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Mucosal immunization with ChAd-SARS-CoV-2-S prevents sequential transmission of SARS-CoV-2 to unvaccinated hamsters

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COVID-19 vaccines have successfully reduced severe disease and death after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Nonetheless, COVID-19 vaccines are variably effective in preventing transmission and symptomatic SARS-CoV-2 infection. Here, we evaluated the impact of mucosal or intramuscular vaccine immunization on airborne infection and transmission of SARS-CoV-2 in Syrian hamsters. Immunization of the primary contact hamsters with a mucosal chimpanzee adenoviral-vectored vaccine (ChAd-CoV-2-S), but not intramuscular messenger RNA (mRNA) vaccine, reduced infectious virus titers ~100-fold and 100,000-fold in the upper and lower respiratory tract of the primary contact hamster following SARS-CoV-2 exposure. This reduction in virus titer in the mucosal immunized contact animals was sufficient to eliminate subsequent transmission to vaccinated and unvaccinated hamsters. In contrast, sequential transmission occurred after systemic immunization with the mRNA vaccine. Thus, immunization with a mucosal COVID-19 vaccine protects against cycles of respiratory transmission of SARS-CoV-2 and can potentially limit the community spread of the virus.

INTRODUCTION

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 was identified in 2019, and multiple vaccines were developed and deployed by the end of 2020. These early COVID-19 vaccines, targeting the spike (S) protein of SARS-CoV-2, were 75 to 95% effective against symptomatic infection, disease severity, and death after exposure to closely matched variants of SARS-CoV-2 (1−6). The emergence of neutralization resistance variants, such as the B.1.1.7 or B.1.5 Omicron variant of SARS-CoV-2, reduced vaccine efficacy, necessitating introduction of regular updates of the COVID-19 vaccine (5, 7−10). The effects of vaccination on SARS-CoV-2 transmission are less well understood.

Transmission of SARS-CoV-2 principally occurs through the air via inhalation of virus-containing droplets and aerosols, although direct contact and fomite transmission have been reported (11). In defined clusters, such as households, cycles of virus transmission can be divided into primary and secondary events; primary transmission is the spread of the virus from the index case to contacts, and secondary transmission denotes spread from these contacts to additional contacts. Transmission of SARS-CoV-2 can be reduced by social distancing, mask wearing, and immunity derived from prior infections and/or vaccinations (12−15). Few studies have measured vaccine efficacy against transmission of SARS-CoV-2. In humans, the efficacy of two doses of BNT162b2 or ChAdOx1, delivered intramuscularly, against infection was between 23 and 57% for the pre-Delta variants of SARS-CoV-2 (16−18). The impact of prior infection or mucosal vaccines on SARS-CoV-2 transmission is not known.

The Syrian hamster is a sensitive model that can be used to assess the impact of vaccination on airborne or direct-contact transmission of SARS-CoV-2. Most studies have evaluated the impact of preclinical vaccines on primary transmission, wherein the donor (or index) hamster is vaccinated and inoculated with a large amount (≥10⁶ infectious units) of virus, and transmission to a vaccinated or unvaccinated recipient hamsters was assessed after prolonged (>1 day) exposure times (19−25). These conditions do not mimic the natural mode or dose of infection, nor do they evaluate sequential or secondary cycles of transmission of the virus from the vaccinated hamsters.

We developed a sequential transmission model of SARS-CoV-2 in Syrian hamsters, using defined exposure times (8 hours), to evaluate the efficacy and impact of a preclinical version of an intranasally delivered COVID-19 mucosal vaccine that is currently used in India (InCovacc) and an intramuscularly delivered mRNA vaccine on primary and secondary transmission. We show that intranasal immunization of recipient animals substantially reduces virus load in the upper and lower respiratory tract and is associated with a lack of secondary airborne transmission and infection from these immunized animals to both unvaccinated and vaccinated animals. Thus, in hamsters, mucosal vaccination with ChAd-CoV-2-S can disrupt transmission cycles of SARS-CoV-2.

RESULTS

Sequential SARS-CoV-2 transmission occurs in the upper and lower airways after a 72-hour incubation of primary contact hamsters

