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Severe Acute Respiratory Syndrome Coronavirus 2 Vaccination Boosts Neutralizing Activity Against Seasonal Human Coronaviruses

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Background. Most of the millions of people that are vaccinated against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (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 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, Middle East Respiratory Syndrome (MERS)-CoV, and hCoV-OC43, hCoV-NL63, and hCoV-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, hCoV-NL63, and hCoV-229E.

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

Keywords. COVID-19 vaccination; seasonal coronaviruses; cross-neutralization.

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 has 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 8000 and 2500 individuals with case-fatality rates of ~10% and 35%, respectively. Fortunately, SARS-CoV-1 has disappeared, whereas MERS-CoV still causes sporadic infections in the Arabian Peninsula. In contrast, the 4 remaining human coronaviruses (hCoVs) have spread around the globe [1, 2]. The endemic α-coronaviruses hCoV-229E and hCoV-NL63 and the β-coronaviruses hCoV-OC43 and hCoV-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 and 40 billion USD in the United States 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 United States estimated the seropositivity of adults for hCoV-229E to be 91.3%, for hCoV-HKU1 59.2%, hCoV-NL63 91.8%, and hCoV-OC43 90.8% [9].

To date (January 14, 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

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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, vaccinations not only decreased incidences, hospitalizations, and deaths, but also allowed reduction of 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 infections compared with previous years [12, 13]. Similar predictions have been made for the current influenza virus season [14]. Readopted security measures because of 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 affects humoral immunity against seasonal coronaviruses. Indeed, it has been reported that anti-spike immunoglobulin M (IgM) and IgG titers against β-coronaviruses hCoV-OC43 and hCoV-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 hCoV-HKU1 spike proteins [21, 27, 28]. Here, we show that SARS-CoV-2 vaccination increases the neutralizing activity against all 3 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 [29]. Presera were taken up to 2 days before prime vaccination and postimmunization sera 2 weeks after the BNT162b2 boost [29]. Antibody titers against SARS-CoV-2 and neutralizing titers against hCoV-OC43 before and after vaccination are provided in supplementary 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 1 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 posttransfection, cells were infected with green fluorescent protein-encoding VSVΔG(green fluorescent protein)*VSV-G particles at a multiplicity of infection (MOI) of 3. Pseudotyped particles were harvested at 16 hours after 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 hCoV-HKU1 spikes, HEK293T cells were transfected with spike expression plasmids together with a pNL1_HIV-1_NL4-3-Δenv-fluc backbone. Forty-eight hours after 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 Dulbecco modified Eagle medium supplemented with 2% fetal calf serum. Cells were incubated at 33°C for 1 day after infection, washed with phosphate-buffered saline and further cultured in fresh medium. Cells were monitored daily under the light microscope until strong cytopathic effects 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.

Tissue Culture Infectious Dose 50

To determine the infectious titer of hCoV-229E, 25,000 Huh-7 cells were seeded 1 day before 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 days after infection, cytopathic effects were observed by light microscopy and the tissue culture infectious dose 50 ($TCID_{50}$) was calculated according to Reed-Münch [30]. For determining the $TCID_{50}$ of hCoV-NL63 and hCoV-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 nonparametric 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 postvaccination sera were analyzed using an unpaired Student *t* test. Serum dilutions causing a 50% reduction of viral RNA production or N protein expression (half maximal inhibitory concentration values), were calculated using a nonlinear regression model, inhibitor vs response, variable slope (4

parameters). All analyses were done by GraphPad Prism version 9.1.1 for Windows, GraphPad Software, San Diego, CA, 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 supplementary 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 3 of the 4 seasonal viruses: hCoV-OC43 (Organ Culture 43), which belongs to the β-coronaviruses, just like SARS-CoV and MERS-CoV, and the two α-coronaviruses NL63 (Netherland 63) and NL-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 quantitative reverse transcriptase polymerase chain reaction 2 days later (Figure 1B). Neutralization was measured as reduction of viral RNA yields. Sera derived from all 24 individuals before and after heterologous COVID-19 immunization efficiently neutralized β-coronavirus OC43 (supplementary Figure 1). Prevaccination sera reduced hCoV-OC43 RNA production with an half maximal inhibitory concentration of 0.09% (Table 1) and achieved complete inhibition at higher concentrations (Figure 1C). Although 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 toward higher efficacies for the post-vaccination sera (Figure 1E).

