SARS-CoV-2 Vaccination boosts Neutralizing Activity against Seasonal Human Coronaviruses

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Summary: About 30% of mild respiratory infections are caused by “seasonal” human coronaviruses that share some homology with SARS-CoV-2, the causative agent of COVID-19. Here, we show that heterologous ChAdOx1 nCoV-19 BNT162b2 prime-boost vaccination against SARS-CoV-2 increases in vitro neutralizing activity against seasonal coronaviruses.
ABSTRACT

**Background.** Most of the millions of people that are vaccinated against SARS-CoV-2, the causative agent of COVID-19, have previously been infected by related circulating human coronaviruses (hCoVs) causing common colds and will experience further encounters with these viruses in the future. Whether or not COVID-19 vaccinations impact neutralization of seasonal coronaviruses is largely unknown.

**Methods.** We analyzed the capacity of sera derived from 24 individuals before and after heterologous ChAdOx1 nCoV-19 BNT162b2 prime-boost vaccination to neutralize genuine OC43, NL63 and 229E hCoVs, as well as viral pseudoparticles carrying the SARS-CoV-1, SARS-CoV-2, MERS-CoV, hCoV-OC43, -NL63 and -229E spike proteins. Genuine hCoVs or spike containing pseudovirions were incubated with different concentrations of sera and neutralization efficiencies were determined by measuring viral RNA yields, intracellular viral nucleocapsid expression, or reporter gene expression in Huh-7 cells.

**Results.** All individuals showed strong preexisting immunity against hCoV-OC43. Neutralization of hCoV-NL63 was more variable and all sera showed only modest inhibitory activity against genuine hCoV-229E. SARS-CoV-2 vaccination resulted in efficient cross-neutralization of SARS-CoV-1 but not of MERS-CoV. On average, vaccination significantly increased the neutralizing activity against genuine hCoV-OC43, -NL63 and -229E.

**Conclusions.** Heterologous COVID-19 vaccination may confer some cross-protection against endemic seasonal coronaviruses.

**Keywords:** COVID-19 vaccination, seasonal coronaviruses, cross-neutralization
INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the current coronavirus disease 2019 (COVID-19) pandemic, is at least the seventh coronavirus that entered the human population [1,2]. The highly pathogenic SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2003 and 2012 and infected about 8,000 and 2,500 individuals with case-fatality rates of ~10% and 35%, respectively. Fortunately, SARS-CoV-1 has disappeared, while MERS-CoV still causes sporadic infections in the Arabian Peninsula. In contrast, the four remaining human coronaviruses (hCoVs) have spread around the globe [1,2]. The endemic α-coronaviruses hCoV-229E and -NL63 and the β-coronaviruses hCoV-OC43 and -HKU1 cause up to 30% of mild respiratory tract infections [1]. However, in infants, elderly or immunocompromised individuals these seasonal coronaviruses may sometimes cause severe bronchiolitis and pneumonia as well as enteric or neurological diseases [1]. The annual economic costs of infections with common cold viruses, including seasonal hCoVs, were estimated to range between 24-40 billion USD in the U.S. alone [3,4]. First exposure to seasonal coronaviruses typically occurs during early childhood [5,6] and induces immune responses that wane within a year allowing regular reinfections [7]. Thus, coronaviruses keep circulating in the human population with annual peaks of infections in the winter months and variations in the dominance of viral species by region and year [8]. A study in the U.S. estimated the seropositivity of adults for hCoV-229E to be 91.3%, for -HKU1 59.2%, -NL63 91.8% and -OC43 90.8% [9].

To date (January 14th, 2022), SARS-CoV-2 has infected more than 310 million people and caused about 5.5 million fatalities worldwide (https://coronavirus.jhu.edu/map.html). To get the COVID-19 pandemic under control, enormous efforts are being made [10] and about 9.5 billion doses of SARS-CoV-2 vaccines have already been administered. COVID-19 vaccines
are highly effective [11]. In countries with high vaccination rates they not only decreased incidences, hospitalizations and deaths but also allowed to reduce restrictive measures. Subsequent increases in social interactions combined with the lack of exposure to regular common cold viruses in the past year resulted in a surge of respiratory syncytial virus (RSV) infections compared to previous years [12,13]. Similar predictions have been made for the current influenza virus season [14]. Readopted security measures due to the emergence of the highly transmissible Omicron variant [15–18] might reduce the frequency of infections with seasonal coronaviruses in the near future. However, the latter will clearly continue to play relevant roles in human health and economics.