We have previously shown that the D614G variant of SARS-CoV-2 transmits efficiently via the airborne route between Syrian hamsters (28). Here, we evaluated the potential of the hamster model to detect secondary airborne transmission of SARS-CoV-2 to model community spread of the virus (Fig. 1A). Donor hamsters were inoculated with 10⁵
plaque-forming units (PFU) of WA1/2020 D614G, and 24 hours later, primary contact hamsters (C1 or contact 1) were exposed to donor hamsters for 8 hours to reflect realistic exposure times, before they were placed back in their original cage. One (Fig. 1B), two (Fig. 1C), or three (Fig. 1D) days later, modeling different incubation times after primary exposure, secondary contact hamsters (C2 or contact 2) were exposed to the contact 1 hamsters for 8 hours. Nasal turbinates, nasal washes, and lungs were collected from all animals for virological analysis (Fig. 1, B to D). All tissues examined from the donor and contact 1 hamsters contained infectious virus and subgenomic RNA (sgRNA; Fig. 1, B to D), except for one nasal wash sample from a contact 1 animal in the 48-hour incubation group (Fig. 1C), confirming efficient primary airborne transmission of SARS-CoV-2 in Syrian hamsters. In contact 2 hamsters that were exposed to contact 1 hamsters 24 hours after the primary transmission event, substantial infectious virus (>10^3 PFU/ml) was detected in the lungs of two of three animals, and low levels of virus were measured in the nasal turbinates (Fig. 1B). At 48 hours, infectious virus was detected in the lungs of all three contact 2 animals and in the nasal wash and nasal turbinate of one animal (Fig. 1C). At 72 hours after the primary exposure event, all tissues examined from all four contact 2 animals contained high levels of infectious SARS-CoV-2 virus (Fig. 1D). Overall, these data show that a secondary transmission event to the upper and lower respiratory tract of unvaccinated contact 2 hamsters is most likely to occur 72 hours after primary exposure of contact 1 hamsters (Fig. 1E).
Mucosal immunization, but not systemic immunization, restricts SARS-CoV-2 replication in the airways of aerosol-exposed hamsters

We next evaluated the impact of a mucosal and systemic COVID-19 vaccine on protection against airborne infection and transmission of SARS-CoV-2. Groups of Syrian hamsters were immunized once intranasally with $10^{10}$ virus particles of a chimpanzee adenoviral-vector SARS-CoV-2 vaccine (ChAd-SARS-CoV-2-S or ChAd-CoV-2-S) or three intramuscularly with 5.0 $\mu$g of remnant mRNA COVID-19 vaccine (BNT162b2). Twenty-one days after the final immunization, serum was collected. Two weeks later, vaccinated and unvaccinated hamsters were exposed for 8 hours to naive donor animals that had been inoculated 24 hours earlier with $10^5$ PFU of WA1/2020 D614G virus (Fig. 2A). Immediately after exposure, the donor animals were euthanized and nasal turbinates, nasal washes, and the lungs were collected to confirm SARS-CoV-2 infection (fig. S1). Three days later, nasal turbinates, nasal washes, and the lungs of the contact hamsters were collected for measurement of infectious virus and viral RNA levels. As expected, the nasal turbinates of the unvaccinated control contact animals contained high amounts of infectious virus ($1 \times 10^7$ PFU/ml) and sgRNA ($6.8 \times 10^7$ copies/ml; Fig. 2B). These were reduced 1700-fold ($P < 0.0001$) and 44-fold ($P < 0.001$), respectively, in the ChAd-CoV-2-S–immunized contact animals (Fig. 2B). Despite the significant reduction in virus titer, only 3 of 14 (21%) ChAd-CoV-2-S–immunized
contact animals were negative for SARS-CoV-2 in both assays. In the mRNA-immunized hamsters, the amount of infectious virus and viral RNA was reduced 11-fold ($P = 0.07$) and 2-fold ($P = 0.81$), respectively, compared to the naïve controls and only 1 of 16 animals (6%) remained SARS-CoV-2 negative. A similar effect of mucosal immunization was observed in the nasal wash of these same animals. Compared to naïve control animals ($2.4 \times 10^{5}$ PFU/ml), the infectious virus titer was reduced 59-fold ($P < 0.0001$) to $4 \times 10^{3}$ PFU/ml and the amount of viral RNA decreased from $1.2 \times 10^{6}$ to $8.6 \times 10^{3}$ copies/ml (14-fold, $P < 0.01$, Fig. 2C) in the nasal wash of ChAd-CoV-2-S–immunized animals. Two animals (14%) remained negative in both assays for SARS-CoV-2 in their nasal wash. Intramuscular immunization with mRNA did not reduce infectious virus ($3.0 \times 10^{2}$ copies/ml) or viral RNA ($2.2 \times 10^{3}$ copies/ml) levels in the nasal wash (Fig. 2C), and only 1 of 16 animals (6%) remained SARS-CoV-2 negative. Last, in the lungs of the ChAd-CoV-2-S–immunized contact animals, the amount of infectious virus ($586,000$-fold, $P < 0.0001$) and sgRNA ($24,000$-fold, $P < 0.0001$) was reduced to the limit of assay detection compared to the control contact animals (Fig. 2D), and 14 of 14 (100%) ChAd-CoV-2-S–immunized animals were protected against lung infection. Systemic immunization with an mRNA vaccine also reduced the infectious virus titer (1500-fold, $P < 0.0001$) and viral RNA load (100-fold, $P < 0.0001$) in the lungs, compared to naïve control animals, albeit the fold reduction was lower ($P < 0.0001$) compared to hamsters that received the mucosal vaccine. In addition, a smaller fraction of the mRNA-vaccinated animals [4 of 16 (25%)] was protected completely against SARS-CoV-2 in the lung. We pooled the data to determine the impact of mucosal and systemic immunization on overall infection and transmission rates to the upper and lower airways (Fig. 2E). Despite the significant reduction in virus load in the respiratory tissues of the immunized hamsters, only 14% (2 of 14) of the ChAd-CoV-2-S–immunized animals were completely protected from infection of the upper airways (Fig. 2E). In contrast, complete protection from airborne infection of the lower airways was achieved after ChAd-CoV-2-S, but not mRNA immunization, suggesting that mucosal vaccines provide superior protection against airborne infection and transmission of SARS-CoV-2. Because immunity, induced by vaccination or previous infection, can affect replication kinetics and virus detection, we repeated the airborne transmission experiment in ChAd-CoV-2-S–immunized and unvaccinated control animals and collected tissues at an earlier time point (48 hours) after exposure (fig. S2). Compared to the unvaccinated control contact 1 animals, the infectious virus titers were reduced in the nasal turbinates (600-fold, $P < 0.05$), nasal washes (84-fold, $P < 0.001$), and lungs (156,000-fold, $P < 0.0001$) of the ChAd-CoV-2-S–immunized animals (fig. S2). The amount of viral RNA was also reduced in the nasal turbinates (9-fold), nasal washes (6-fold), and lungs (5700-fold), albeit the difference was only significant in the lower airways ($P < 0.0001$). Similar to previous studies (Fig. 2), limited protection against infection was observed in the upper airways, but near-complete protection was detected in the lower airways (fig. S2).

