Pre-existing and de novo humoral immunity to SARS-CoV-2 in humans

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Abstract

Several related human coronaviruses (HCoVs) are endemic in the human population, causing mild respiratory infections\(^1\). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiologic agent of Coronavirus disease 2019 (COVID-19), is a recent zoonotic infection that has quickly reached pandemic spread\(^2,3\). Zoonotic introduction of novel coronaviruses is thought to occur in the absence of pre-existing immunity in the target human population. Using diverse assays for detection of antibodies reactive with the SARS-CoV-2 Spike (S) glycoprotein, we demonstrate the presence of pre-existing immunity in uninfected and unexposed humans to the new coronavirus. SARS-CoV-2 S-reactive antibodies, exclusively of the IgG class, were readily detectable by a sensitive flow cytometry-based method in SARS-CoV-2-uninfected individuals with recent HCoV infection and targeted the S2 subunit. In contrast, SARS-CoV-2 infection induced higher titres of SARS-CoV-2 S-reactive IgG antibodies, as well as concomitant IgM and IgA antibodies throughout the observation period of 6 weeks since symptoms onset. HCoV patient sera also variably reacted with SARS-CoV-2 S and nucleocapsid (N), but not with the S1 subunit or the receptor binding domain (RBD) of S on standard enzyme immunoassays. Notably, HCoV patient sera exhibited specific neutralising activity against SARS-CoV-2 S pseudotypes, according to levels of SARS-CoV-2 S-binding IgG and with efficiencies comparable to those of COVID-19 patient sera. Distinguishing pre-existing and de novo antibody responses to SARS-CoV-2 will be critical for serology, seroprevalence and vaccine studies, as well as for our understanding of susceptibility to and natural course of SARS-CoV-2 infection.
Results

Immune cross-reactivity among seasonally spreading human coronaviruses (HCoVs) has long been hypothesised to provide cross-protection, albeit transient, against infection with distinct HCoV types\textsuperscript{1,4,5}. To determine the degree of cross-reactivity between HCoVs and the recently introduced zoonotic coronavirus SARS-CoV-2, we developed a sensitive flow cytometry-based assay for detection of SARS-CoV-2-binding antibodies. Sera from COVID-19 patients at University College London Hospitals (UCLH) (Table S1), contained high levels of IgG, IgM and IgA antibodies recognising the wild-type Spike (S) glycoprotein of SARS-CoV-2 expressed on the surface of HEK293T cells, whereas control sera did not (Fig. 1a). Notably, sera from a proportion of patients with confirmed HCoV infection collected before or during the early spread of SARS-CoV-2 in the UK (Table S1), also contained SARS-CoV-2 S-specific antibodies (Fig. 1a). However, the latter sera contained only lower levels of S-specific IgG and no IgM or IgA antibodies, which clearly distinguished them from COVID-19 patient sera (Fig. 1a).

The SARS-CoV-2 S protein is proteolytically processed into the S1 and S2 subunits that mediate target cell attachment and entry, respectively\textsuperscript{6,7}. S2 exhibits a higher degree of homology among coronaviruses than S1 (Extended data Fig. 1) and it was likely to be the main target of cross-reactive antibodies\textsuperscript{6}. Indeed, whereas the addition of recombinant soluble S1 completely abolished binding of the S1-reactive CR3022 antibody\textsuperscript{8} to SARS-CoV-2 S-expressing cells, it did not affect the binding of HCoV patient sera (Fig. 1b,c). Binding of COVID-19 patient sera was reduced by approximately 30% by soluble S1, indicating recognition of both S1 and S2 (Fig. 1b,c). Consistent with these observations, sera from both COVID-19 and HCoV patients reacted comparably in a SARS-CoV-2 S-coated ELISA, whereas only those from COVID19 patients reacted in a SARS-CoV-2 S1-coated ELISA (Fig. 1d). These data highlighted extensive cross-reactivity of HCoV patient sera with SARS-CoV-2 S, and specific recognition of the S1 subunit only by COVID-19 patient sera.

