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Long-term, infection-acquired immunity against the SARS-CoV-2 Delta variant in a hamster model

Graphical abstract

Highlights

- Human vaccinee serum-neutralizing titers are reduced against SARS-CoV-2 Delta
- Similar results are obtained with serum from previously infected hamsters
- Prior infection with an early isolate protects hamsters from reinfection with Delta
- Prior infection of hamsters prevents Delta transmission to naive hamsters

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In brief

As SARS-CoV-2 variants accumulate mutations, there is a risk of ineffective neutralizing antibodies against new variants and potential re-infection. Halfmann et al. report that, in the hamster model, previous infection with an early prototypical SARS-CoV-2 isolate prevents re-infection of the Delta variant and its transmission to naive hamsters.

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Long-term, infection-acquired immunity against the SARS-CoV-2 Delta variant in a hamster model

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SUMMARY

The emergence of the SARS-CoV-2 Delta variant (B.1.617.2) raises concerns about potential reduced sensitivity of the virus to antibody neutralization and subsequent vaccine breakthrough infections. Here, we use a live virus neutralization assay with sera from Pfizer- and Moderna-vaccinated individuals to examine neutralizing antibody titers against SARS-CoV-2 and observe a 3.9- and 2.7-fold reduction, respectively, in neutralizing antibody titers against the Delta variant compared with an early isolate bearing only a D614G substitution in its spike protein. We observe similar reduced sensitivity with sera from hamsters that were previously infected with an early isolate of SARS-CoV-2. Despite this reduction in neutralizing antibody titers against the Delta variant, hamsters previously infected (up to 15 months earlier) with an early isolate are protected from infection with the Delta variant, suggesting that the immune response to the first infection is sufficient to provide protection against subsequent infection with the Delta variant.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Delta variant (B.1.617.2) was first detected in India in December 2020, and since then has become the dominant variant circulating throughout many parts of the world (WHO, 2021). The dominance of the Delta variant may be linked to its higher transmissibility compared with the former dominant variant Alpha (B.1.1.7) (Li et al., 2021; Liu and Rocklov, 2021).

Given its transmissibility, the Delta variant has raised concerns regarding the protective efficacy of the current vaccines against SARS-CoV-2. The SARS-CoV-2 spike protein is a viral surface receptor and the only viral antigen component of the mRNA vaccines from Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) and the viral vector-based vaccines from Johnson & Johnson/Janssen (JNJ-78436735) and AstraZeneca (ChAdOx1). The Delta variant is characterized by the spike protein amino acid mutations T19R, D614G, and D950N along with a deletion of two amino acids in the N-terminal domain at positions 157–158, antigenic mutations in the receptor binding domain (L452R and T478K), and a P681R mutation at the S1-S2 furin cleavage site. This latter mutation has been linked to higher viral loads and increased transmission (Liu et al., 2021b).

Breakthrough Delta variant infections in vaccinated populations have been reported (Brown et al., 2021). Yet data from in vitro neutralization assays with the Delta variant show only modest decreases in neutralization titers in sera samples obtained from individuals fully vaccinated (i.e., twice) with vaccines such as BNT162b2 or ChAdOx1 (Liu et al., 2021a; Pegu et al., 2021; Planas et al., 2021).

RESULTS

Here, we performed a live virus neutralization assay using serum samples from individuals vaccinated with either BNT162b2 or mRNA-1273. A prototypical virus (SARS-CoV-2/UT-HP095-1N/Human/2020/Tokyo; HP095 S-614G) with only the D614G substitution in its spike protein was used as a reference virus to compare changes in neutralization titers against an isolate of the Delta variant (hCoV-19/USA/WI-UW-5250/2021). We selected 30 serum samples from individuals vaccinated twice with BNT162b2 and 17 serum samples from individuals vaccinated twice with mRNA-1273 (Table S1). For both sets of samples, the sera were collected 7–25 days after the second dose. Neutralization titers were higher when the HP095 S-614G virus was used in the assay compared with the Delta variant.
(Figure 1A). With samples from BNT162b2-vaccinated individuals, we observed a 3.9-fold decrease in neutralization titers with the Delta variant compared with the HP095 S-614G reference virus (Figure 1B). Similarly, we observed a 2.7-fold decrease in neutralization titers with serum samples collected from individuals vaccinated with mRNA-1273 for the Delta variant when compared against the HP095 S-614G virus (Figure 1B). These observations are similar to other reported findings and demonstrate that both mRNA vaccines elicit neutralizing antibody responses that are effective against the Delta variant (Liu et al., 2021a; Planas et al., 2021).