**Immunization with ChAd-CoV-2-S vaccine disrupts secondary transmission of SARS-CoV-2 to unvaccinated hamsters**

Although immunization with ChAd-CoV-2-S or mRNA did not prevent SARS-CoV-2 infection after airborne exposure, the viral load in the upper and lower airways was reduced, suggesting that it might prevent secondary or sequential cycles of transmission of SARS-CoV-2. To evaluate the impact of nasal or systemic immunization on secondary transmission, vaccinated and unvaccinated contact 2 animals were exposed to ChAd-CoV-2-S– and mRNA-vaccinated hamsters 72 hours after the initial exposure to an unvaccinated donor animal (Fig. 3A). As a control, we used age-matched unvaccinated or naïve contact 1 and 2 hamsters. Nasal turbinates, nasal washes, and lungs were collected from the contact 2 hamsters 48 hours after the secondary exposure, for measurement of infectious virus and viral RNA. In the unvaccinated control hamsters, secondary airborne transmission resulted in high virus titers in the nasal turbinates ($1.3 \times 10^{5}$ PFU/ml and $1.2 \times 10^{4}$ RNA copies/ml, Fig. 3B), nasal washes ($1.1 \times 10^{4}$ PFU/ml and $1.4 \times 10^{5}$ RNA copies/ml, Fig. 3C), and lungs ($1.2 \times 10^{6}$ PFU/ml and $1.3 \times 10^{4}$ RNA copies/ml, Fig. 3D) of the contact 2 animals. Overall, 10 of 15 (67%) nasal turbinates, 8 of 15 (53%) nasal washes, and 12 of 15 (80%) lungs of the unvaccinated contact 2 animals were positive for SARS-CoV-2. In contrast, the upper and lower airways from ChAd-CoV-2-S–immunized contact 2 hamsters exposed to ChAd-CoV-2-S–immunized contact 1 hamsters did not contain any measurable infectious virus or viral RNA in any of the tissues collected, and 100% of the animals were protected from secondary transmission (Fig. 3, B to D). We also failed to detect SARS-CoV-2 in the respiratory tissues of age-matched unvaccinated contact 2 hamsters exposed to ChAd-CoV-2-S–immunized contact 1 hamsters, as all animals were protected from secondary transmission. In a separate cohort of ChAd-CoV-2-S–immunized animals, we detected a similar level of protection from secondary airborne transmission to naïve and ChAd-CoV-2-S–immunized animals when the incubation time, i.e., time between primary and secondary exposure, was reduced from 72 to 48 hours (fig. S2).

Unlike the mucosal vaccine, immunization of contact 1 animals with mRNA did not eliminate secondary airborne transmission to vaccinated or unvaccinated contact 2 hamsters. SARS-CoV-2 was detected in the nasal turbinates of three of eight (38%) unvaccinated (3.3 $\times 10^{3}$ PFU/ml and $1.4 \times 10^{5}$ RNA copies/ml) and mRNA-vaccinated (3.8 $\times 10^{3}$ PFU/ml and $3.5 \times 10^{4}$ RNA copies/ml) contact 2 animals (Fig. 3B). Similarly, three of eight (38%) and four of eight (50%) unvaccinated (8.0 $\times 10^{4}$ PFU/ml and $2.1 \times 10^{6}$ RNA copies/ml) and vaccinated (4.0 $\times 10^{4}$ PFU/ml and $3.8 \times 10^{4}$ RNA copies/ml) contact 2 hamsters, respectively, were positive for SARS-CoV-2 in the nasal wash (Fig. 3C). Last, the lungs of four of eight unvaccinated (3.1 $\times 10^{3}$ PFU/ml and $1.5 \times 10^{5}$ RNA copies/ml) and three of eight (38%) mRNA-vaccinated (2.4 $\times 10^{4}$ PFU/ml and $3.1 \times 10^{5}$ RNA copies/ml) contact 2 hamsters were SARS-CoV-2 positive (Fig. 3D).