A concurrent IgG, IgM and IgA response to SARS-CoV-2 S was not detected by FACS in any of the 95 samples from SARS-CoV-2 uninfected individuals (SARS-CoV-2 -), which included earlier samples of unknown HCoV status and more recent samples of known HCoV status (Fig. 2a), underscoring its specificity to COVID-19 patients. However, 8 of the 95 control samples had S-specific IgG, but not IgM or IgA antibodies detectable by FACS or IgG antibodies detectable on an S-coated ELISA (Fig. 2a,b). In contrast, none of the control samples had IgG antibodies detectable on ELISAs coated with the less conserved SARS-CoV-2 S1 or receptor binding domain (RBD) (Fig. 2c), indicating cross-reactivity with conserved epitopes of S. Moreover, the 3 control samples with the highest cross-recognition of S (on FACS or ELISA) and a further 4 control samples also tested positive on ELISA coated with recombinant SARS-CoV-2 nucleocapsid (N) (Fig. 2d,e), which is also highly conserved among coronaviruses (CoVs). Collectively, these results suggested that a total of 12 of 95 control samples exhibited IgG antibodies cross-reactive with conserved epitopes in SARS-CoV-2 proteins (S2 and N). In contrast, IgG antibodies to SARS-CoV-2 S1 or RBD and IgG, IgM and IgA antibodies to S detected by FACS were exclusive to COVID-19 patients (Fig. 2e).

Whilst a concurrent IgG, IgM and IgA response was present only in COVID-19 patients, 6 out of 35 samples in this COVID-19 patient cohort were negative by the FACS-based assay (Fig. 2a). Of these, 5 were collected between days 5 and 10 post onset of symptoms and likely represented the pre-seroconversion period (Extended data Fig. 2), as seropositivity marked all the samples collected at later time-points, in agreement with current reports\textsuperscript{9-20}. Of note, a sample from an 81-year-old COVID-19 patient collected on day 16 post mild COVID-19 symptoms exhibited only IgG reactivity to SARS-CoV-2 S, but not to S1, which would be more characteristic of pre-existing antibody memory to HCoVs, than a de novo response to SARS-CoV-2 (Extended data Fig. 2). Rates of IgG seropositivity determined by SARS-CoV-2 S1-coated ELISA in the same samples were congruent with, but overall lower than those determined by FACS, particularly for the early time-points, indicating higher sensitivity of the FACS-based assay (Extended data Fig. 2).
To validate these findings, we tested samples from an additional cohort of 50 SARS-CoV-2-uninfected pregnant women, all of which were negative when considering the presence of IgG, IgM and IgA SARS-CoV-2 S-reactive antibodies (Extended data Fig. 3). However, at least 5 of these samples (all collected in 2018) showed evidence for lower levels of SARS-CoV-2 S-reactive IgG antibodies only, which could not have been elicited by SARS-CoV-2 infection (Extended data Fig. 3). An additional 2 samples reacted with N, but not S or S1 on ELISA (Extended data Fig. 3). These data suggested that a minimum of 10% of this cohort had pre-existing antibodies cross-reactive with SARS-CoV-2 proteins.

We also tested an extended set of sera from 135 COVID-19 patients (Table S1). The vast majority of these (128 of 135) had readily detectable IgG, IgM and IgA antibodies to SARS-CoV-2 S (Extended data Fig. 4). Of the remaining 7 samples, 6 were collected between days 2 and 15 post symptoms onset and seropositivity, assessed by FACS, was 100% at later time-points. Importantly, the concurrent IgG, IgM and IgA response to SARS-CoV-2 S that distinguished COVID-19 patients was present at the latest time-point examined (day 43 post onset of symptoms), with no evidence of decline during this period (Extended data Fig. 5).

The sensitivity of ELISAs run on the same samples was considerably lower than that of the FACS-based assay, with a further 20 samples testing negative on ELISA coated the specific S1 subunit, 8 of which also tested negative on the less specific S-coated or N-coated ELISAs (Extended data Fig. 4d-e). The discordant samples were collected, on average, at earlier time-points (Extended data Fig. 5) or displayed weaker responses (Extended data Fig. 6), emphasising the higher sensitivity of the FACS-based assay, particularly at these early time-points.

Of note, a single 60-year-old patient in this cohort, sampled on day 29 post onset of symptoms, at a time-point when all other COVID-19 patients had seroconverted, had SARS-CoV-2 S-reactive IgG, but not IgM or IgA antibodies, that were detected by FACS, but not by ELISAs (Extended data Fig. 5).