We then asked, does previous SARS-CoV-2 infection provide protection from re-infection with the Delta variant? A study by Planas et al. using human convalescent serum showed similarly reduced serum antibody sensitivity to the Delta variant as that seen with serum obtained from vaccinated individuals (Planas et al., 2021). To examine whether previous infection with an early isolate of SARS-CoV-2 provides protection from an infection with the Delta variant, we used Syrian hamsters, a robust animal model of infection that has characteristics similar to SARS-CoV-2 USA-WA1/2020, an early isolate with an aspartic acid (D) at amino acid position 614 of the spike protein (WA-1 S-614D). Two months after this primary infection, the hamsters were inoculated with 1,000 plaque-forming units (pfu) of SARS-CoV-2/UT-NCGM02/Human/Tokyo, another early isolate of SARS-CoV-2 with 614D (NCGM02 S-614D) (Imai et al., 2020). Fifteen months after this primary infection, the hamsters were inoculated with 1,000 pfu of NCGM02 S-614D (n = 3) or the Delta variant (n = 3). Age-matched (female, 17 months old) naive animals were also infected with both viruses (3 animals for each virus) as control animals to measure virus replication in these aged hamsters.

We observed high virus titers in these older naive hamsters in both the lungs and nasal turbinates (Figure 2C), similar to the titers in the younger naive hamsters (Figure 2A). In contrast, no replicating virus was detected in the lungs of any of the previously infected hamsters (Figure 2A). Virus was detected in only one of the previously infected hamsters, although virus was detected in the nasal turbinates of two of the four animals infected with WA-1 S-614D and two of the four animals infected with the Delta variant (Figure 2B).

Our data demonstrate that prior infection of hamsters with an early isolate of SARS-CoV-2 (WA-1 S-614D) offers protection against re-infection with the Delta variant within 10 weeks. Next, we examined the long-term durability of this protection against the Delta variant elicited by prior infection. Hamsters (female, 2 months old, n = 6) were infected with SARS-CoV-2/UT-NCGM02/Human/Tokyo, another early isolate of SARS-CoV-2 with 614D (NCGM02 S-614D) (Imai et al., 2020). Fifteen months after this primary infection, the hamsters were inoculated with 1,000 pfu of NCGM02 S-614D (n = 3) or the Delta variant (n = 3). Age-matched (female, 17 months old) naive animals were also infected with both viruses (3 animals for each virus) as control animals to measure virus replication in these aged hamsters.

We observed high virus titers in these older naive hamsters in both the lungs and nasal turbinates (Figure 2C), similar to the titers in the younger naive hamsters (Figure 2A). In contrast, no replicating virus was detected in the lungs of any of the previously infected hamsters (Figure 2D). Interestingly, in the nasal turbinates, replicating virus was observed in all three older previously infected hamsters that were re-infected with NCGM02, whereas virus was detected in only one of the previously infected hamsters that was re-infected with the Delta variant (Figure 2D). The nasal turbinate titers of NCGM02 S-614D and the Delta variant in the previously infected hamsters were not, however, significantly different (p = 0.1298).

We next examined the transmission of the Delta variant between hamsters. Naive (female, 5 months old, n = 4) were infected with 1,000 pfu of the Delta variant. Twenty-four hours after inoculation, infected hamsters were paired with age-matched naive hamsters in cages separated by a 5-cm divider to prevent direct contact between the hamsters. Virus was detected in the nasal washes of all infected hamsters on day 3 after infection, confirming infection (Figure 3A). On day 6 after infection, virus
was still detectable in the lungs of all of the infected animals, and in the nasal turbinates of two of the infected animals (Figure 3A). The Delta variant efficiently transmitted to all four contact hamsters after 24 h of exposure. We detected virus in nasal swabs on day 2 after exposure in the lungs and nasal turbinates on day 4 after exposure (Figure 3B).

However, when previously infected hamsters (female, 5 months old, previously infected with WA-1 S-614D 4 months prior, n = 4) were re-inoculated with 1,000 pfu of the Delta variant, we did not detect virus transmission to the naive hamsters under the same transmission conditions. No virus was detected in the nasal washes (day 3) or respiratory tissues (lungs and nasal turbinates; day 6) of the re-infected hamsters (Figure 3C). Similarly, virus was not detected in the nasal swabs (day 2) or respiratory tissues (day 4) of the naive contact hamsters after 24 h of exposure (Figure 3D).

**Figure 2.** Virus titers of the Delta variant in hamsters (A–D) Virus replication in groups of naive hamsters (A and C) or groups of hamsters previously infected with WA-1 S-614D (B) or NCGM02 S-614D (D) and re-infected with the same virus or the Delta variant. Black dots represent individual titers from one study and those on the x axis represent virus titers below the limit of detection (10 pfu/g). The bars represent the mean titer for each group. There were no significant differences between virus titers in any of the groups shown (p < 0.05).