Analysis of the cumulative rates of secondary airborne transmission revealed that the mucosal ChAd-CoV-2-S vaccine conferred significant protection from secondary transmission to both vaccinated ($P < 0.01$) and unvaccinated ($P < 0.001$) hamsters with odds ratios (ORs) of 0.0 (95% CI 0.00 to 0.179) and 0.0 (95% CI 0.00 to 0.262). Systemic immunization with mRNA also reduced secondary transmission rates to both vaccinated and unvaccinated (OR = 0.42, 95% CI 0.07–2.34) hamsters, albeit the difference was not statistically significant (Fig. 3E).

**Serum antibody responses correlate with virus titers in ChAd-CoV-2-S, but not mRNA-immunized hamsters**

To begin to define the immune correlates of protection from airborne SARS-CoV-2 infection and transmission, S-specific immunoglobulin G (IgG) and neutralizing antibody responses in the serum 21 days after the final immunization were measured. As expected, serum
from unvaccinated control hamsters that received phosphate-buffered saline (PBS) did not bind to the S protein by enzyme-linked immunosorbent assay (ELISA) or neutralized WA1/2020 D614G virus (Fig. 4, A and B). In comparison, serum collected from ChAd-CoV-2-S–immunized hamsters contained high levels of anti–Wuhan-1 S–specific IgG antibodies [geometric mean titer (GMT)] of ~1:12,000, \( P < 0.0001 \) and neutralized WA1/2020 D614G effectively (GMT of ~1:4000, \( P < 0.0001 \); Fig. 4, A and B). Serum collected from mRNA-immunized animals contained 2-fold higher levels of Wuhan-1 S binding antibodies (GMT of ~1:30,000, \( P < 0.001 \)) and <2-fold lower virus neutralization titers (GMT of ~1:2400, \( P < 0.05 \)) compared to the ChAd-CoV-2-S–immunized animals. Correlation analysis between
were measured by ELISA (A) and focus reduction neutralization titer (FRNT) (B), respectively, and analyzed by one-way ANOVA with multiple comparisons correction on 

Correlation analysis with Bonferroni's multiple comparison correction.

Serum antibody titers correlate with virus titers in ChAd-CoV-2-S–immunized hamsters. (A and B) Hamsters were immunized with a single intranasal (IN) dose of ChAd-CoV-2-S (ChAd-S, 10⁸ virus particles, n = 14) or three intramuscular (IM) doses of remnant mRNA vaccine (BNT162b2, 5 μg, n = 16). PBS was used as a control (naive, n = 15). Serum was collected 3 weeks after the final immunization and Wuhan-1 spike protein binding titers and neutralization of titers against WA1/2020 D614G were measured by ELISA (A) and focus reduction neutralization titer (FRNT) (B), respectively, and analyzed by one-way ANOVA with multiple comparisons correction on In-transformed data (***, P < 0.0001, * P < 0.05). (C to H) Analysis of neutralization (IC₅₀) and serum binding (EC₅₀) titers against infectious viral titers in the nasal turbinate ([C] and [D]), nasal wash (E and F), and lungs (G) and (H) of unvaccinated (open symbols), ChAd-S–immunized (green symbols), or mRNA-immunized (purple symbols) primary contact hamsters 3 days after exposure. The results are from two to six independently repeated experiments, and each symbol is a different animal. Spearman correlation analysis with Bonferroni's multiple comparison correction.

The serum binding and virus neutralization antibody titers and virus titer detected in the nasal turbinates of the vaccinated and unvaccinated contact 1 animals (Fig. 2) revealed a significant negative correlation in the ChAd-CoV-2-S–immunized animals (r = −0.78, P < 0.0001 for neutralization titer and r = −0.82, P < 0.0001 for ELISA titer). No significant correlation was observed in mRNA-vaccinated hamsters in either assay (r = −0.28, P = 0.36 for neutralization titer and r = −0.33, P = 0.23 for ELISA titer; Fig. 4, C and D). In the ChAd-CoV-2-S–vaccinated animals, virus titers in the nasal wash also correlated with neutralizing and S binding antibody titers (r = −0.54, P < 0.05 for neutralization titer and r = −0.60, P < 0.01 for ELISA titer). Similar to the nasal turbinate, no significant correlation was detected in the nasal wash of mRNA-immunized hamsters (r = −0.22, P = 1.0 for neutralization titer and r = 0.15, P = 1.0 for ELISA titer; Fig. 4, E and F). Last, in the lungs, serum neutralization and binding (both with an r = −0.83 and P < 0.0001) titers correlated well with virus titers in the ChAd-CoV-2-S–immunized hamsters. In contrast to the upper airways, significant correlations between neutralizing antibody and lung virus titers were also found for the mRNA-immunized (r = −0.22, P < 0.01). However, there was no correlation seen in binding antibody titers in the lungs of mRNA hamsters (r = −0.15, P = 0.23; Fig. 4, G and H).