Again, this profile was more consistent with an anamnestic response to HCoVs, than a de novo response to SARS-CoV-2. Inspection of patient records indicated that this was a bone marrow transplant recipient, who tested positive for HCoV infection in February 2020 and subsequently contracted SARS-CoV-2, testing positive by RT-qPCR the following month (Extended data Fig. 7).

Of note, serum samples taken around the diagnosis of HCoV and SARS-CoV-2 infections were negative for SARS-CoV-2 S-reactive antibodies, likely due to the immunosuppressed state of this patient (Extended data Fig. 7). However, serum taken 3 weeks later contained SARS-CoV-2 S-reactive IgG, in the absence of other antibody classes, which was not consistent with a de novo response (Extended data Fig. 7). This patient described only mild COVID-19 symptoms that did not necessitate hospitalisation, but appeared chronically infected with SARS-CoV-2, having tested repeatedly positive for viral RNA for over a month (Extended data Fig. 7).

These observations support a model whereby exposure to HCoVs elicits humoral immunity that cross-reacts with conserved protein domains in other coronaviruses, including SARS-CoV-2. Infection with SARS-CoV-2 additionally induces de novo antibody responses to variable domains unique to this virus, specifically S1. Consistent with this model, SARS-CoV-2 S-reactive class-switched antibodies were detected by FACS concurrently with IgM antibodies in our COVID-19 patients, kinetics typically associated with anamnestic responses. A similar flow assay was recently used to demonstrate cross-reactivity of COVID-19 patient sera with the S proteins, but not the RDs of the other two zoonotic CoVs, SARS-CoV and MERS\(^1\). Sera from SARS-CoV-recovered patients have been suggested to neutralise, to a degree, SARS-CoV-2 S pseudotypes\(^7\). Moreover, infection with SARS-CoV-2 has recently been shown to increase IgG seroreactivity to HCoVs\(^2\), supporting the existence of shared epitopes. Numerous serology assays that are being developed have also detected SARS-CoV-2-reactive antibodies in a considerable proportion of SARS-CoV-2-uninfected individuals, which has often been considered as unspecific binding, rather than HCoV-elicited specific antibodies\(^9\).\(^10\).\(^20\).\(^22\)

Based on our data, we would argue that this is the result of cross-reactivity between HCoVs. Indeed, some samples from HCoV-OC43-infected donors tested positive on a commercial ELISA coated with SARS-CoV-2 S1 in one such serology study\(^16\). SARS-CoV-2 S-reactive T helper cells, a prerequisite for
class-switched antibody responses, are being detected in the majority of COVID-19 patients, as well as in one-third of healthy individuals, albeit at lower clonal frequencies. In agreement with the specificity of the antibody response we describe here, T helper cells from COVID-19 patients targeted both subunits of SARS-CoV-2 S, whereas those from healthy individuals targeted the S2 subunit.

Although the data presented here and emerging in the recent and unrefereed literature suggest extensive immune cross-reactivity between SARS-CoV-2 and HCoVs, the potential consequences of pre-existing immunity for the course of SARS-CoV-2 infection or susceptibility to COVID-19 have not been fully considered. One of the ways in which HCoV-elicited antibodies might protect against SARS-CoV-2 infection is inhibition of entry into the target cell. SARS-CoV-2 binding to one identified cellular receptor, Angiotensin-converting enzyme 2 (ACE2), is mediated by the RBD. As HCoV-induced antibodies cross-react with S2, but not the RBD, they would not be expected to block ACE2-RBD interactions. However, reports of SARS-CoV-2-neutralising antibodies that do not block RBD-ACE2 interaction are emerging and neutralising antibodies targeting the S2 subunit of SARS-CoV have already been described.

It is therefore plausible that HCoV patient sera targeting the S2 also neutralise, without affecting binding to ACE2, by interfering with alternative routes of viral entry. Indeed, entry of SARS-CoV-2 can also be facilitated by the alternative receptor CD147, also known as Basigin (BSG) and possibly also by receptor-independent mechanisms, as has been described for other CoVs.