**Figure 3.** Transmission of the Delta variant between hamsters (A–D) Virus replication in naive hamsters infected with the Delta variant (A) and their exposed cage mates (B), or hamsters previously infected with WA-1 S-614D and re-infected with the Delta variant (C) and their exposed cage mates (D). Black dots represent individual titers from one study and those on the x axis represent virus titers below the limit of detection (10 pfu/g). The bars represent the mean titer for each group. Nasal wash (NW); day (D); nasal turbinate (NT). There were no significant differences between virus titers in any of the groups shown (p < 0.05).
DISCUSSION

With reports of vaccine breakthrough infections linked to the Delta variant, and the reduced sensitivity of serum neutralizing antibodies against the Delta variant (Brown et al., 2021; Liu et al., 2021a; Pegu et al., 2021; Planas et al., 2021), here we focused on the protective efficacy afforded by previous SARS-CoV-2 infection both in the short and long term, by using a hamster model of infection. Similar to other studies, we found a reduction, albeit modest, in the sensitivity of neutralizing antibodies against the Delta variant when we tested human sera collected after two doses of either mRNA vaccine and sera collected from hamsters previously infected with early SARS-CoV-2 isolates (S-614D). But, when previously infected hamsters were re-infected with a late isolate of SARS-CoV-2 (S-614D) or the Delta variant, replicating virus was not detected in the lungs and was only detected in the nasal turbinates of some animals, most likely due to limited immunity in the mucosal tissues. Moreover, while the Delta variant could easily transmit between naive hamsters, previously infected hamsters re-inoculated with the Delta variant could not transmit the virus to naive hamsters.

Limitations of the study

While the disease phenotype of SARS-CoV-2 infection in hamsters is similar to human disease (Imai et al., 2020), infection of hamsters is controlled and homogeneous. Laboratory Syrian hamsters are genetically relatively homogeneous and our experiments were performed under controlled conditions. In contrast, humans are genetically heterogeneous, differ in their comorbidities, and are exposed to different amounts of virus. The difference between a controlled animal infection model and the diversity of human infections, including the variability of antibody and cellular immune responses in the human population, may explain why we did not really observe breakthrough infections after re-infection of hamsters with the Delta variant.

Nevertheless, our results in the hamster model do demonstrate that while the Delta variant is antigenically different from early S-614D isolates of SARS-CoV-2, a robust immune response generated from a first infection will protect from a subsequent infection with the Delta variant.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Ethics statements
  - Animal studies
  - Cells
- **METHOD DETAILS**
  - Virus propagation
  - Hamster infections

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2022.110394.

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AUTHOR CONTRIBUTIONS

Conceptualization, P.J.H., A.G., and Y.K.; methodology, P.J.H., W.R., A.G., and Y.K.; investigation, P.J.H., M.K., M.A., R.A., and C.G.; writing – original draft, P.J.H., A.G., and Y.K.; funding acquisition, A.G., and Y.K.; resources, M.A., R.V., C.G., W.R., and A.G.; and supervision, P.J.H., A.G., and Y.K.

DECLARATION OF INTERESTS

Y.K. has received unrelated funding support from Daiichi Sankyo Pharmaceutical, Toyama Chemical, Taus Laboratories, Inc., Shionogi & Co. LTD, Otsuka Pharmaceutical, KM Biologics, Kyoritsu Seiyaku, Shinya Corporation, and Fuji Rebio. The remaining authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** | | |
| SARS-CoV-2/UT-HP095-1N/Human/2020/Tokyo | In-house; upon request | S-614G |
| USA-WA1/2020 | BEI | NR52281 |
| hCoV-19/USA/WI-UW-5250/2021 | In-house; upon request | Delta variant |
| **Biological samples** | | |
| Human serum samples; vaccinated Pfizer or Moderna | In house; isolated from volunteers | IASO study |
| **Experimental models: Cell lines** | | |
| Vero E6/TMPRSS2 cells | JCRB Cell Bank | 1819 |
| **Experimental models: Organisms/strains** | | |
| Syrian hamsters; females | Envigo | 8903F |
| **Software and algorithms** | | |
| Graphpad Prism 9 | Graphpad Prism 9 | N/A |
| **Other** | | |
| Virus transmission system | Custom built | N/A |

RESOURCE AVAILABILITY

**Lead contact**
Requests for resources and reagents should be directed to the Lead Contact Author Yoshihiro Kawaoka (yoshihiro.kawaoka@wisc.edu).

**Materials availability**
All unique reagents generated in this study are available from the Lead Contact with completed Material Transfer Agreements.

**Data and code availability**
- The datasets supporting the current study are available from the lead contact on request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

**Ethics statements**
After informed consent was obtained, blood samples were collected from individuals who received the mRNA vaccine BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna) as part of the Immunity Associated with SARS-CoV-2 study under the institutional review board-approved protocol of the University of Michigan Medical School (protocol number HUM00184533). The age and sex of participants is available in Table S1.