Seasonal coronaviruses share about 23-29% sequence homology in the spike protein to SARS-CoV-2 [19] and even higher homology in the S2 subunit that mediates fusion between the viral and cellular membranes [20,21]. This homology may result in overlapping immune epitopes [20,22], posing the question whether spike-based SARS-CoV-2 vaccination affect humoral immunity against seasonal coronaviruses. Indeed, it has been reported that anti-spike IgM and IgG titers against β-coronaviruses hCoV-OC43 and -HKU1 increase upon SARS-CoV-2 infection [23–26]. Similarly, SARS-CoV-2 vaccination might boost IgM, IgG and IgA titers against hCoV-OC43 and -HKU1 spike proteins [21,28,29]. Here, we show that SARS-CoV-2 vaccination increases the neutralizing activity against all three tested genuine seasonal coronaviruses.
METHODS

Study design and patient samples. The study design and cohort of individuals aged 25-46 (median 30.5) years, who received a ChAdOx1 nCoV-19 prime followed by a BNT162b2 boost has been reported [30]. Presera were taken up to 2 days before prime vaccination and post-immunization sera 2 weeks after the BNT162b2 boost [30]. Antibody titers against SARS-CoV-2 and neutralizing titers against hCoV-OC43 before and after vaccination are provided in supplemental Table 1. Prior SARS-CoV-2 infection was excluded by medical history and by measuring anti-SARS-CoV-2 nucleocapsid antibody levels for all but one reconvalescent individual (#22).

Pseudoparticle stock production. Vesicular stomatitis virus (VSV) based pseudoparticles were generated by transfection of HEK293T cells with spike expression plasmids and subsequent infection with VSV-glycoprotein (G) deleted but complemented (*) VSV particles. To this end, cells were transfected with plasmids encoding hCoV-229E, hCoV-NL63, MERS-CoV, SARS-CoV-1, SARS-CoV-2 spike proteins, or VSV-G using LT1 transfection reagent (Mirus, MIR 2306). 24 hours post-transfection, cells were infected with GFP-encoding VSVΔG(GFP)*VSV-G particles at a multiplicity of infection (MOI) of 3. Pseudotyped particles were harvested at 16 h post-infection. Residual particles carrying VSV-G were blocked by adding 10% (v/v) of I1 Hybridoma supernatant (I1, mouse hybridoma supernatant from CRL-2700; ATCC). To produce lentiviral particles containing hCoV-OC43 or -HKU1 spikes, HEK293T cells were transfected with spike expression plasmids together with a pNL1_HIV-1_NL4-3-Δenv-fluc backbone. 48 hours post-transfection, supernatants were harvested and stored at -80°C.
Virus strains and propagation. HCoV-229E was obtained from ATCC (VR-740TM). For propagation, Huh-7 cells were inoculated with a MOI of 0.1 in DMEM medium supplemented with 2% FCS. Cells were incubated at 33°C for one day after infection, washed with PBS and further cultured in fresh medium. Cells were monitored daily under the light microscope until strong cytopathic effects (CPE) became visible on day 5. Supernatant was harvested, aliquoted and stored at -80°C. HCoV-NL63 was propagated as described for hCoV-229E but using LLC-MK2 cells. HCoV-OC43 was obtained from ATCC (CR-1558TM) and propagated as described for hCoV-229E but using HCT-8 cells and harvesting viral stocks on day 7.

\( \text{TCID}_{50} \). To determine the infectious titer of hCoV-229E, 25,000 Huh-7 cells were seeded one day prior infection in a 96 well plate. The following day, cells were inoculated with a 10-fold serial dilution of the respective virus stock. At 7 dpi, CPE was observed by light microscopy and the tissue culture infectious dose 50 (TCID\(_{50}\)) was calculated according to Reed-Münch [31]. For determining the TCID\(_{50}\) of hCoV-NL63 and -OC43 stocks, 10,000 LLC-MK2 cells or 30,000 HCT-8 cells were seeded, respectively, and treated as described for hCoV-229E. TCID\(_{50}\) values were used to normalize the MOIs in neutralization assays.

