-specific and other pathogen immunity plays a limited role in shaping SARS-CoV-2 humoral immune responses.

DISCUSSION

The recent outbreak of SARS-CoV-2 has altered the globe due to its unprecedented speed of dissemination. Treatment of infection has been hampered by our lack of knowledge related to the underlying mechanisms that drive heterogeneous disease
outcomes. While the majority of individuals appear to experience mild disease, it remains unclear why a fraction of those infected go on to develop severe and lethal disease. Comorbidities including obesity, heart disease, etc., have been clearly linked to poor disease outcomes (19). However, given the prevalence of other CoVs in the population, hypotheses have emerged related to the potential for cross-CoV immunity. Yet, little is known about the prevalence of CoV-specific immunity at a population level and how it may influence the evolution of SARS-CoV-2 immunity. Similar to previous reports, we observed the development of robust virus-specific IgG, IgM, and IgA1 responses within the first 2 weeks of symptoms (20). This humoral evolution was marked by the rapid evolution of Fc-receptor binding antibodies (Fig. 2 and 3A), highlighting the functional nature of the humoral immune response to SARS-CoV-2. In contrast, the humoral response to the RBDs of more common CoVs was equivalent across SARS-CoV-2 RNA⁺ and RNA⁻ individuals and did not shift with the evolution of infection. While unlikely, based on clinical presentation, it is possible that some of the SARS-CoV-2 RNA⁻ individuals were infected but were not captured by the PCR. However, if this is the case, these individuals would likely be early in their infection course, based on their humoral profile, with low antibody levels similar to the SARS-CoV-2 RNA⁺ individuals within the first 6 days (Fig. 1A). Given the lack of sequence similarity across SARS-CoV-2 and the common CoVs, these data point to the limited influence of cross-CoV immunity on shaping SARS-CoV-2 responses.

HKU1 and NL63 share 20%/26% and 2%/19% similarity with SARS-CoV-2 (RBD/S) (7), respectively, are structurally remarkably distinct, and are therefore unlikely to contribute strongly to cross-reactivity. However, due to enhanced similarity in other genes, including the nucleocapsid, cross-protective immunity may emerge not only at the level of antibody cross-reactivity. Specifically, the presence of cross-reactive T cell immunity (21, 22), targeting conserved linear regions of the virus, could preexist and support the more rapid selection and boosting of humoral immune responses that could then drive enhanced control/protection against SARS-CoV-2. However, if T cell boosting could propagate more effective SARS-CoV-2 immune responses, a shift in the original CoV-humoral immune profile might be observable. No changes were observed in CoV immunity, other than SARS-CoV-2 responses, highlighting the remarkably restricted evolution of the humoral immune response to this CoV alone. Yet, here we used only the RBD from several CoVs, due to its immunologic importance in neutralizing antibody-mediated blockade of infection, which is likely to be key to cross-CoV immunity. Instead, cross-CoV immunity may emerge outside the RBD and happen in a genus-restricted manner potentially explained by conservation of the S2 subunit (23). Further analysis may be required to rule out the possibility of the influence of cross-reactivity on shaping SARS-CoV-2 immunity.

Beyond the potential role of cross-CoV immunity in shaping the initial response to the virus, it is plausible that cross-CoV responses could evolve following SARS-CoV-2 infection to more similar CoVs, such as SARS-CoV-1 (70% identity) and MERS-CoV (50% identity) (24). These similarities may translate to the development of cross-reactive neutralizing antibodies (25). Recent studies have demonstrated that neutralizing antibodies develop in most individuals and seem to be biomarkers of disease progression, with higher neutralizing antibody levels in older individuals and individuals with more severe disease (26). Beyond neutralization, antibodies can also drive innate immune functions, including antibody-dependent cellular cytotoxicity (ADCC), by binding to FcRs which appear to be more sensitive at picking up infection. Here, we find that SARS-CoV-2-specific FcγR binding emerged rapidly following symptom onset, potentially emerging as a more sensitive marker of infection. Therefore, since antibody functions are correlated with FcγR binding, it is likely that individuals induce functional antibodies early in infection. Future studies should explore the timing of functional antibody induction and whether certain antibody functions are important for clearance of SARS-CoV-2 infection. Moreover, in recent nonhuman primate rechallenge and vaccine studies, neutralizing and functional antibodies to RBD and S were shown to
predict protection (27, 28), indicating an important immunological role of these anti-
gens in immune protection.