Isolation of the SARS-CoV-2 Delta variant from de-identified residual nasopharyngeal swab samples was reviewed by the Human Subjects Institutional Review Board at the University of Wisconsin.

**Animal studies**
All experiments with hamsters were performed after approval from the Animal Care and Use Committee at the University of Wisconsin-Madison in accordance with the approved protocol (V6426). The study included female hamsters that ranged from 1 to 17 months in age as indicated within the main text.

**Cells**
Vero E6/TMPRSS2 cells from the Japanese Collection of Research Bioresources (JCRB) Cell Bank (1819) were propagated in DMEM containing 10% fetal bovine serum, antibiotics, and 1 mg/ml geneticin (G418) at 37°C with 5% CO2. Cells were verified to be negative for mycoplasma contamination by monthly PCR analysis.
METHOD DETAILS

Virus propagation
WA-1 S-614D, NCGM02 S-614D, HP095 S-614G, and the Delta variant were propagated on Vero E6/TMPRSS2 cells. Virus titers in the tissue samples were determined by performing plaque assays on Vero E6/TMPRSS2 cells with a 1% methylcellulose overlay to allow for plaque formation.

All experiments with SARS-CoV-2 were performed under biosafety level-3 agriculture (BSL-3 Ag) containment at the University of Wisconsin-Madison in laboratory space approved by the Centers for Disease Control and Prevention and by the US Department of Agriculture.

Hamster infections
Female Syrian hamsters (Envigo) were used in these studies at the ages indicated in the text. While under isoflurane anesthesia, hamsters were intranasally (re-)infected with 1000 pfu of virus in 50 μl of inoculum. Animals were checked daily to evaluate their health. Three days after infection, the animals were humanely euthanized, and their tissues were collected.

Titration of virus from hamster tissue samples
Three days after infection, hamsters were humanely euthanized, and respiratory tissue samples (nasal turbinate and lung) were collected. For lung samples, a piece of each lobe was collected and pooled. After being frozen at -80°C for at least 24 hours, the tissue samples were homogenized in 1 ml of media and clarified by centrifugation. Undiluted and a 10-fold dilution series of the clarified tissue samples (100 μl per well) were used to infect a monolayer of Vero E6/TMPRSS2 cells for 30 minutes at 37°C. The cells were then washed once to remove unbound virus and then overlayed with 1% methylcellulose media for four days. Crystal violet solution was added directly to the wells overnight to fix and visualize the plaques. Titration of the pooled lung lobe samples was performed in duplicate.

Virus neutralization assay
Virus neutralization assays were performed with different isolates of SARS-CoV-2 on Vero E6/TMPRSS2. Sera samples were incubated at 56°C for at least 30 minutes and then diluted two-fold for a final concentration after the addition of an equal amount of virus of 1:20 to 1:10240. After the virus (approximately 100 plaque-forming units) and diluted sera mixture was incubated at 37°C for 30 minutes, the mixture was added to confluent Vero E6/TMPRSS2 cells that had been plated at 30,000 cells per well the day prior in 96-well plates. The cells were then incubated for an additional 3 days at 37°C. Virus neutralization titers were determined as the highest serum dilution that completely prevented cytopathic effects.

Transmission study in hamsters
To evaluate indirect virus transmission between hamsters, hamsters were infected with 1,000 pfu of the Delta variant intranasally under isoflurane anesthesia. Infected animals were housed in wire cages inside an isolator unit (Hou et al., 2020; Imai et al., 2012). Twenty-four hours later, naive hamsters were placed in the other side of the cage; a double-layered wire mesh separated the hamsters by 5 cm to prevent direct contact. The infected hamster was position in the front of the isolator unit, which provide unidirectional airflow during the study. Metal shrouds were placed over the cages so that only the front and back of the cages were open for airflow. After pairing the hamsters for 24 h, the animals were removed from the wire cages and housed in separate cages for the rest of the study. Nasal washes were collected from the infected hamsters 3 days after infection and from the exposed contact hamsters 2 days after exposure. Tissue samples (lung and nasal turbinate) were collected from the infected hamsters 6 days after infection and from the exposed contact hamsters 4 days after exposure.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis
The sample sizes for the hamster studies were determined from previous studies that demonstrated significant differences among groups. The researchers were not blinded to the group allocations during the experiments. Virus titers from animals are expressed as scatter plots with bars and individual datapoints, obtained by using Graphpad Prism 9. Statistical analyses were performed using two-tailed unpaired Student’s t-tests (Graphpad Prism 9). Neutralization titers and fold-changes are displayed as scatter plots with individual datapoints for each serum sample. The geometric mean is shown by the line in each graph and the mean fold changes are shown in parentheses.