**Mucosal vaccination with ChAd-CoV-2-S leads to increased IgG and IgA antibodies in the nose**

To evaluate mucosal antibody immunity in our hamster cohort, we measured the amount of S-specific IgG and IgA antibodies in the nasal wash of contact 2 hamsters that had no detectable virus by plaque assay or quantitative reverse transcription polymerase chain reaction (RT-qPCR). As expected, nasal wash from unvaccinated control hamsters did not contain IgG or IgA antibodies that bound to the S protein of SARS-CoV-2 (fig. S3). In contrast, ChAd-CoV-2-S–vaccinated hamsters had significantly higher levels of S-specific IgG (P = 0.0001) and IgA (P < 0.01) compared to the naive control hamsters (fig. S3). Nasal washes from hamsters that received three doses of mRNA intramuscularly contained low levels of S-specific IgG, but no detectable IgA antibodies (P < 0.05 and not significant compared to ChAd-CoV-2-S and control hamsters, respectively). Collectively, these data show that intranasal immunization induces stronger mucosal and local immune responses compared to intramuscular mRNA vaccines.

**Sequential airborne transmissions of SARS-CoV-2 does not induce selection of viral variants**

To assess whether airborne transmission of SARS-CoV-2 was associated with genetic adaptations of SARS-CoV-2 during sequential...
transmission, we utilized next-generation sequencing of the S gene in a selection of the nasal turbinate (Table 1) of naïve and immunized contact 1 \((n = 13)\) and contact 2 \((n = 5)\) animals. No amino acid mutations were detected in the majority \((14 of 18)\) of the contact 1 and 2 hamsters (Table 1). However, single amino acid changes were identified in four hamsters with an allele frequency ranging from 25 to 54%. None of these changes were in the receptor binding domain of the S protein. Collectively these data suggest that airborne transmission of SARS-CoV-2 and breakthrough infections are not associated with any amino acid changes in the S protein of the virus.

**DISCUSSION**

Here, we developed a sequential airborne SARS-CoV-2 transmission model in Syrian hamsters and compared the impact of a mucosal and systemic vaccine on airborne infection and transmission. We demonstrated that intranasal immunization with a Chimpanzee adenoviral-vectored mucosal COVID-19 vaccine \((\text{ChAd-CoV-2-S})\) prevented primary SARS-CoV-2 transmission and infection of the lungs and blocked sequential transmission to both vaccinated and unvaccinated hamsters. In comparison, systemic immunization with a COVID-19 mRNA vaccine did not block virus replication in the lungs or sequential SARS-CoV-2 transmission. Overall, these data in hamsters demonstrate the potential impact of mucosal vaccines on preventing lower respiratory tract infections in a setting of airborne SARS-CoV-2 transmission and disrupting transmission.

Our study shows that a mucosal, but not systemic, COVID-19 vaccine blocks sequential airborne transmission from exposed and infected primary contact animals to unvaccinated secondary contacts. This observation has implications for vaccine development aimed at reducing the community spread of SARS-CoV-2 and other respiratory viruses. Although a positive impact of mucosal vaccines on sequential transmission was reported previously \((26, 27)\), these studies used very few animals, measured direct contact instead of sequential airborne transmission, or demonstrated a lack of SARS-CoV-2 infection in the primary contact animals. Despite these caveats, the studies highlight the potential of mucosal vaccines in reducing the household and community spread of the virus, which could lessen the impact of the pandemic by limiting sequential transmission cycles. The mechanism of the enhanced protection of the mucosal vaccine against primary and sequential transmission is not completely understood. Using a limited number of samples, we showed that mucosal IgA is detected in intranasally immunized hamsters, but not in naïve or mRNA-immunized hamsters, suggesting that the ChAd-CoV-2-S vaccine induced local immune responses including mucosal antibody and T cell responses as reported previously in mouse models \((29, 30)\). The local immune response is associated with reduced virus titers in the upper and lower airways, effectively reducing the amount of virus transmitted to levels below the minimal infectious dose of hamsters. The reduction in peak titer could be due to an early control of virus replication or a reduction in the effective infectious inoculum. Future experiments with genetically tagged viruses may be able to differentiate between these two possibilities. Alternatively, SARS-CoV-2–specific immunity modulates the spread of the virus, transmitted particle size, or the virus particle itself, effectively reducing virus transmission.

The experimental design used in this study has several advantages compared to other SARS-CoV-2 transmission studies \((21, 22, 26, 27, 31, 32)\). First, the use of a naïve donor or index animal and measuring sequential transmission mimic more closely the natural route and dose of infection of SARS-CoV-2. Second, our exposure time of 8 hours is more like that of a natural exposure in a community household setting, although it might overestimate the protective effects of transmission where more prolonged periods of exposure occur. Third, we used large numbers of animals in a sequential transmission chain.