Slowing the spread of the SARS-CoV-2 pandemic will require widespread immune
testing and the development of a vaccine. Since antibodies against SARS-CoV-2 N seem
to arise earlier than antibodies against RBD and S, N-specific responses may provide
easier diagnostic value. However, some cross-reactivity to N, in RNA− individuals, may
render these responses less reliable. However, together N and S/RBD immunity may
help guide early diagnosis.

While high-quality and precise serological assays have now emerged, defining the
potential influence of cross-CoV immunity on assay performance but also with respect
to potential cross-immunity is of utmost importance. However, due to low sequence
identity between SARS-CoV-2 and more common CoVs, the data reported here point to
limited cross-CoV overlap in humoral responses. Additionally, the lack of relationship
between other respiratory pathogens and SARS-CoV-2 additionally suggests that no
intrinsic biases exist between the abilities to mount immunity to respiratory pathogens.
Thus, although preexisting immunity or enhanced respiratory immunity has been
postulated to potentially lower peak responses and shorten durability (29), the data
presented here argue that preexisting immunity does not influence SARS-CoV-2 and
thus should not influence diagnostics or vaccine-induced immunity. Given the similarity
between SARS-CoV-1 and the possibility for future bat-derived CoVs, a multivalent
vaccine able to drive immunity to distinct RBDs may ultimately be necessary to protect
different CoV pathogens. Therefore, further research into cross-reactivity of
antibodies, especially postvaccination, is needed to decipher the humoral protective
immune profile needed not only to end this pandemic but also to prevent future
outbreaks caused by CoVs.

**MATERIALS AND METHODS**

**Sample set.** Blood samples from SARS-CoV-2 RNA− (n = 26) and RNA− (n = 17) individuals who were
admitted to Massachusetts General Hospital were collected in this study between 13 March 2020 and 31
March 2020. Clinical information on their disease outcome (deceased/discharged), gender, age, and
symptom onset were collected (see Table S1 in the supplemental material). The RNA− patients had fever
and or symptoms consistent with a respiratory viral infection. This research was approved by the
Institutional Review Board of Massachusetts General Hospital, IRB approval no. 2007P002451.

**Subclassing and isotyping via Luminex.** In order to quantify the antigen-specific antibody titer per
subclass and isotype as well as Fcγ-receptor levels, a customized Luminex subclassing assay was used
(30). Due to the sample-sparing and multiplex-able nature of the assay, we used a Luminex assay to
capture data on these samples, following the confirmation of Luminex performance to a qualified
enzyme-linked immunosorbent assay (ELISA). Fluorescent carboxyl-modified microspheres (Luminex) were
coupled with different antigens: SARS-CoV-2 S (kindly provided by Bing Chen), SARS-CoV-2 RBD,
CoV-HKU1 RBD (accession no. AY597011, amino acid [aa] residues 310 to 677), CoV-NL63 RBD (accession
no. AKT07952, aa residues 481 to 616) (kindly provided by Aaron Schmidt), SARS-CoV-2 N (Aalto Bio
Reagents), influenza virus antigen mix [HA(ΔTM)(A/California/04/2009)(H1N1), HA1(B/Massachusetts/2/
2012), and HA1(A/Texas/50/2012)(H3N2)—all from ImmuneTech], and RSV postfusion (NIH). The sequences of HKU1 and NL63 RBD were cloned into the pVRC vector with a C-terminal SBP
(streptavidin-binding peptide) tag and produced in 293F cells. Luminex bead regions were coupled
via covalent N-hydroxysuccinimide (NHS)—ester linkages utilizing EDC [1-ethyl-3-(3-dimethylamino-
propyl)carbodiimide hydrochloride] (Thermo Scientific) and sulfo-NHS (Thermo Scientific) according to
the manufacturer’s instructions. Beads (1.2 × 106 per Luminex region) were added in Luminex assay
buffer containing 0.1% bovine serum albumin (BSA) and 0.05% Tween 20 to each well of a 384-well plate
(Greiner Bio-one). Five microliters of diluted plasma samples or phosphate-buffered saline (PBS) for
background assessment (FcγR binding and IgG at a 1:500 dilution, other subclasses/isotypes at 1:100)
was added in duplicate and incubated for 16 h at 4°C while shaking at 900 rpm. The immune-complexed
microspheres were washed six times with 60 μl of Luminex assay buffer with an automated plate washer
(Tecan). Phycoerythrin (PE)-coupled IgG1-, IgG2-, IgG3-, IgG4, IgA1-, or IgM-specific detection reagents
(Southern Biotech) were added at 1.3 μg/ml in Luminex assay buffer and incubated for 1 h at room
temperature while shaking at 900 rpm. The coated beads were then washed and read on an iQue
Screener (IntelliCyt) using a robotic arm (PAA). Similarly, for the FcγR binding profiles, recombinant
FcγRIIA, FcγRIIB, FcγRIIA, and FcγRIIB (Duke Protein Production Facility) were biotinylated (Thermo
Scientific), conjugated to streptavidin-PE for 10 min (Southern Biotech) in Luminex buffer, and added at
1 μg/ml. Samples were run in duplicate for each secondary detection agent.