To examine the ability of HCoV patient sera to inhibit viral entry, we used HEK293T cells as targets. These cells lack ACE2 expression, but are nevertheless permissive to entry of lentiviral particles pseudotyped with SARS-CoV-2 S (Extended data Fig. 8). Moreover, transduction efficiency of HEK293T cells by SARS-CoV-2 S pseudotypes was not further increased by ACE2 overexpression (Extended data Fig. 8), highlighting ACE2-independent entry. In contrast, HEK293T cells expressed high levels of BSG, encoding CD147 (Extended data Fig. 8). Sera from seroconverted (Ab +) COVID-19 patients efficiently neutralised SARS-CoV-2 S pseudotypes, consistent with prior reports, whereas those from COVID-19 patients that had not yet produced binding antibodies (Ab -) did not (Fig. 3a). Surprisingly, sera from HCoV-infected patients that contained SARS-CoV-2 S-reactive antibodies neutralised these pseudotypes, with efficiencies comparable with sera from seroconverted COVID-19 patients (Fig. 3a). None of the sera neutralised lentiviral particles pseudotyped with the control glycoprotein of vesicular stomatitis virus (VSV) (Fig. 3a). Titres of neutralising antibodies in COVID-19 patient sera correlated well with titres of SARS-CoV-2 RBD-binding antibodies, determined by ELISA, but better with titres of SARS-CoV-2 S-binding antibodies, determined by FACS (Fig. 3b). Despite the low numbers, the correlation between neutralising antibodies in HCoV patient sera with SARS-CoV-2 S-binding IgG antibodies determined by FACS was even stronger (Fig. 3c). Collectively, these data indicated that HCoV-elicited cross-reactive antibodies interfere with at least one mode of SARS-CoV-2 entry into target cells.

Conclusions

Cross-reactivity between seasonal HCoVs and the pandemic SARS-CoV-2 needs to be carefully considered in the development and interpretation of assays for precise detection of SARS-CoV-2-specific antibodies. The flow cytometry-based method we employed demonstrated the highest degree of specificity and sensitivity. A similar method was also recently used in an independent study, reaching comparable results, except no cross-reactivity with HCoV was suggested. Although not specifically investigated in the latter study, detection of cross-reactivity with HCoV was deliberately avoided by higher dilution of serum samples. Whilst clearly detectable, titres of SARS-CoV-2 S-reactive antibodies are one order of magnitude lower in HCoV patient sera than in COVID-19 patient sera and drop below detection limits upon further serum dilution also in our assay.

Running the assay in sensitive mode still allows the distinction between pre-existing and de novo antibody responses to SARS-CoV-2, based on the levels of SARS-CoV-2 S-reactive IgG and parallel
detection of IgM and IgA. These distinguishing features were maintained and, indeed, appear to increase during the first 43 days post onset of COVID-19 symptoms (the latest time-point we have examined). It is possible that these features are maintained for longer periods post SARS-CoV-2 infection, but it is also expected that the discriminating ability will be lost over time.

Increasing specificity of the assays will be at expense of sensitivity. This is evident in the use of more variable SARS-CoV-2 protein domains such as S1 and RBD, which exhibited approximately 85% sensitivity during the first 43 days post onset of COVID-19 symptoms in our study, and which is likely to drop further with waning antibody titres over longer periods.

The apparent ability of HCoV patient sera to neutralise SARS-CoV-2 S pseudotypes raise similar concerns regarding the specificity of neutralisation assays. For example, 5 of 1,000 samples from healthy Scottish blood donors collected in March 2020 neutralised SARS-CoV-2 S pseudotypes and 1 or 100 of samples collected in 2019 also had neutralising activity in the absence of a strong ELISA signal\textsuperscript{31}. Notably, these samples were described to have low or no SARS-CoV-2 S-reactive IgM antibodies\textsuperscript{31}, a feature we would associate with immune memory of HCoVs.

In addition to its implications for serology assay development and interpretation or for the design of vaccination studies, potential cross-reactivity between seasonal HCoVs and the pandemic SARS-CoV-2 has important ramifications for natural infection. Thorough epidemiological studies of HCoV transmission suggest that cross-protective immunity is unlikely to be sterilising or long-lasting\textsuperscript{32}, which is also supported by repeated reinfection of all age groups\textsuperscript{4}, sometimes even with homologous HCoVs\textsuperscript{33}. Nevertheless, prior immunity induced by one HCoV has also been reported to reduce the transmission of homologous and, importantly, heterologous HCoVs, and to ameliorate the symptoms where transmission is not prevented\textsuperscript{1,4,5}. A possible modification of COVID-19 severity by prior HCoV infection might account for the age distribution of COVID-19 susceptibility, where higher HCoV infection rates in children than in adults\textsuperscript{5,34,35}, correlates with relative protection from COVID-19\textsuperscript{36}.