**Table 1. Amino acid changes detected in the spike protein of primary or secondary contact hamsters.**

<table>
<thead>
<tr>
<th>Contact</th>
<th>Condition</th>
<th>Animal no.</th>
<th>Amino acid mutation</th>
<th>Allele frequency</th>
</tr>
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<tr>
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<td>2</td>
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<td></td>
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<td>12</td>
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<td></td>
<td></td>
<td>13</td>
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to evaluate the impact of mucosal and mRNA vaccines on primary and secondary transmission. A caveat of the study is that we compared two different vaccine platforms, each with its own route of administration. We chose this study design because we wanted to compare clinical vaccines that are currently in human use via the approved route. Moreover, previous studies had shown that intranasal administration of ChAd-CoV-2-S was more protective against upper airway infection compared to intramuscular administration (30, 33). The differences in the number of immunizations between ChAd-CoV-2-S and mRNA aimed to obtain a comparable serum antibody response and to standardize the time (4 to 5 weeks) between the last vaccination and exposure to SARS-CoV-2.

Limitations of the study
(i) Transmission was assessed by virological analysis and not seroconversion at 14 to 21 days after exposure, which is the more stringent measurement for assessing virus infection and transmission. (ii) All studies were done using male hamsters as male hamsters are more susceptible to SARS-CoV-2 infection (34). Although we were unable to assess the role of sex differences on vaccine efficacy and airborne SARS-CoV-2 transmission here, we understand this to be important and should be included in future studies. (iii) We did not evaluate the role of T cells on protection from primary and secondary transmission in hamsters. (iv) These studies were not performed with antigenically shifted variants of SARS-CoV-2 because of the reduction in hamsters (28).

Together, our study showed that mucosal immunization by an intranasal route, but not systemic intramuscular immunization, decreased viral burden in the upper airways, eliminated viral replication in the lower airways, and prevented sequential transmission to unvaccinated and vaccinated hamsters. This study highlights the possibility that SARS-CoV-2 transmission can be controlled in a population that is partially protected by prior infection or through mucosal vaccination.

MATERIALS AND METHODS
Experimental design
The goal of this study was to evaluate mucosal and systemic immunization on SARS-CoV-2 transmission in a preclinical model. Syrian hamsters were vaccinated with ChAd-CoV-2-S (30) intranasally or with remnant BNT162b2 intramuscularly and then exposed to SARS-CoV-2–infected hamsters or to previously exposed and infected hamsters. Viral titers were determined for the upper and lower airways to establish positive or negative transmission events. Secondary contact hamsters were excluded from analysis if the primary contact hamster was uninfected. Hamsters were randomly assigned to the vaccination or donor/contact group. Sample sizes were chosen on previous experience with SARS-CoV-2 transmission.

Cells and viruses
Vero cells expressing human angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2; Vero-hACE2-hTMPRSS2, a gift of A. Creanga and B. Graham, NIH) were cultured at 37°C in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 mM Hepes (pH 7.3), 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and puromycin (10 μg/ml). Vero E6 cells expressing human TMPRSS2 (Vero-hTRMPRSS2) were cultured at 37°C in DMEM supplemented with 10% FBS, 10 mM Hepes (pH 7.3), 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and blasticidin (5 μg/ml). Vero-hACE2-hTRMPRSS2 cells are used to titrate stocks and tissues and Vero-hTRMPRSS2 cells are used to generate virus stocks. Recombinant SARS-CoV-2 (strain 2019-nCoV/USA-WA1/2020) plus D614G (WA1/2020 D614G) was generated (35), and the virus sequence was confirmed by next-generation sequencing before in vitro or in vivo use. All work with infectious SARS-CoV-2 was performed in Institutional Biosafety Committee–approved BSL-3 and ABSL3 facilities at the Washington University School of Medicine using appropriate positive pressure air respirators and protective equipment.

Hamster experiments
Animal studies were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (assurance number A3381-01). Male hamsters (5 to 6 weeks old) were obtained from Charles River Laboratories and housed at Washington University.

Primary transmission
Male donor hamsters (5 to 6 weeks old) were inoculated with 10⁵ PFU of WA1/2020 D614G, and 24 hours later, donor and contact hamsters were placed in separate porous stainless steel (isolator) cages that were then placed in a single biocontainment unit (BCU) cage with 50 to 60 air changes per hour allowing for directional airflow from the donor to the contact hamster. The contact hamster was exposed for 8 hours, after which the donor and contact hamsters were placed back into their original cage. Donor and contact hamsters were euthanized and tissues were collected immediately following exposure (donor hamsters) or 48 to 72 hours after exposure (contact hamsters).