**Statistics.** Twice the PBS control was subtracted from each measurement, negative values were set
to 0, and subsequently values were log10(x + 1) transformed and z-scored. For the heatmap in Fig. 1A,
each row is a feature and each column corresponds to one blood sample. The columns are clustered
using complete linkage clustering within the columns of RNA− and RNA− individuals. The principal-
component analysis (PCA) was performed using the R package ‘ropls’. For the comparison of antibody responses for SARS-CoV-2 RNA° and RNA+ individuals, Mann-Whitney U tests were performed, and P values were corrected for multiple testing using the Benjamini-Hochberg correction. To assess correlations between antigens, we used Spearman rank correlations and P values were corrected for multiple testing using the Benjamini-Hochberg correction. We calculated significances for the correlation coefficients using all samples and samples for which both values are above the background. When the correlation was significant only when using all samples, we indicated it with ° in the heatmap in Fig. 1A. For the individuals with multiple time points, we used the mean levels for the difference and correlation analysis shown in Fig. 3A and Fig. 4. All analyses were performed using R version 3.6.1.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TABLE S1, TIF file, 0.7 MB.

DATA SET S1, CSV file, 0.1 MB.

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C.L., C.A., S.F., and G.A. designed the study. C.A., S.F., J.B., and M.D.S. performed all experiments. R.C.C. and E.T.R. collected and selected the clinical specimens. C.L. and D.L. performed all analyses. C.L., C.A., S.F., and G.A. wrote the manuscript. All authors contributed to the final version of the manuscript.

REFERENCES

1. Corman VM, Muth D, Niemeyer D, Drosten C. 2018. Hosts and sources of endemic human coronaviruses. Adv Virus Res 100:163–188. https://doi.org/10.1016/bs.avir.2018.01.001.

2. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J, Xiang Z, Mu Z, Chen X, Chen J, Hu K, Jin Q, Wang J, Qian Z. 2020. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 11:1620. https://doi.org/10.1038/s41467-020-15562-9.

3. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RA. 2007. The proximal origin of SARS-CoV during an outbreak in Iran: comparison with SARS and MERS. Rev Med Virol 17:189–202. https://doi.org/10.1002/rmv.1195.

4. Ye ZW, Yuan S, Yuan KS, Fung SY, Chan CP, Jin DY. 2020. Zoonotic origins of human coronaviruses. Int J Biol Sci 16:1686–1697. https://doi.org/10.7150/ijbs.45472.

5. Tang F, Quan Y, Xin Z-T, Wrammert J, Ma M-J, Lu H, Wang T-B, Yang H, Richards JH, Liu W, Cao W-C. 2011. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J Immunol 186:7264–7268. https://doi.org/10.4049/jimmunol.0903490.

6. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. 2020. The evolution of SARS-CoV-2 and cross-coronavirus immunity. mSphere 5:1038/e00622-20. https://doi.org/10.1128/mSphere.00622-20.

7. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. Nucleic Acids Res 41:D36–42. https://doi.org/10.1093/nar/gks1195.

8. Denner J. 2020. SARS-CoV-2 and enhancing antibodies. J Clin Virol 128:104424. https://doi.org/10.1016/j.jcv.2020.104424.

9. Haveri A, Smura T, Kuivanen S, Österlund P, Hepojoki J, Ikonen N, Pitkäpaasi M, Blomqvist S, Rönkkö E, Kantele A, Strandin T, Kallio-Kokko H, Mannonen L, Puumalainen T, Mäkinen M, Aalto S, Särkänen M, Salminen M, Vuolteenaho T, Nissinen M, Salminen L, Salmela H, Vapalahti O, Savolainen-Kopra C. 2020. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland. January to February 2020. Euro Surveill. 25:2000266. https://doi.org/10.2807/1560-7917.ES.2020.25.11.2000266.

