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SARS-CoV-2 Omicron BA.1 Challenge after Ancestral or Delta Infection in Mice

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We assessed cross-reactivity to BA.1, BA.2, and BA.5 of neutralizing antibodies elicited by ancestral, Delta, and Omicron BA.1 SARS-CoV-2 infection in mice. Primary infection elicited homologous antibodies with poor cross-reactivity to Omicron strains. This pattern remained after BA.1 challenge, although ancestral- and Delta-infected mice were protected from BA.1 infection.

The SARS-CoV-2 Omicron variant (B.1.1.529, BA.1 sublineage) emerged nearly 2 years after the ancestral strain was identified (1). The Omicron BA.1 variant contains ≈50 mutations in the spike protein (2), resulting in substantial antigenic change. The strain was more infectious than prior variants of concern (VOCs) and escaped immunity, causing infections in persons who were previously vaccinated with ancestral strain–based vaccines (3) or infected with the ancestral virus or Delta (B.1.617.2) VOC. Since January 2022, additional Omicron sublineages (BA.2 to BA.5) have been detected worldwide. BA.4/BA.5 have identical spike proteins, most similar to BA.2, with additional spike mutations (4).

We sought to mimic the human scenario and selected a mouse model from available animal models (5) to assess the cross-reactivity of neutralizing antibody elicited by ancestral, Delta, and BA.1 viruses and to assess the effect of primary homologous and heterologous infection on secondary infection with the Omicron BA.1 strain. We also compared antibody cross-reactivity to BA.2 and BA.5 in serum samples from mice infected with ancestral, Delta, and BA.1 strains.

We first compared the associated illness, mortality rates, and kinetics of replication of 10^4 50% tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2/Australia/Vic/01/20 (ancestral strain–like),

SARS-CoV-2/Australia/Vic/18440/2021 (Delta), and SARS-CoV-2/Australia/NSW/RPAH-1933/2021 (Omicron BA.1) strains in 7- to 9-week-old female K18hACE2 transgenic mice (Appendix Figure, https://wwwnc.cdc.gov/EID/article/28/11/22-0718-App1.pdf). We infected groups of 15 K18hACE2 mice with intranasally delivered ancestral, Delta, or Omicron BA.1 strains by using a low dose of each virus (10^2 $TCID_{50}$), selected so that the mice would survive primary infection (Figure, panel A). We mock-infected 15 mice with phosphate-buffered saline (PBS). We collected blood on day 27 after primary infection and then challenged mice with 10^4 $TCID_{50}$ of Omicron BA.1 virus. We collected lungs and nasal turbinates (NTs) 2 and 4 days after challenge; we weighed and monitored 5 mice per group for clinical signs for 14 days (Figure, panel B). We collected blood samples on day 28 after Omicron BA.1 challenge (day 56 from primary infection).

After primary infection, all Omicron BA1–infected mice survived without major weight loss, but 1 ancestral strain–infected and 5 Delta-infected mice died during days 8–13. After challenge with 10^4 $TCID_{50}$ of Omicron, all mice, including the PBS group (naive control), survived without weight loss. The control group had mean virus titers of $10^{2.6}$ (day 2) and $10^{2.7}$ (day 4) in NTs and $10^{3.7}$ (day 2) and $10^{3.5}$ (day 4) $TCID_{50}$/organ in lungs after Omicron BA.1 challenge.

Consistent with other reports (6), we found the titers of BA.1 to be lower than those for ancestral and Delta viruses (Appendix Figure, panel C). Virus was not recovered from the tissues of mice challenged with BA.1 that had prior primary infection with ancestral, Delta, or BA.1 viruses (Figure C), except 1 mouse in each of the ancestral and Delta primary infection groups.

The homologous responses were strongest to ancestral (geometric mean titer [GMT] 709), followed by Delta (GMT 129), and were lowest to BA.1 (GMT 83) (Table). The low titer neutralizing antibody response to Omicron BA.1 infection is probably attributable to less robust replication of BA.1 virus in mouse tissues (Appendix Figure, panel C). Mice recovered from primary BA.1 infection were fully protected from rechallenge with the higher dose of BA.1, and no boost in homologous neutralizing antibody titers occurred (day 56 GMT 62).

Primary Omicron BA.1 infection did not induce heterologous neutralizing activity against ancestral, Delta, BA.2, or BA.5 viruses (Table). In contrast, primary ancestral infection elicited an 8-fold reduced titer against Delta and 21-fold reduced titer against the BA.1 virus, and primary Delta infection elicited a 2-fold reduced titer against ancestral strain. None of the mice first infected with BA.1,

ancestral, or Delta viruses developed neutralizing antibodies against BA.5.