Secondary transmission
Male hamsters (5 to 6 weeks old) were inoculated intranasally with 10⁵ PFU of WA1/2020 D614G, and 24 hours later, naïve contact hamsters (contact 1) were exposed for 8 hours according to our primary airborne transmission setup (see above). The primary contact hamsters were placed back in their original cage, and 48, 48, or 72 hours later, they were used as donor hamsters to measure secondary or onward airborne transmission to a second naïve contact hamster (contact 2). Following exposure, the secondary contact hamsters were placed back in their original cages. Lungs, nasal turbinates, and nasal washes were collected from the donor and primary contact hamsters immediately after exposure or from the secondary contact animals 48 hours after the secondary exposure for virological analysis. Detection of sgRNA or infectious virus in any of the three tissues from the primary or secondary contact hamsters was considered a positive transmission and infection event.

At time of tissue collection, nasal wash, whole lung, and nasal turbinate were collected. The nasal wash was performed with 1 ml of PBS with 0.1% bovine serum albumin (BSA) and clarified at 1200 rpm for 10 min at 4°C. The whole lung was homogenized in 1.0 ml of DMEM and clarified by centrifugation at 1000g for 5 min. Nasal turbinates were collected by removing the skin along the nose and cheeks and then cutting the jaw to expose the upper palate. A sagittal incision through the palate exposed the nasal turbinates, which were then removed using blunt forceps. The nasal turbinates were homogenized in 1.0 ml of DMEM supplemented with 2% FBS, 10 mM Hepes (pH 7.3), and 2 mM L-glutamine and clarified by centrifugation at 1000g for...
Immunization and viral challenge

Male hamsters (5 to 6 weeks old) were either immunized intranasally with a single dose of $10^{10}$ viral particles (vp) of a chimpanzee adenovirus vector expressing a prefusion stabilized S protein of the ancestral SARS-CoV-2 (ChAd-CoV-2-S) (30, 33, 36) in 100 μl of PBS or immunized three times intramuscularly with 5 μg of recombinant mRNA COVID-19 vaccine (BNT162b2) in 100 μl of PBS (50 μl in each hindlimb) at 3- and 10-week intervals. Control hamsters received PBS alone. Twenty-one days after the last immunization, a serum sample was obtained for antibody analysis. Five to eight weeks after final immunization, the animals were transferred to the ABSL-3 and exposed to age-matched male donor hamsters inoculated intranasally with $10^5$ PFU of WA1/2020 D614G 24 hours earlier per our airborne transmission setup. Eight hours after exposure, the contact hamsters were placed back in their original BCU cages. Lungs, nasal turbinates, and nasal wash of the donor and contact hamsters were collected immediately after exposure (32 hours after inoculation) or 48 or 72 hours after exposure, respectively.

Virus titration assays

Plaque assays were performed on Vero-hACE2-hTRMPSS2 cells in 24-well plates. Nasal washes, lung tissue homogenates, or nasal turbinate homogenates were serially diluted in cell infection medium [DMEM supplemented with 2% FBS, 10 mM Heps (pH 7.3), and 2 mM l-glutamine]. Two hundred microliters of the diluted virus was added to a single well per dilution per sample. After 1 hour at 37°C, the inoculum was aspirated, the cells were washed with PBS, and a 1% methylcellulose overlay in MEM supplemented with 2% FBS was added. Seventy-two hours after virus inoculation, the cells were fixed with 10% formalin, and the monolayer was stained with crystal violet (0.5% w/v in 25% methanol in water) for 1 hour at 20°C. The number of plaques was counted and used to calculate the plaque-forming units per milliliter. Infectious virus titer detected in any of the contact hamster organs was considered a positive transmission event.

To quantify viral load in nasal washes, lung tissue homogenates, or nasal turbinate homogenates, RNA was extracted from 100-μl samples using E.Z.N.A. Total RNA Kit I (Omega) and eluted with 50 μl of water. Four microliters of RNA was used for real-time RT-qPCR to detect and quantify sgRNA of SARS-CoV-2 using TaqMan RNA-to-CT 1-step Kit (Thermo Fisher Scientific) as previously described (37) using the following primers and probes: forward: ACCAACCAACTTTCGATCTCTT; reverse: TCTGTTACTGCGAGTTGATCTCT; probe: ACGTCTTGGTGGCACCTCAGATTTCA; 5’Dye/3’ Quencher: 6-FAM/ZEN/IBFQ. sgRNA copy numbers per milligram for lung tissue homogenates or copy numbers per milliliter for nasal washes and nasal turbinate homogenates, based on a standard included in the assay, which was created via in vitro transcription of a synthetic DNA molecule containing the target region of the N gene. Viral RNA titer detected in any of the contact hamster organs was considered a positive transmission event.