Preexisting neutralization of hCoV-NL63 was more variable and on average less efficient compared with hCoV-OC43 (supplementary Figures 2 and 3). For example, sera from individuals 6, 15, and 16 reduced hCoV-NL63 RNA yields by ~98% at the lowest concentration (0.2%), whereas other sera showed little if any neutralizing activity (supplementary 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 prevaccination sera (< 50%; Figures 1E-F). 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 (supplementary Figures 3 and 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 hCoV-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).

Although the strength of preexisting humoral immunity against the 3 seasonal coronaviruses OC43, NL63, and 229E varied, the boosting effect of COVID-19 vaccination was observed for all 3 of them (Table 1). To challenge this finding in an independent experimental system, we established an in-cell enzyme-linked immunosorbent assay (ELISA)-based neutralization assay that quantifies the levels of the nucleocapsid (N) protein (Figure 2A). Such assays have been reported for SARS-CoV-2 [31,32], and titration studies verified that they allow to quantify OC43 and 229E infection (supplementary Figure 5). We found that this ELISA-based assay allows to readily measure neutralization of hCoV-OC43 and hCoV-229E, albeit with reduced sensitivity compared with the quantitative reverse transcriptase polymerase chain reaction assay. However, it failed to yield reproducible results for hCoV-NL63. The ELISA-based assay confirmed efficient neutralization of hCoV-OC43 by all sera (supplementary Figure 6), as well as the modest increase of inhibitory activity upon COVID-19 vaccination (Figure 2B–D; Table 1). In agreement with our previous data (Figure 1E), the efficiencies of the 2 sets of sera correlated but shifted toward higher activity after vaccination (Figure 2D).

Predictably, neutralizing activity against hCoV-229E was also low in the in-cell ELISA-based assay (supplementary Figures 3 and 7). Even at the highest concentration of 5%, the sera only reduced the levels of N protein expression by ~40% before and by ~55% after COVID-19 vaccination (Figure 2E). Differences between pre- and postvaccination 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% (supplementary Figure 8A). Under other conditions, correlations were not detected because effects were saturated at 1% for hCoV-OC43 (quantitative polymerase chain reaction; supplementary Figure 8*A*) or generally small for hCoV-229E (ELISA; supplementary Figure 8*B*). 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 (supplementary Figure 8*C*). Most importantly, however, both assays consistently showed that COVID-19 vaccination enhances the neutralizing activity against seasonal coronaviruses (Table 1).