Statistics. A non-parametric Spearman rank test was used to assess possible correlations. Differences between sera obtained before and after vaccination were analyzed with the Wilcoxon matched-pairs signed rank test. Differences between the average activities of pre- and post-vaccination sera were analyzed using an unpaired students t-test. Serum dilutions causing a 50% reduction of viral RNA production or N protein expression (IC\(_{50}\) values), were calculated using a non-linear regression model, inhibitor vs. response, variable slope (four parameters). All analyses were done by GraphPad Prism version 9.1.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com, R (version 4.0.1) and SAS (version 9.4).
**Study approval.** Blood samples from individuals were obtained after recruitment of participants and written informed consent as approved by the ethics committee of Ulm University (99/21).

Detailed methods are described in the Supplemental Methods.

**RESULTS**

*COVID-19 vaccination enhances neutralization of genuine seasonal coronaviruses.* To determine the neutralizing activity of sera obtained before and after COVID-19 vaccination under the most physiological conditions possible, we established cell culture models allowing efficient production of genuine seasonal coronaviruses. We achieved this for three of the four seasonal viruses: hCoV-OC43 (Organ Culture 43), which belongs to the β-coronaviruses, just like SARS- and MERS-CoVs, and the two α-coronaviruses NL63 (Netherland 63) and -229E (Figure 1A). To determine the neutralizing capacity of sera, we mixed them with the respective hCoVs, inoculated Huh-7 cells, and determined viral RNA production by qRT-PCR two days later (Figure 1B). Neutralization was measured as reduction of viral RNA yields. Sera derived from all 24 individuals prior to and after heterologous COVID-19 immunization efficiently neutralized β-coronavirus OC43 (supplemental Figure 1). Prevaccination sera reduced hCoV-OC43 RNA production with an IC₅₀ of 0.09% (Table 1) and achieved complete inhibition at higher concentrations (Figure 1C). While the preexisting neutralizing activity against hCoV-OC43 was already high, it further increased upon COVID-19 vaccination (Figure 1D; Table 1). The hCoV-OC43 neutralizing capacity of the sera obtained before and after COVID-19 vaccination correlated but shifted towards higher efficacies for the post-vaccination sera (Figure 1E).

Preexisting neutralization of hCoV-NL63 was more variable and on average less efficient compared to hCoV-OC43 (supplemental Figures 2, 3). For example, sera from individuals 6,
15 and 16 reduced hCoV-NL63 RNA yields by ~98% at the lowest concentration (0.2%), while other sera showed little if any neutralizing activity (supplemental Figure 2). At a concentration of 0.2%, sera obtained after COVID-19 vaccination neutralized hCoV-NL63 with significantly higher efficacy (on average by almost 80%) than the pre-vaccination sera (less than 50%; Figures 1F-H). On average, the neutralizing activity against hCoV-NL63 increased about 2-fold after vaccination (Table 1).

Preexisting humoral immunity was lowest against the α-coronavirus 229E (supplemental Figures 3, 4). At a serum concentration of 5% the reduction of viral RNA production varied between 0% and 95% with an average of 60% (Figure 1I). Similar to the results obtained for hCoV-OC43 and -NL63, neutralization efficiency increased upon heterologous COVID-19 vaccination (Figures 1I-K), on average by almost 4-fold (Table 1). Neutralization of hCoV-229E did not saturate and significant increases after ChAdOx1 nCoV-19 BNT162b2 prime-boost vaccination were observed at all serum concentrations investigated (Figure 1J).