10. Damelang T, Rogerson SJ, Kent SJ, Chung AW. 2019. Role of IgG3 in infectious diseases. Trends Immunol 40:197–211. https://doi.org/10.1016/j.it.2019.01.005.

11. Vidarsson G, Dekkers G, Rispens T. 2014. IgG subclasses and allotypes: from structure to effector functions. Front Immunol 5:520. https://doi.org/10.3389/fimmu.2014.00520.

12. Sun B, Feng Y, Li X, Zheng P, Wang Q, Li P, Peng P, Liu X, Chen Z, Huang H, Zhang F, Luo W, Niu X, Hu P, Wang L, Peng H, Huang Z, Feng L, Li F, Zhang F, Li F, Zhong N, Chen L. 2020. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes Infect 9:940–948. https://doi.org/10.1080/22221751.2020.1762515.

13. Mkaddem SB, Benhamou M, Monteiro RC. 2019. Understanding Fc receptor involvement in inflammatory diseases: from mechanisms to new therapeutic tools. Front Immunol 10:811. https://doi.org/10.3389/fimmu.2019.00811.

14. Rokni M, Ghasemi V, Tavakoli Z. 2020. Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: comparison with SARS and MERS. Rev Med Virol 30:e2107. https://doi.org/10.1002/rmv.2107.

15. Huang AT, Garcia-Carreras B, Hitchings MDT, Yang B, Katzelnick L, Rattigan SM, Borgert B, Moreno C, Solomon BD, Rodriguez-Barraquer I, Lessler J, Salje H, Burke DS, Wosiewolski A, Cummings DAT. 2020. A systematic review of antibody mediated immunity to coronaviruses: antibody kinetics, correlates of protection, and association of antibody responses with severity of disease. medRxiv 2020.04.14.20065771. https://doi.org/10.1101/2020.04.14.20065771.

16. Sun ZF, Meng XJ. 2004. Antigenic cross-reactivity between the nucleocapsid protein of severe acute respiratory syndrome (SARS) coronavirus and polyclonal antisera of antigenic group I animal coronaviruses: implication for SARS diagnosis. J Clin Microbiol 42:2351–2352. https://doi.org/10.1128/JCM.42.5.2351-2352.2004.

17. Vaslova AN, Zhang X, Hasokusz M, Nagesha HS, Haynes LM, Fang Y, Lu S, Sall LF. 2007. Two-way antigenic cross-reactivity between severe acute respiratory syndrome coronavirus (SARS-CoV) and group 1 animal CoVs is mediated through an antigenic site in the N-terminal region of the SARS-CoV nucleoprotein. J Virol 81:13365–13377. https://doi.org/10.1128/JVI.01169-07.
18. Krammer F, Simon V. 2020. Serology assays to manage COVID-19. Science 368:1050–1065. https://doi.org/10.1126/science.abc1227.

19. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, Ji R, Wang H, Wang Y, Zhou Y. 2020. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: a systematic review and meta-analysis. Int J Infect Dis 94:91–95. https://doi.org/10.1016/j.ijid.2020.03.017.

20. Iyer AS, Jones FK, Nodoushani A, Kelly M, Becker M, Slater D, Mills R, Teng E, Kamruzzaman M, Garcia-Beltran WF, Astudillo M, Yang D, Miller TE, Oliver E, Fischinger S, Atyeo C, Iafrate AJ, Cedarwood SB, Lauer SA, Yu J, Li Z, Feldman J, Hauser BM, Cardonna TM, Branda JA, Turbett SE, LaRocque RC, Mellon G, Barouch DH, Schmidt AG, Azman AS, Alter G, Ryan ET, Harris JB, Charles RC. 2020. Dynamics and significance of the antibody response to SARS-CoV-2 infection. medRxiv 2020.07.18.20155374. https://doi.org/10.1101/2020.07.18.20155374.

21. Braun J, Loyal L, Frentsch M, Wendisch D, Georg P, Kurth F, Hippenstiel S, Feldman J, Lauster R, Mall MA, Beyer K, Röhmel J, Voigt S, Schmitz J, Miltenyi S, Demuth I, Müller MA, Hocke A, Wittenrath M, Suttorp N, Kern F, Reimer U, Wenschuh H, Drosten C, Corman VM, Giesecke-Thiel C, Sander LE, Thiel A. 2020. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature https://doi.org/10.1038/s41586-020-2598-9.