Enzyme-linked immunosorbert assay

Hamster serum or concentrated nasal wash was evaluated by ELISA for IgG and/or IgA antibody binding. Seven-hundred microliters of nasal wash collected from SARS-CoV-2–uninfected contact 2 hamsters were concentrated using Amicon Ultra-0.5 centrifugal filter units to 50 μl. Ninety-six-well microtiter plates (Nunc MaxiSorp; Thermo Fisher Scientific) were coated with 100 μl of recombinant SARS-CoV-2 S protein (2019-nCoV/USA-WA1/2020) at a concentration of 1 μg/ml in PBS (Gibco) at 4°C overnight; negative control wells were coated with BSA (1 μg/ml; Sigma-Aldrich). The plates were washed three times with T-PBS (1× PBS supplemented with 0.05% Tween 20) and then blocked for 90 min at room temperature with 280 μl of blocking solution [PBS supplemented with 0.05% Tween 20 (Sigma-Aldrich) and 10% FBS (Corning)]. Sera or concentrated nasal washes from hamsters were diluted serially six- or threefold with a starting dilution of 1:100 or 1:10, respectively, in blocking solution and incubated for 90 min at room temperature. The plates were washed three times with T-PBS. Horseradish peroxidase (HRP)– conjugated anti-hamster IgG(H + L) antibody (Southern Biotech catalog no. 6061-05), diluted 1:500 in blocking solution (for IgG studies), or biotinylated anti-hamster IgA antibody (Brookwood Biomedical, catalog no. sab3002a), diluted 1:1000 in the blocking solution (for IgA studies), was added to all wells and incubated for 1 hour at room temperature. For IgA studies, plates were washed three times with T-PBS and incubated with HRP-conjugated streptavidin (Zymed) diluted 1:5000. Plates were washed three times with T-PBS and three times with 1× PBS, and 100 μl of one-step Ultra TMB-ELISA substrate solution (Thermo Fisher Scientific) was added to all wells. The reaction was stopped after 5 min using 100 μl of 1 M HCl or 1 N H₂SO₄, and the plates were analyzed at a wavelength of 490 nm using a microtiter plate reader (BioTek).

Focus reduction neutralization titer assay

Serial dilutions of serum samples, starting at 1:100, were incubated with $10^2$ focus-forming units of SARS-CoV-2 WA1/2020-D614G for 1 hour at 37°C. Antibody–virus complexes were added to Vero-hTRMPSS2 cell monolayers in 96-well plates and incubated at 37°C for 1 hour. Subsequently, cells were overlaid with 1% (w/v) methycellulose in Eagle’s minimal essential medium (MEM, Thermo Fisher Scientific). Plates were harvested 30 hours later by fixing with 10% formalin in PBS for 20 min at room temperature. Plates were washed and sequentially incubated with a pool of anti-S murine antibodies (SARS2-02, -08, -09, -10, -11, -13, -14, -17, -20, -26, -27, -28, -31, -38, -41, -42, -44, -49, -57, -62, -64, -65, -67 and -71) (38) and HRP-conjugated goat antimouse IgG (Sigma-Aldrich catalog no. A98294) in PBS supplemented with 0.1% saponin and 2% FBS. SARS-CoV-2–infected cell foci were visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot microanalyzer (Cellular Technologies).

Next-generation sequencing of S gene

RNA extracted from 18 contact 1 and contact 2 hamsters was used to prepare double-stranded DNA (dsDNA) for S gene sequencing. The primers were designed to tile the entire S gene of SARS-CoV-2. Amplicons tiling the S gene were generated using the SuperScript IV One-Step RT-PCR System (catalog no. 12594100). The following primers were used to make two pools of amplicons using two sets of nonoverlapping primers: primer set 1: forward: CTAAGTGTATCTTTGTTAACAAC, reverse: GTCTACAGCAGCTGATTGGA; primer set 2: forward: CAGAGAATTGGAGACTAC, reverse: CACTATTAAATGTTGTTGGAATC; primer set 3: forward: GATTGTCTTCTCAGTTGGACAG, reverse: GAACAGCAACTGTTTGAAG; and primer set 4: forward: GTCAACTCATTCTGTGGTG, reverse: CAGTTTCAATTGGGAAGT. For the first pool, primer sets 1 and 2, and for the second pool, primer sets 3 and 4 were used. The two
polls of amplicons were combined and purified to generate a library. The Nextera XT DNA Library Preparation Kit (Illumina) was used to prepare the sequencing libraries. Final PCR amplification products were size selected using Agencourt AMPure XP Beads (Beckman Coulter). Libraries were quantified using a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, catalog no. Q32851) to determine the concentration. The quality was then assessed with the Agilent High Sensitivity DNA kit (Agilent Technologies) on a Bioanalyzer 2100 System.

**Statistical analysis**

Statistical significance was assigned when P values were <0.05 using GraphPad Prism version 10.1. Tests, number of animals, and statistical comparison groups are indicated in the figure legends. Changes in infectious virus titer, viral RNA levels, or serum antibody responses were compared by one-way analysis of variance (ANOVA) with a multiple comparisons correction or unpaired t test dependent on the number of comparisons using natural log (ln) transformed data. Statistical differences in the percent SARS-CoV-2–positive contact 1 or 2 hamsters were determined by Fisher’s exact test.

**Supplementary Materials**

This PDF file includes:

Figs 5 to 11

**REFERENCES AND NOTES**


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