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

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**Fig. 1.** Flow cytometric detection and specificity of antibodies reactive with SARS-CoV-2 S. (A) Detection of IgG, IgA, and IgM in five individuals from each indicated group. IgM levels are indicated by a heatmap. (B to D) Inhibition of SARS-CoV-2 S binding of sera from SARS-CoV-2–infected (SARS-CoV-2⁺, n = 10) or SARS-CoV-2–uninfected (SARS-CoV-2⁻ HCoV⁺, n = 6) patients by soluble S1 or S2. (B) Flow cytometry profile of one representative patient per group. (C) Mean frequency of positive cells. *P = 0.013; **P = 0.006, one-way analysis of variance (ANOVA) on ranks. (D) Mean staining intensity [mean fluorescence intensity (MFI) of sample as a percentage of negative control MFI]. In (C) and (D), dots represent individual samples from one of three similar experiments.

**Fig. 2.** Prevalence of SARS-CoV-2 S–cross-reactive antibodies detected by different methods. (A) Flow cytometry and ELISA results for each sample in cohorts A and C to E listed in table S1. (B) Flow cytometry and ELISA results for serum samples from SARS-CoV-2–uninfected pregnant women. (C to E) SARS-CoV-2 S–cross-reactive antibodies in healthy children and adolescents. (C) Representative flow cytometry profiles of seronegative donors (Negative) or COVID-19 patients (Positive) and of SARS-CoV-2–uninfected adolescents with SARS-CoV-2 cross-reactive antibodies. (D) Frequency of cells stained with all three antibody classes (IgG⁺ IgM⁺ IgA⁺) or only with IgG (IgG⁺) ranked by their IgG⁺ IgM⁺ IgA⁺ frequency. The dashed line denotes the assay sensitivity cutoff. (E) Flow cytometry and ELISA results for each sample. (F) Prevalence of SARS-CoV-2 S–cross-reactive antibodies in the indicated age groups (line) and frequency of cells that stained only with IgG (dots) in all samples for which the date of birth was known. The heatmaps in (A), (B), and (E) represent the quartile values above each assay’s technical cutoff.
SARS-CoV-2 S–reactive IgM and IgA were also detected in two of these donors, albeit at considerably lower levels than in COVID-19 patients (fig. S11), suggestive of recent or ongoing response. In an additional cohort of 13 donors recently infected with HCoVs, only one had SARS-CoV-2 S–reactive IgG antibodies, and these were at very low levels (fig. S12). This suggested that their emergence was not simply a common transient event after each HCoV infection in this age group (median age 51 years; table S1). Instead, given that HCoV-reactive antibodies are present in virtually all adults (3–5), the rarity of SARS-CoV-2 S cross-reactivity (16 of 302; 5.29%) indicates additional requirements such as random B cell receptor repertoire focusing or frequency of HCoV infection rather than time since the last HCoV infection. Indeed, the frequency of HCoV infection displays a characteristic age distribution, being the highest in children and adolescents (1, 4–8). We therefore examined a cohort of younger SARS-CoV-2–uninfected healthy donors (age 1 to 16 years; table S1) sampled between 2011 and 2018. At least 21 of these 48 donors had detectable levels of SARS-CoV-2 S–reactive IgG antibodies (Fig. 2, C to E), whereas only one of an additional cohort of 43 young adults (age 17 to 25 years; table S1) had such antibodies (Fig. 2F). Staining with sera from SARS-CoV-2–uninfected children and adolescents was specific to HEK293T cells expressing SARS-CoV-2 S, but not the unrelated HERV-K113 envelope glycoprotein, and was outcompeted by soluble SARS-CoV-2 S2 (fig. S13). The prevalence of SARS-CoV-2 S–reactive IgG antibodies peaked at 62% between 6 and 16 years of age (Fig. 2F), when HCoV seroconversion in this age group also peaks (3, 4, 6, 7), and was significantly higher than in adults (P < 0.00001, Fisher’s exact test).

To determine the potential consequences of antibody cross-reactivity, we examined the ability of preexisting antibodies to inhibit SARS-CoV-2 entry into HEK293T cells (fig. S14 and supplementary text). Although not expected to directly inhibit RBD-mediated cell attachment, S2-targeting antibodies that can neutralize SARS-CoV-2 have recently been discovered (9, 10). HEK293T cell infection with SARS-CoV-2 S pseudotypes was efficiently inhibited by sera from seroconverted (Ab+) COVID-19 patients, but not from those who had not yet seroconverted (Ab–) (Fig. 3A). Sera from SARS-CoV-2–uninfected donors with SARS-CoV-2 S–reactive antibodies also neutralized these pseudotypes, whereas none of the sera neutralized vesicular stomatitis virus (VSV) glycoprotein pseudotypes (Fig. 3A). Comparable neutralization of SARS-CoV-2 S pseudotypes was also observed with sera from SARS-CoV-2–uninfected adolescents (Fig. 3A). Moreover, most of the sera from SARS-CoV-2–uninfected donors with flow cytometry–detectable cross-reactive antibodies also neutralized authentic SARS-CoV-2 infection of Vero E6 cells, albeit on average less potently than COVID-19 patient sera (Fig. 3B). By contrast, sera from SARS-CoV-2–uninfected patients without cross-reactive antibodies exhibited no
neutralizing activity (Fig. 3B). Antiviral antibodies may also enhance viral entry by Fc receptor–mediated antibody-dependent enhancement. However, entry of SARS-CoV-2 S pseudotypes was not enhanced by either COVID-19 patient sera or SARS-CoV-2–uninfected patient sera in FcγRIIA-expressing K562 cells (fig. S15).