Public health measures intended to prevent the spread of SARS-CoV-2 will also prevent the spread of and, consequently, maintenance of herd immunity to HCoVs, particularly in children. It is, therefore, imperative that any effect, positive or negative, of pre-existing HCoV-elicited immunity on the natural course of SARS-CoV-2 infection is fully delineated.
Methods

Patients and clinical samples

The following patient groups were studied: 31 SARS-CoV-2-uninfected patients without recent HCoV infection (SARS-CoV-2 - HCoV -). These were all haematology patients at University College Hospitals (UCLH) testing negative for recent HCoV infection and sampled between August 2019 and September 2019; 34 SARS-CoV-2-uninfected patients with recent HCoV infection (SARS-CoV-2 - HCoV +). These were also UCLH haematology patients testing positive for recent HCoV infection by RT-qPCR between October 2019 and March 2020 and sampled between December 2019 and March 2020 (Table S1); 30 SARS-CoV-2-uninfected UCLH patients of unknown HCoV status, sampled between August 2019 and September 2019, prior to presumed SARS-CoV-2 circulation; 50 SARS-CoV-2-uninfected visitors of antenatal clinics, sampled in May 2018; SARS-CoV-2-infected patients testing positive for SARS-CoV-2 infection by RT-qPCR and sampled between March 2020 and April 2020 (Table S1). An initial cohort of 35 patients (31 annotated) and an extended cohort of 135 patients were tested between 2 and 43 days after the onset of COVID-19 symptoms (Table S1). All patient sera and sera remaining after antenatal screening of healthy pregnant women were from residual samples prior to discarding, in accordance with Royal College Pathologists guidelines and the UCLH Clinical Governance for assay development. All serum or plasma samples were heat-inactivated at 56°C for 30 min prior to testing.

Viral infection RT-qPCR diagnosis

SARS-CoV-2 nucleic acids were detected at Health Services Laboratories, London, UK, by an RT-qPCR method as recently described. HCoV nucleic acids were detected by RT-qPCR, as part of a diagnostic panel for respiratory viruses, run by Health Services Laboratories (HSL), London, UK.

Cell lines

HEK293T cells were obtained from the Cell Services facility at The Francis Crick Institute, verified as mycoplasma-free and validated by DNA fingerprinting. HEK293T cells overexpressing ACE2 were generated by transfection, using GeneJuice (EMD Millipore), with a plasmid containing the complete human ACE2 transcript variant 1 cDNA sequence (NM_001371415.1) cloned into the mammalian expression vector pcDNA3.1-C’ FLAG by Genscript. Cells were grown in Iscove’s Modified Dulbecco’s Medium (Sigma Aldrich) supplemented with 5% fetal bovine serum (Thermo Fisher Scientific), L-glutamine (2 mmol/L, Thermo Fisher Scientific), penicillin (100 U/mL, Thermo Fisher Scientific), and streptomycin (0.1 mg/mL, Thermo Fisher Scientific).

Flow cytometry

HEK293T cells were transfected with an expression plasmid expressing wild-type SARS-CoV-2 S (NC_045512.2) and were transferred into V-bottom 96-well plates (20,000 cells/well). Cells were incubated with sera (diluted 1:50 in PBS) for 30 min, washed with FACS buffer (PBS, 5% BSA, 0.02% Tween 20, 0.05% sodium azide), and stained with BV421 anti-IgG (Biolegend), APC anti-IgM (Biolegend) and PE anti-IgA (Miltenyi Biotech) for 30 min (all antibodies diluted 1:200 in FACS buffer). Cells were washed with FACS buffer and fixed for 20 min in CellFIX buffer (BD Bioscience). Samples were run on a LSR Fortessa with a high-throughput sampler (BD Biosciences) running BD FACSDiva v8.0, and analysed using FlowJo v10 (Tree Star Inc.) analysis software.