22. Grifoni A, Weiskopf D, Ramirez SJ, Mateus J, MODERBACHER JM, Rawlings CR, Sutherland SA, Premkumar A, Jadi L, Marrasa RS, Silva D, De AM, Frazier A, Carlin A, Greenbaum JA, Peters B, Krammer F, Smith DM, Crotty S, Sette A. 2020. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 181: 1489–1501.e15. https://doi.org/10.1016/j.cell.2020.05.015.

23. Prévost J, Gasser R, Beaucaire-Cassieres G, Richard J, Duerr R, Laumaea A, Anand SP, Goyette G, Ding S, Medjahed H, Lewin A, Perreault J, Tremblay T, Gendron-Lepage G, Gauthier N, Carrier M, Marcoux D, Piché A, Lavoie M, Benoit A, Loungnarath V, Brochu G, Desforges M, Talbot PJ, Gould Maule GT, Côté M, Therrien C, Serhir B, Bazin R, Roger M, Finzi A. 2020. Cross-sectional evaluation of humoral responses against SARS-CoV-2 Spike. bioRxiv 2020.06.08.140244. https://doi.org/10.1101/2020.06.08.140244.

24. Wang H, Li X, Li T, Zhang S, Wang L, Wu X, Liu J. 2020. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur J Clin Microbiol Infect Dis 39:1629–1627. https://doi.org/10.1007/s10096-020-03899-4.

25. Poh CM, Carissimo G, Wang B, Amrun SN, Lee CY-P, Chee RS-L, Fong S-W, Yeo NK-W, Lee W-H, Torres-Ruesta A, Leo Y-S, Chen MI-C, Tan S-Y, Chai LYA, Kalimuddin S, Kheng SSG, Thien S-Y, Young BE, Lye DC, Hanson BJ, Wang C-I, Renia L, Ng LFP. 2020. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. Nat Commun 11:2806. https://doi.org/10.1038/s41467-020-16638-2.

26. Xun J, Lu L, Jiang S, Lu H, Wen Y, Huang J. 2020. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv 2020.03.30.20047365. https://doi.org/10.1101/2020.03.30.20047365.

27. Chandrashekar A, Liu J, Martinot AJ, McMahan K, Mercado NB, Peter L, Tostanoski LH, Yu J, Maliga Z, Nityanandam R, Nikolaou JP, Sanders AG, Miller AD, Baric RS, Alter G, Sorger PK, Estes JD, Andersen H, Lewis MG, Barouch DH. 2020. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. Science 369:812–817. https://doi.org/10.1126/science.abc4776.

28. Yu J, Tostanoski LH, Peter L, Mercado NB, McMahan K, Mahrokhian SH, Nkolola JP, Liu J, Li Z, Chandrashekar A, Martinez DR, Loos C, Atyeo C, Fischinger S, Burke JS, Slein MD, Pessaint L, Van Ry A, Greenhouse J, Taylor T, Blade K, Cook A, Finneyfrock B, Brown R, Teow E, Velasco J, Zahn R, Wegmann F, Abbink P, Bondzie EA, Dagotto G, Gebre MS, He X, Jacob-Dolan C, Kordana N, Li Z, Lifton MA, Mahrokhian SH, Maxfield LF, Nityanandam R, Nikolaou JP, Schmidt AG, Miller AD, Baric RS, Alter G, Sengor PK, Estes JD, Andersen H, Lewis MG, Barouch DH. 2020. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science 369:806–811. https://doi.org/10.1126/science.abc6284.

29. Zhu F-C, Li Y-H, Guan X-H, Lou Y-H, Wang W-J, Li J-X, Wu S-P, Wang B-S, Wang Z, Wang L, Jia S-Y, Jiang H-D, Wang L, Jiang T, Hu Y, Gou J-B, Xu S-B, Xu J-J, Wang X-W, Wang W, Chen W. 2020. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 395:1845–1854. https://doi.org/10.1016/S0140-6736(20)31208-3.

30. Brown EP, Licht AF, Dugast AS, Choi I, Bailey-Kellogg C, Ackerman ME. 2012. High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples. J Immunol Methods 386:117–123. https://doi.org/10.1016/j.jim.2012.09.007.