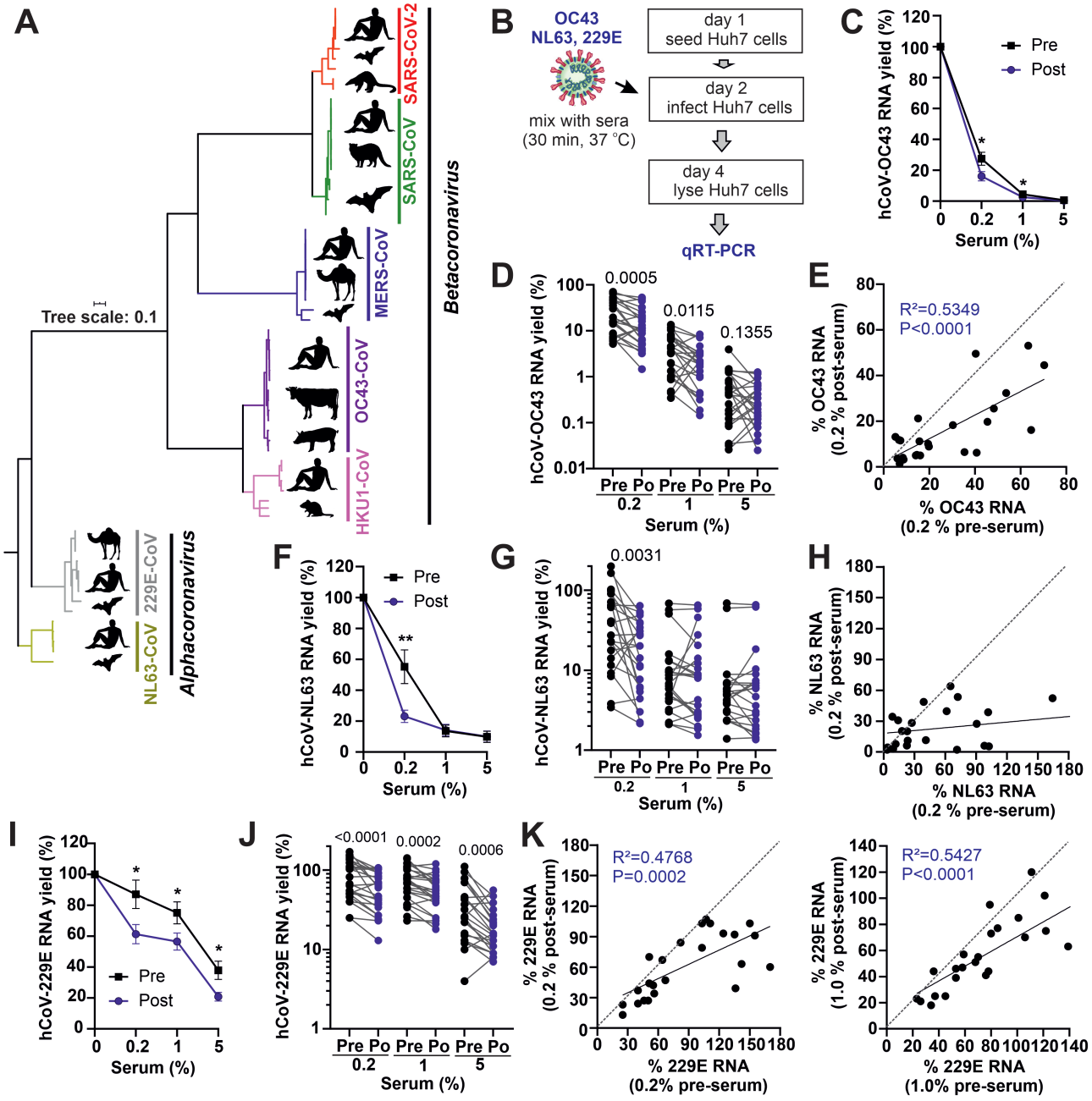

Figure 1. Effect of heterologous COVID-19 vaccination on neutralizing activity against genuine seasonal coronaviruses. (*A*) Phylogenetic relationship between hCoVs and their animal relatives based on representative full-genome nucleotide sequences (modified from [50]). (*B*) Schematic outline of the quantitative reverse transcriptase polymerase chain reaction-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 (supplementary 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 before 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 supplementary Figure 2. (*G*) Grouped comparisons of neutralization efficacies and (*H*) correlation between neutralization by pre- and postimmunization 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 supplementary Figure 4. (*J*) Grouped comparisons of viral RNA yield and (*K*) correlation between neutralization efficiencies of pre- and postimmunization sera. Refer to panels *C–E* for details. Differences were analyzed with the Wilcoxon matched-pairs signed-rank test (**P* < .05; ***P* < .01). COVID-19, coronavirus disease 2019; hCoV, human coronavirus; SEM, standard error of the mean.