Recombinant protein production

The SARS-CoV-2 RBD and S1 constructs, spanning SARS-CoV-2 S (UniProt ID P59594) residues 319-541 (RVQPT…KCVNF) and 1-530 (MFVFL…GPKKS), respectively, were produced with C-terminal twin Strep tags. To this end, the corresponding codon-optimised DNA fragments were cloned into
mammalian expression vector pQ-3C-2xStrep. A signal peptide from immunoglobulin kappa gene product (METDTELLVWLLÄWPGSTGD) was used to direct secretion of the RBD construct. Stabilized ectodomain of the SARS-CoV-2 S glycoprotein (residues 1-1208) with inactivated furin cleavage site (RRAR, residues 682-685 mutated to GSAS) and a double proline substitution (K986P/V987P) was produced with a C-terminal T4 fibrin trimerization domain and a hexahistidine (His$_6$) tag from pcDNA3 vector. Exp293F cells growing at 37°C in 5% CO$_2$ atmosphere in shake flasks in FreeStyle 293 medium were transected with the corresponding plasmids using ExpiFectamine reagent (Thermo Fisher Scientific). Conditioned medium containing secreted proteins was harvested twice, 3-4 and 6-8 days post-transfection. Twin Strep- and His$_6$-tagged proteins were captured on Streptactin XT (IBA LifeSciences) or Talon (Takara) affinity resin, respectively, and purified to homogeneity by size exclusion chromatography through Superdex 200 (GE Healthcare) in 150 mM NaCl, 1 mM EDTA, 20 mM Tris-HCl, pH 8.0. Full-length SARS CoV2 N gene product was produced with an N-terminal His$_6$ tag from pOPTH-1124 plasmid (kindly provided by J. Luptak and L. James, Laboratory for Molecular Biology, Cambridge, UK). Escherichia coli C43(DE3) cells (Lucigen) transformed with pOPTH-1124 were grown in terrific broth medium, and expression was induced by addition of 1 mM Isopropyl β-D-1-thiogalactopyranoside at 37°C. Bacteria, harvested 4 h post-induction, were disrupted by sonication in core buffer (1M NaCl, 10 mM imidazole, 20 mM HEPES-NaOH, pH 8.0) supplemented with BaseMuncher nuclease (Expediton; 1 ml per 40 ml cell suspension) and Complete EDTA-free protease inhibitor mix (Roche). The extract was precleared by centrifugation, and His$_6$-tagged protein was captured on NiNTA agarose (Qiagen). Following extensive washes with core buffer supplemented with 20 mM imidazole, the protein was eluted with 500 mM imidazole. SARS CoV2 N, was further purified by cation exchange and heparin affinity chromatography prior polishing by gel filtration through a Superdex 200 16/40 column (GE Healthcare), which was operated in 300 mM NaCl, 20 mM HEPES-NaOH, pH 8.0. Purified SARS CoV2 antigens, concentrated to 1-5 mg/ml by ultrafiltration using appropriate VivaSpin devices (Sartorius), were snap-frozen in liquid nitrogen in small aliquots and stored at -80°C.

ELISA

96-well plates were coated overnight at 4°C with purified protein in borate-buffered saline (3 μg/μl per well in 50 μl) and blocked for 2 hr at 37°C in blocking buffer (PBS, 5% milk, 0.05% Tween 20, 0.01% sodium azide). Sera was diluted in blocking buffer (1:50) and 50 μl added to the plate then incubated for 1 hr at room temperature. After washing 4 times with PBS-T, plates were incubated with alkaline phosphatase-conjugated goat anti-human IgG, IgM, or IgA (1:1000, Jackson ImmunoResearch) for 1 hr. Plates were developed by adding 100 μl of alkaline phosphatase substrate (Sigma Aldrich) for 30 min after 4 washes with PBS-T. Optical densities were measured at 405 nm on a microplate reader (Tecan). CR3022 (Absolute Antibodies) was used as a positive control for ELISAs coated with S, S1, and RBD.