While the strength of preexisting humoral immunity against the three seasonal coronaviruses OC43, NL63 and 229E varied, the boosting effect of COVID-19 vaccination was observed for all three of them (Table 1). To challenge this finding in an independent experimental system, we established an in-cell ELISA-based neutralization assay that quantifies the levels of the nucleocapsid (N) protein (Figure 2A). Such assays have been reported for SARS-CoV-2 [32,33] and titration studies verified that they allow to quantify OC43 and 229E infection (supplemental Figure 5). We found that this ELISA-based assay allows to readily measure neutralization of hCoV-OC43 and -229E, albeit with reduced sensitivity compared to the qRT-PCR assay. However, it failed to yield reproducible results for hCoV-NL63. The ELISA-based assay confirmed efficient neutralization of hCoV-OC43 by all sera (supplemental Figure 6), as well as the modest increase of inhibitory activity upon COVID-19 vaccination (Figures 2B-D; Table 1). In agreement with our previous data (Figure
1E), the efficiencies of the two sets of sera correlated but shifted towards higher activity after vaccination (Figure 2D).

Predictably, neutralizing activity against hCoV-229E was also low in the in-cell ELISA-based assay (supplemental Figures 3, 7). Even at the highest concentration of 5%, the sera only reduced the levels of N protein expression by ~40% prior to and by ~55% after COVID-19 vaccination (Figure 2E). Differences between pre- and post-vaccination sera were modest but highly significant (Figure 2F). Altogether, SARS-CoV-2 vaccination clearly increased hCoV-229E neutralizing activity (Figure 2G; Table 1). The results of the RNA- and ELISA-based neutralization assays correlated significantly for hCoV-OC43 at a serum concentration of 0.2% (supplemental Figure 8A). Under other conditions correlations were not detected because effects were saturated at 1% for hCoV-OC43 (qPCR; supplemental Figure 8A) or generally small for hCoV-229E (ELISA; supplemental Figure 8B). In addition, the neutralizing titers against hCoV-OC43 detected before and after immunization did not correlate with the efficiency of the neutralizing response against SARS-CoV-2 spike-mediated infection (supplemental Figure 8C). Most importantly, however, both assays consistently showed that COVID-19 vaccination enhances the neutralizing activity against seasonal coronaviruses (Table 1).

*COVID-19 vaccination cross-neutralizes SARS-CoV-1 spike pseudoparticles and enhances inhibition of hCoV-OC43 spike-mediated infection.* Experiments with genuine coronaviruses are most relevant for the *in vivo* situation. However, they came with the caveat that highly pathogenic SARS-CoV-1 and MERS-CoV isolates could not be analyzed for biosafety reasons. For more comprehensive analyses of the effect of COVID-19 vaccination on neutralization of human coronaviruses, we thus used VSV- or HIV-based pseudoparticles containing the spike proteins of SARS-CoV-1, SARS-CoV-2, MERS-CoV and hCoV-OC43,
-NL63 and -229E (Figure 3A). We also attempted to generate pseudoparticles containing the spike of hCoV-HKU1 but the titers were too low for meaningful analysis. Sera from six individuals were selected based on adequate availability. Predictably, COVID-19 immunization strongly enhanced neutralization of SARS-CoV-2 (Figure 3B; supplemental Table 1) [30]. In agreement with published data [26], sera obtained after vaccination also inhibited infection mediated by the SARS-CoV-1 spike, which shares about 76% homology with that of SARS-CoV-2, but not infection mediated by the MERS-CoV spike showing only 35% homology [19]. Concordant with the results obtained with genuine hCoV-OC43, vaccination further enhanced the strong preexisting humoral immunity against pseudovirions carrying the hCoV-OC43 spike protein (Figure 3B). Despite the limited sample size, the increases in neutralization capacity against SARS-CoV-1 and hCoV-OC43 after COVID-19 vaccination were significant (Figures 3B, C). Pre-vaccination sera showed no activity against MERS-CoV but modest inhibition of SARS-CoV-1 and -2 spike containing pseudoparticles (Figures 3B, supplemental Figure 9) indicating possible cross-neutralization due to previous exposure to seasonal hCoVs. Sera obtained prior to vaccination also neutralized pseudotypes carrying the NL63 or 229E spike proteins and their efficacy was slightly increased after COVID-19 vaccination (Figures 3B, C). For the most part, the results obtained with the pseudoparticles confirmed and expanded the results obtained with genuine seasonal coronaviruses. However, genuine hCoV-229E was substantially less sensitive to neutralization than viral pseudoparticles carrying the 229E spike.