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

Table 1. Average Neutralization Activities Before and After SARS-CoV-2 Immunization

	vRNA			N ELISA		
CoV strain	IC_{50} pre	IC_{50} post	Ratio	IC_{50} pre	IC_{50} 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	NA	NA	NA

Values specify the average (n = 24) serum concentration (%) required to inhibit vRNA production or N antigen expression of the indicated seasonal coronaviruses by 50%. Number in parentheses gives the corresponding serum dilution. Ratios indicate the average increase in neutralizing activity after severe acute respiratory syndrome coronavirus 2 immunization.

Abbreviations: CoV, coronavirus; IC_{50}, half maximal inhibitory concentration; NA, not available; vRNA, viral RNA.

neutralization of human coronaviruses, we thus used VSV- or human immunodeficiency virus-based pseudoparticles containing the spike proteins of SARS-CoV-1, SARS-CoV-2, MERS-CoV, and hCoV-OC43, hCoV-NL63, and hCoV-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 6 individuals were selected based on adequate availability. Predictably, COVID-19 immunization strongly enhanced neutralization of SARS-CoV-2 (Figure 3B; supplementary Table 1) [29]. 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 (Figure 3B and 3C). Prevaccination sera showed no activity against MERS-CoV

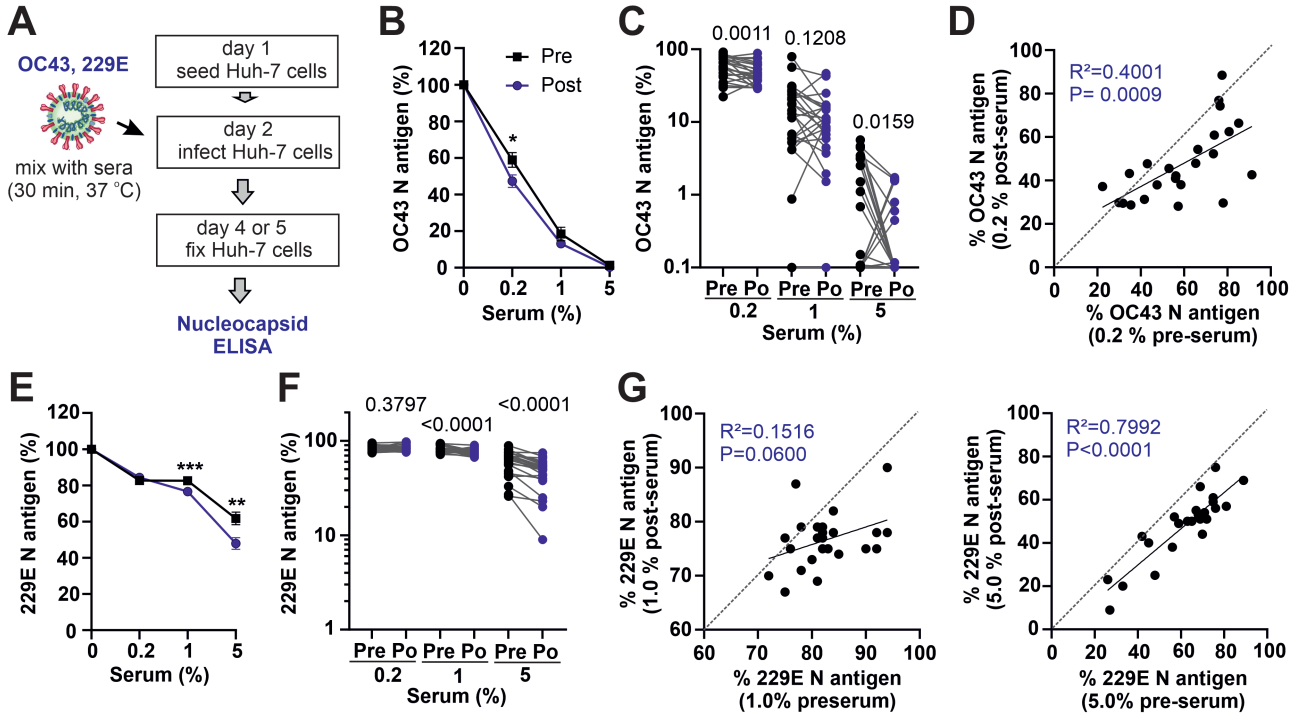

Figure 2. Neutralizing activity of pre- and postvaccination sera against hCoV-OC43 and hCoV-229E in an in-cell ELISA-based assay. (*A*) Schematic outline of the in-cell ELISA-based neutralization assay. (*B–D*) hCoV-OC43 neutralizing activity of sera obtained before and after heterologous COVID-19 vaccination. (*B*) Neutralization of hCoV-OC43 by sera obtained before (pre) and after (post) vaccination compared with the untreated control (100%). Shown are mean values (±SEM) obtained from the data shown in supplementary 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 supplementary Figure 7. (*G*) Grouped comparisons of mean hCoV-229E N levels and (*G*) correlation between the effect of pre- and postimmunization 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* < .05; ***P* < .01; ****P* < .001). COVID-19, coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; hCoV, human coronavirus; SEM, standard error of the mean.