Collectively, these findings highlight functionally relevant antigenic epitopes conserved within the S2 subunit. Over its entire length, SARS-CoV-2 S exhibits marginally closer homology with the S proteins of the betacoronaviruses HCoV-OC43 and HCoV-HKU1 than with the alphacoronaviruses HCoV-NL63 and HCoV-229E (fig. S16A). To probe shared epitopes, we constructed overlapping peptide arrays spanning the last 743 amino acids of SARS-CoV-2 S (fig. S16B). Multiple putative epitopes were differentially recognized by sera with cross-reactive antibodies (Ab+), were reasonably conserved, and most mapped to the surface of S2 (Fig. 4, A and B, and table S2). An epitope overlapping the S2 fusion peptide was also recently identified as being cross-reactive with the corresponding peptides from HCoV-OC43 and HCoV-229E (11). Cross-reactivity with the identified epitopes was further supported by ELISAs coated with synthetic peptides (fig. S17).

As expected (3–5), reactivity with one or more HCoVs was detectable by flow cytometry in all sera (Fig. 4D and fig. S18). However, IgG and IgA reactivity against HCoVs was higher in SARS-CoV-2–uninfected adults with SARS-CoV-2–reactive IgG compared with those without ($P = 1.4 \times 10^{-16}$ for IgG and $P = 0.017$ for IgA, Student’s t test) and in SARS-CoV-2–uninfected children or adolescents with SARS-CoV-2–reactive IgG compared with those without ($P = 0.010$ for IgG and $P = 0.021$ for IgA, Student’s t test) (Fig. 4D), supporting a direct link between the two. Accordingly, IgG reactivity against each HCoV type was independently correlated with the presence of SARS-CoV-2–reactive antibodies (Fig. 4D).

Our results from multiple independent assays demonstrate the presence of preexisting antibodies recognizing SARS-CoV-2 in uninfected individuals. Identification of conserved epitopes in S2 targeted by neutralizing antibodies may hold promise for a universal vaccine protecting against current as well as future CoVs. Together with preexisting T cell (12–14) and B cell (10, 15) memory, antibody cross-reactivity between seasonal HCoVs and SARS-CoV-2 may have important ramifications for natural infection. Epidemiological studies of HCoV transmission suggest that cross-protective immunity is unlikely to be sterilizing or long-lasting (8), which is also supported by repeated reinfection (2, 16). Nevertheless, prior immunity induced by one HCoV can reduce the transmission of homologous and heterologous HCoVs and ameliorate the symptoms when transmission is not prevented (1, 2). A possible modification of COVID-19 severity by prior HCoV infection may account for the age distribution of COVID-19 susceptibility, in which higher HCoV infection rates in children than in adults (4, 6) correlate with relative protection from COVID-19 (17) and may also shape seasonal and geographical patterns of transmission. It is imperative that any effect, positive or negative, of preexisting
HCoV-elicited immunity on the natural course of SARS-CoV-2 infection be fully delineated.

REFERENCES
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SUPPLEMENTARY MATERIALS
science.sciencemag.org/content/370/6522/1339/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S18
Tables S1 and S2
References (18–40)
MDAR Reproducibility Checklist
View/request a protocol for this paper from Bio-protocol.

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Antibodies predating infection

Immunological memory after infection with seasonal human coronaviruses (hCoVs) may potentially contribute to cross-protection against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Ng et al. report that in a cohort of 350 SARS-CoV-2–uninfected individuals, a small proportion had circulating immunoglobulin G (IgG) antibodies that could cross-react with the S2 subunit of the SARS-CoV-2 spike protein (see the Perspective by Guthmiller and Wilson). By contrast, COVID-19 patients generated IgA, IgG, and IgM antibodies that recognized both the S1 and S2 subunits. The anti-S2 antibodies from SARS-CoV-2–uninfected patients showed specific neutralizing activity against both SARS-CoV-2 and SARS-CoV-2 S pseudotypes. A much higher percentage of SARS-CoV-2–uninfected children and adolescents were positive for these antibodies compared with adults. This pattern may be due to the fact that children and adolescents generally have higher hCoV infection rates and a more diverse antibody repertoire, which may explain the age distribution of COVID-19 susceptibility.

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