DISCUSSION

Analyzing genuine coronaviruses as well as spike containing viral pseudoparticles, we found that heterologous ChAdOx1 nCoV-19 BNT162b2 prime-boost vaccination significantly increases the neutralizing activity against seasonal coronaviruses. While the sensitivity of the
two assays used to determine neutralization varied, the magnitudes of the increases due to vaccination were highly similar (Table 1). We observed strong preexisting neutralization activity against hCoV-OC43 that belongs to the β-coronavirus genus just like SARS-CoV-2. On average, neutralizing potency against hCoV-OC43 increased about 1.5-fold after vaccination. In comparison, preexisting immunity against the α-coronavirus hCoV-229E was modest but neutralization activity increased about 4-fold upon vaccination, although the spike proteins of SARS-CoV-2 and hCoV-229E show only very limited homology.

We found that VSVpp carrying the 229E spike were substantially more susceptible to neutralization compared to the genuine virus. It has been reported that hCoV-229E shows antigenic drift to escape humoral immunity [34]. hCoV-229E was isolated more than 50 years ago [35] and the virus isolate used has already been deposited in 1973. To assess whether differences between the spike proteins of the genuine 229E virus and the viral pseudoparticles might contribute to the differential neutralization sensitivity, we compared their sequences. We found that both 229E spike amino acid sequences differ at only two positions (F230C and N714K) outside of the receptor-binding domain. This strongly suggests that the differential sensitivity is independent of changes in spike and may be due to the higher intrinsic infectiousness of genuine hCoV-229E compared to viral pseudoparticles. In either case, our data show that results obtained using pseudotype neutralization assays do not always fully recapitulate neutralization activities against genuine coronaviruses.

Current data regarding the effect of SARS-CoV-2 immunization on neutralization of seasonal coronaviruses are contradictory. One previous study did not observe differences in the antibody titers against seasonal coronaviruses after SARS-CoV-2 infection [36]. Our results, however, agree with results showing that SARS-CoV-2 infection induces neutralizing antibodies against SARS-CoV-1 and hCoV-OC43 and that sera from humans who received SARS-CoV-2 vaccines exert protective effects against various coronaviruses in mice [23–
One possible reason for the discrepancies is the utilization of different types of SARS-CoV-2 vaccines. For example, accumulating evidence shows that the heterologous ChAdOx1 nCoV-19 BNT162b2 prime-boost vaccination regimen applied in the present study induces stronger humoral immune responses than homologous COVID-19 vaccination [37–39]. Notably, we did not observe significant correlations between the neutralizing titers against SARS-CoV-2 spike VSVpp and circulating coronaviruses. Thus, our results add to the evidence that increased neutralizing activity against seasonal coronaviruses observed after SARS-CoV-2 vaccination may be due to reactivation of memory hCoV B cells rather than cross-neutralizing activity of SARS-CoV-2 antibodies [23,40,41].

Our study comes with the limitation that only 24 individuals were examined. Advantages are that we used genuine hCoV-OC43, -NL63 and -229E viruses and confirmed and expanded the analyses using pseudovirions covering six of the seven human coronaviruses. Induction of cross-neutralizing activity against SARS-CoV-1 was expected because its spike shows high homology to SARS-CoV-2 and agrees with published data [26,42–44]. In contrast, COVID-19 immunization did not induce definitive protective effects against MERS-CoV pseudoparticles, although its spike protein shows higher homology to the SARS-CoV-2 spike amino acid sequence than the seasonal coronaviruses. Although it is conceivable that vaccination with a β-CoV spike protein is more likely to induce cross-neutralization against other β-CoVs than to highly divergent α-CoVs, some domains in the S2 subunit of the α-CoV spike proteins show a higher degree of conservation [20]. Notably, hCoV-NL63 also uses ACE2 as receptor and it is tempting to speculate that the RBD of this α-CoV may share some conserved features with that of SARS-CoV-2.