Lentiviral particle production and neutralisation

Lentiviral particles pseudotyped with either SARS-CoV-2 S or Vesicular Stomatitis Virus glycoprotein (VSVg) were produced by co-transfection of HEK293T cells with plasmids encoding either of these glycoproteins together a plasmid encoding the SIVmac Gag-Pol polyprotein and a plasmid expressing an HIV-2 backbone with a GFP encoding gene, using GeneJuice (EMD Millipore). Virus-containing supernatants were collected 48 hr post-transfection and stored at -80°C until further use. For neutralisation assays, lentiviral pseudotypes were incubated with serial dilutions of patient sera at 37°C for 30 minutes and were subsequently added to HEK293T cells seeded in 96-well plates (3,000 cells/well). Polybrene (4 μg/mL, Sigma Aldrich) was also added to the cells and plates were spun at 1,200 rpm for 45 min. The percentage of transduced (GFP+) cells was assessed by flow cytometry 72 hours later.
Data analysis

Data were analyses and plotted in GraphPad Prism 7 (GraphPad Software) or SigmaPlot 14.0.
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References


Figure legends

**Figure 1.** Flow cytometric detection and specificity of antibodies reactive with SARS-CoV-2 S. a, FACS profiles of IgG, IgA and IgM antibodies in 5 individual patient sera from each indicated group. Levels of IgM are indicated by a heatmap. b, Ability of soluble S1 to inhibit binding to SARS-CoV-2 S of the S1-specific CR3022 antibody or antibodies in the sera of SARS-CoV-2-infected (SARS-CoV-2 +) or HCoV-infected (SARS-CoV-2 - HCoV +) patients. One representative of two patients is show. c, Quantitation of the inhibitory effect of soluble S1 on binding to SARS-CoV-2 S of sera from patients described in b. Each dot is an individual patient serum tested at 1:50 and 1:200 dilution. d, Optical densities from ELISAs coated with S or S1 of sera from patients described in b. Each dot is an individual patient serum tested at 1:50 dilution. The dashed lines represents the optical densities obtained with the CR3022 antibody.

**Figure 2.** Comparison of antibody detection methods using a panel of patient samples. Serum samples from the following groups were compared in all panels: SARS-CoV-2-uninfected without recent HCoV infection (SARS-CoV-2 - HCoV -); SARS-CoV-2-uninfected with recent HCoV infection (SARS-CoV-2 - HCoV +); SARS-CoV-2-uninfected with unknown history of recent HCoV infection (SARS-CoV-2 -); SARS-CoV-2-infected (SARS-CoV-2 +). a, Frequency of cells that stained with all three antibody classes (IgM+IgG+IgA+) or only with IgG (IgG+) in each of these samples, ranked by their IgM+IgG+IgA+ frequency. b-d, Optical densities from ELISAs coated with S (b), S1 or RBD (c) or N (d) of the same samples. Dashed lines in a-d denote the assay sensitivity cut-offs. e, Summary of the results from a-d, represented as a heatmap of the quartile values.

**Figure 3.** Neutralisation of SARS-CoV-2 S pseudotypes by SARS-CoV-2-infected and -uninfected patient sera. a, Percent inhibition of transduction efficiency of SARS-CoV-2 S (left) or control VSVg (right) lentiviral pseudotypes by SARS-CoV-2-infected patient sera without (SARS-CoV-2 + Ab -) or with (SARS-CoV-2 + Ab +) detectable SARS-CoV-2 S-binding antibodies and those from SARS-CoV-2-uninfected patients with recent HCoV infection and with detectable SARS-CoV-2 S-binding antibodies (SARS-CoV-2 - HCoV + Ab +). Each line is an individual serum sample. b, Correlation of neutralising antibody titres in SARS-CoV-2-infected patient sera with optical densities from RBD-coated ELISAs (left) or IgM+IgG+IgA+ staining frequencies, determined by FACS (right). c, Correlation of neutralising antibody titres in sera from SARS-CoV-2-uninfected patients with recent HCoV infection with IgG+ staining frequencies, determined by FACS. In b-c, each dot represents an individual sample.
Figure 2

SARS-CoV-2 - HCoV -
n=31
SARS-CoV-2 - HCoV +
n=34
SARS-CoV-2 -
n=30
SARS-CoV-2 +
n=35

Optical density at 405 nm

S IgG
IgM
IgG
IgA
FACS
S1 IgG
RBD IgG
N IgG
ELISA

Quartiles

IgM/IgG/IgA = FACS
IgG only FACS
S IgG ELISA
S1 IgG ELISA
RBD IgG ELISA
N IgG ELISA

Optical density at 405 nm

Sample number

Positive cells, %

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Figure 3

a) SARS-CoV-2 S pseudotype

b) SARS-CoV-2 +

c) SARS-CoV-2 – HCoV +

R2 = 0.910

Optical density at 405 nm

Positive cells, %

Neutralising Ab titre

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