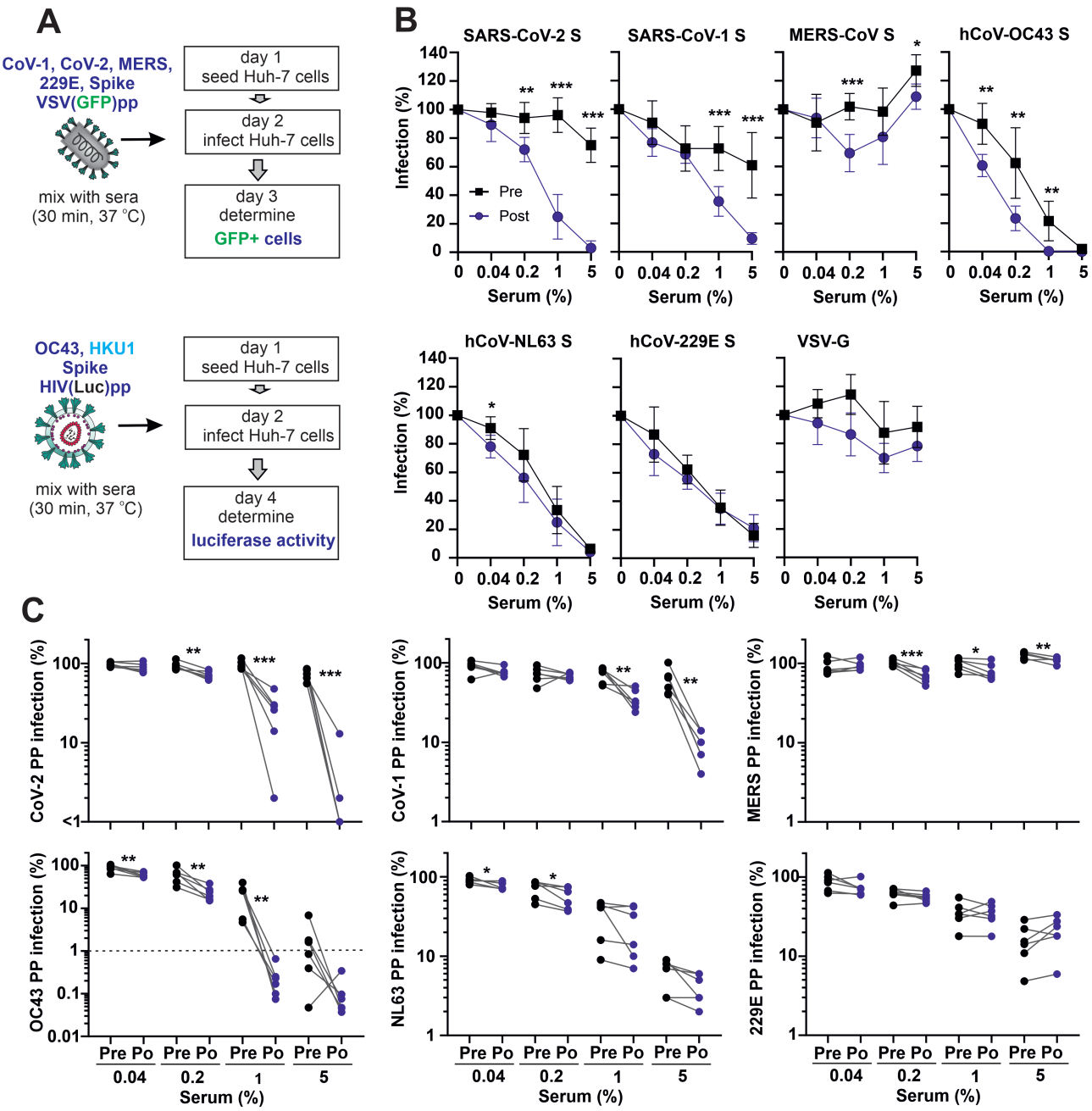

Figure 3. Neutralization of pseudoparticles containing the spike proteins of highly pathogenic or circulating seasonal coronaviruses. (*A*) Schematic presentation of the assays to assess spike-mediated VSVpp or HIVpp infection. (*B*) Neutralization of VSVpp or HIVpp containing the indicated spike (S) 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 supplementary 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 (*$P < .05$; **$P < .01$; ***$P < .001$). COVID-19, coronavirus disease 2019; HIVpp, human immunodeficiency virus pseudoparticles; SEM, standard error of the mean; VSVpp, vesicular stomatitis virus pseudoparticles.

but modest inhibition of SARS-CoV-1 and -2 spike containing pseudoparticles (Figure 3B, supplementary Figure 9), indicating possible cross-neutralization from previous exposure to seasonal hCoVs. Sera obtained before vaccination also neutralized pseudotypes carrying the NL63 or 229E spike proteins and their efficacy was slightly increased after COVID-19 vaccination (Figure 3B and 3C). 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. Although the sensitivity of the 2 assays used to determine neutralization varied, the magnitudes of the increases from 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 VSV particles (pp) carrying the 229E spike were substantially more susceptible to neutralization compared with the genuine virus. It has been reported that hCoV-229E shows antigenic drift to escape humoral immunity [33]. hCoV-229E was isolated more than 50 years ago [34] and the virus isolate used was 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 2 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 with 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 [35]. 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 [23–26]. 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 [36–38]. 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, 39, 40].

Our study comes with the limitation that only 24 individuals were examined. Advantages are that we used genuine hCoV-OC43, hCoV-NL63, and hCoV-229E viruses and confirmed and expanded the analyses using pseudovirions covering 6 of the 7 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, 41–43]. 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 receptor-binding domain 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 (ie, 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 [44]. Others reported that antibodies against circulating coronaviruses are boosted upon SARS-CoV-2 infection but not associated with protection [45] or even exerting negative effects on the protective response to SARS-CoV-2 [46]. 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 preexisting 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 [47]. 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 [48, 49]. Thus, although 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.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. M., J. A. M., and F. K. conceived the research, supervised the experiments, interpreted data, and wrote the manuscript. L. v. d. H. provided resources. Q. X. performed the quantitative polymerase chain reaction-based, J. L. the enzyme-linked immunosorbent assay-based, and F. Z., Q. X., and A. S. the pseudoparticle-based neutralization assays. F. Z. and T. W. supervised experiments. D. K. collected blood samples for neutralization assays.

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Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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