Our goal was to clarify whether heterologous vaccination against SARS-CoV-2 affects humoral immunity against circulating coronaviruses. Some recent studies addressed the opposite question, i.e. does preexisting immunity against circulating coronaviruses affect the
efficacy of COVID-19 vaccination. In support of a protective role, it has been documented that recent infection with circulating coronaviruses is associated with less severe COVID-19 [45]. Others reported that antibodies against circulating coronaviruses are boosted upon SARS-CoV-2 infection but not associated with protection [46] or even exerting negative effects on the protective response to SARS-CoV-2 [47]. We did not observe a significant correlation between the preexisting neutralizing activity against hCoV-OC43 and the efficiency of SARS-CoV-2 S neutralization upon vaccination. Altogether, more comprehensive studies seem required to clarify whether pre-existing immunity to seasonal coronaviruses has beneficial or detrimental effects on the immune response to SARS-CoV-2 infection or vaccines.

Seasonal coronaviruses seem well adapted to their human host and have become endemic. It is tempting to speculate that SARS-CoV-2 might develop in a similar way [48]. Accumulating evidence suggests that the omicron variant of concern spreads substantially faster and escapes antibodies more readily than previous variants but may be less pathogenic [49,50]. Thus, while the omicron variant poses enormous challenges to health systems around the globe, it may also initiate the transition from pandemic to endemic. Given that COVID-19 vaccination clearly induces some cross-neutralization of hCoV-OC43 and (to a lesser extent) -NL63 and -229E, it might impact common cold infections. Strong protection against infection seems unlikely because the boosting effect of COVID-19 vaccination was usually modest and common cold viruses cause repeated infections despite preexisting immunity. However, it will be of interest to examine whether the course of seasonal coronaviruses infections is affected by COVID-19 vaccination.
NOTES

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Author contributions

JM, JAM and FK conceived the research, supervised the experiments, interpreted data, and wrote the manuscript. LvdH provided resources. QX performed the qPCR-based, JL the ELISA-based and FZ, QX and AS the pseudoparticle-based neutralization assays. FZ and TW supervised experiments. DK collected blood samples for neutralization assays.

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Conflict of interest

The authors declare that no conflict of interest exists
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FIGURE LEGENDS

Figure 1. Effect of heterologous COVID-19 vaccination on neutralizing activity against genuine seasonal coronaviruses. (A) Phylogenetic relationship between hCoVs and their animal relatives based on representative full-genome nucleotide sequences (modified from [51]). (B) Schematic outline of the qRT-PCR-based neutralization assay. (C-E) hCoV-OC43 neutralizing activity of sera obtained before and after vaccination. (C) Average hCoV-OC43 RNA copies detected after viral treatment with sera obtained before (pre) and after (Post) COVID-19 vaccination compared to the untreated control (100%). Shown are mean values (±SEM) obtained from 24 serum donors (Figure S1). (D) Differences between viral RNA yields were analyzed with the Wilcoxon matched-pairs signed rank test. (E) Spearman correlation between the neutralizing activity of sera obtained prior to and after vaccination. (F-H) hCoV-NL63 neutralizing activity of sera obtained before and after vaccination. (F) Mean hCoV-NL63 RNA copies (±SEM) derived from the neutralization assays shown in supplemental Figure 2. (G) Grouped comparisons of neutralization efficacies and (H) correlation between neutralization by pre- and post-immunization sera. Refer to panels C-E for details. (I-K) hCoV-229E neutralizing activity of sera obtained before and after vaccination. (I) Mean hCoV-229E RNA copies (±SEM) derived from the neutralization assays shown in supplemental figure 4. (J) Grouped comparisons of viral RNA yield and (K) correlation between neutralization efficiencies of pre- and post-immunization sera. Refer to panels C-E for details. Differences were analyzed with the Wilcoxon matched-pairs signed rank test (* p<0.05; ** p<0.01).

Figure 2. Neutralizing activity of pre- and post-vaccination sera against hCoV-OC43 and hCoV-229E in an in-cell ELISA-based assay. (A) Schematic outline of the in-cell ELISA-based neutralization assay. (B-D) hCoV-OC43 neutralizing activity of sera obtained
before and after heterologous COVID-19 vaccination. (B) Neutralization of hCoV-OC43 by sera obtained before (Pre) and after (Post) vaccination compared to the untreated control (100%). Shown are mean values (±SEM) obtained from the data shown in supplemental Figure 6. (C) Differences in the levels of hCoV-OC43 nucleocapsid (N) antigen expression were analyzed with the Wilcoxon matched-pairs signed rank test. (D) Correlation between the levels of hCoV-OC43 N antigen expression after virus treatment with sera obtained prior to and after vaccination. (E-G) hCoV-229E neutralizing activity of sera obtained before and after vaccination. (E) Mean hCoV-229E N levels (±SEM) derived from the neutralization assays shown in supplemental Figure 7. (G) Grouped comparisons of mean hCoV-229E N levels and (G) correlation between the effect of pre- and post-immunization sera on hCoV-229E N expression levels. Refer to panels B-D for detail. Differences were analyzed with the Wilcoxon matched-pairs signed rank test (* p<0.05; ** p<0.01; *** p<0.001).

**Figure 3. Neutralization of pseudoparticles containing the spike-proteins of highly pathogenic or circulating seasonal coronaviruses.** (A) Schematic presentation of the assays to assess spike-mediated VSVpp or HIVpp infection. (B) Neutralization of VSVpp or HIVpp containing the indicated spike (S) proteins or the VSV-G for control by sera obtained before (Pre) and after (Post) vaccination compared to the untreated control (100%). Shown are mean values (±SEM; n=6) obtained from the data shown in supplemental Figure 9. (C) Comparison between S-mediated VSVpp or HIVpp infection rates in the presence of different concentrations of sera obtained before and after heterologous COVID-19 vaccination. Differences were analyzed with the Wilcoxon matched-pairs signed rank test (* p<0.05; ** p<0.01; *** p<0.001).
Table 1. Average neutralization activities before and after SARS-CoV-2 immunization.

| CoV strain | vRNA |         | N ELISA |         |
|------------|------|---------|---------|---------|
|            | IC₅₀ pre | IC₅₀ post | ratio | IC₅₀ pre | IC₅₀ post | ratio |
| OC43       | 0.09% (1:1062) | 0.05% (1:1883) | 1.77 | 0.27% (1:367) | 0.18% (1:545) | 1.49 |
| 229E       | 3.01% (1:33) | 0.77% (1:130) | 3.90 | 19.99% (1:5) | 4.89% (1:20) | 4.09 |
| NL63       | 0.18% (1:559) | 0.09% (1:1152) | 2.06 | n.a. | n.a. | n.a. |

Values specify the average (n=24) serum concentration (%) required to inhibit viral RNA production or N antigen expression of the indicated seasonal coronaviruses by 50%. Number in parentheses give the corresponding serum dilution. Ratios indicate the average increase in neutralizing activity after SARS-CoV-2 immunization. N.a., not available.
Figure 2

A

B

C

D

E

F

G

Figure 2: [Diagram and graphs showing the results of experiments related to OC43 and 229E antigens in serum samples.]

Day 1: seed Huh-7 cells
Day 2: infect Huh-7 cells
Day 4 or 5: fix Huh-7 cells

Nucleocapsid ELISA

Pre and Post serum samples are compared for antigen levels.

R² = 0.1516
P = 0.0600

R² = 0.7502
P < 0.0001

R² = 0.4001
P = 0.0009
Figure 3

**A**
- CoV-1, CoV-2, MERS, 229E, Spike VSV(GFP)pp
  - day 1: seed Huh-7 cells
  - day 2: infect Huh-7 cells
  - day 3: determine GFP+ cells
- OC43, HKU1, Spike HIV(ΔLuc)pp
  - day 1: seed Huh-7 cells
  - day 2: infect Huh-7 cells
  - day 4: determine luciferase activity

**B**
- SARS-CoV-2 S
- SARS-CoV-1 S
- MERS-CoV S
- hCoV-OC43 S

**C**
- CoV-2 PP infection (%)
- CoV-1 PP infection (%)
- MERS PP infection (%)
- NL63 PP infection (%)
- 229E PP infection (%)