Despite the absence of detectable BA.1 virus in the respiratory tract tissues after secondary infection in mice previously infected with ancestral or Delta (Figure, panel C), we observed a boost in homologous GMTs 1,338 (ancestral) and >453 (Delta), and cross-
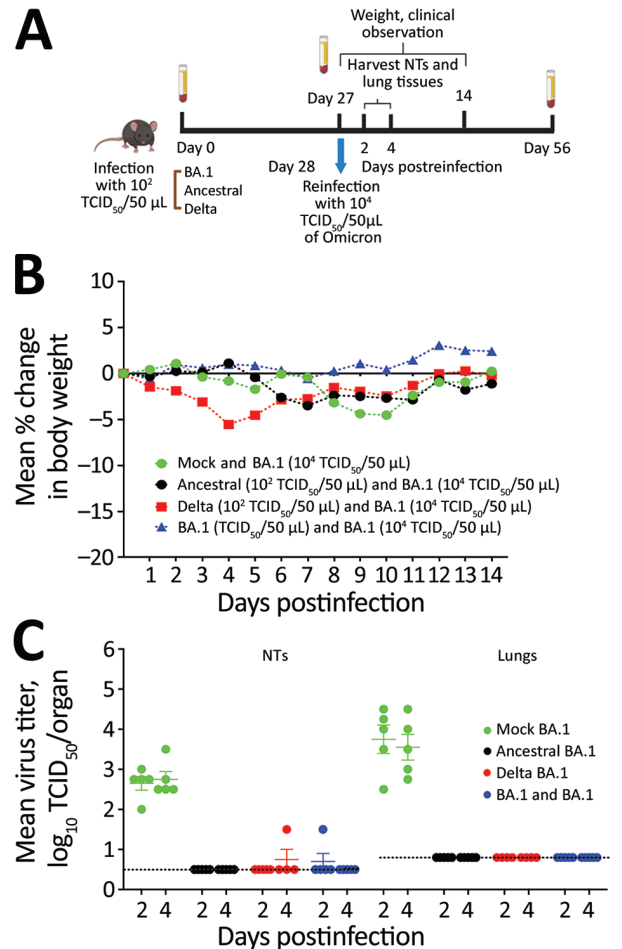
Figure. Primary infection with ancestral, Delta, or Omicron BA.1 SARS-CoV-2 strains as protection in mice from BA.1 reinfection. A) Flowchart of 6- to 8-week-old female hACE2K18 transgenic mice who received primary infection with low doses (10^2 $TCID_{50}$) of Omicron BA.1, ancestral, or Delta viruses and were reinfected with a higher dose (10^4 $TCID_{50}$) of BA.1. B) Weight loss in mice reinfected intranasally with 50 μL containing 10^4 $TCID_{50}$ of Omicron on day 28 after primary infection with each SARS-CoV-2 strain. Animals were monitored daily for weight loss, and deaths were recorded over a period of 14 days. Mice were euthanized when they lost 20% of their original bodyweight. C) Replication kinetics of Omicron BA.1 virus in mice after reinfection with 10^4 $TCID_{50}$/virus. Virus titers in the NTs and lungs of 5 mice per group euthanized on days 2 and 4 postinfection are expressed as $\log_{10}$ $TCID_{50}$/mL (NTs) and $\log_{10}$ $TCID_{50}$/organ (lungs). Horizontal bars represent mean titers, and symbols represent titers from individual mice. The dashed horizontal line indicates the lower limit of detection, $10^{0.5}$ $TCID_{50}$ per mL for the NTs and $10^{0.8}$ $TCID_{50}$ per organ for lungs. NTs, nasal turbinates; $TCID_{50}$, 50% tissue culture infectious dose.

Table. Homologous and heterologous serum neutralizing antibody titers on days 27 and 56 after primary and secondary SARS-CoV-2 infection in hACE2K18 transgenic mice*

Primary infection, 10^2 $TCID_{50}$	Secondary infection, 10^4 $TCID_{50}$	Serum neutralizing antibodies (GMT) against indicated virus after primary and secondary infection				
		BA.1	BA.2†	BA.5†	Ancestral	Delta
BA.1	BA.1	**83/62**	10/10	10/10	7‡/7‡	7‡/8‡
Ancestral	BA.1	34‡/27‡	10/10	10/10	**709/1,338**	90‡/>440‡
Delta	BA.1	16/60	10/35	10/53	55‡/124‡	**129/>453**

*Bold indicates homologous titers. GMT, geometric mean titer; $TCID_{50}$, 50% tissue culture infectious dose.
†Lower limit of detection in indicated assays is 10. In other assays, the lower limit of detection is 5.
‡Serum samples from different sets of 5 mice from the group were tested on days 27 and 56.

reactive neutralizing antibody titers GMTs >440 (ancestral) and 124 (Delta), and vice versa (GMTs of 27 and 60, respectively), with no improvement in cross-reactivity to BA.1. Mice first infected with Delta and rechallenged with BA.1 had low but detectable neutralizing antibody titers against BA.5 (Table).

Our observations are consistent with BA.1 being antigenically distinct from the ancestral and Delta strains (K. van der Straten K et al., unpub. data, https://doi.org/10.1101/2022.01.03.2126858. A boost occurred in preexisting SARS-CoV-2 neutralizing antibodies to ancestral and Delta but not in cross-reactivity to Omicron, probably because more epitopes are shared between ancestral and Delta than between those strains and Omicron. Serologic data from humans suggest that ≥3 exposures to ancestral strains as infection or vaccination or a combination are needed to induce cross-reactive antibodies to BA.1 (7). Although data from antigenic cartography using human serum suggest that BA.2 is antigenically closer to the ancestral and Delta strains (A. Rössler et al., unpub. data, https://doi.org/10.1101/2022.05.10.22274906), we did not detect cross-reactive neutralizing antibodies after primary infection with ancestral and Delta strains. Protection from replication of the Omicron BA.1 strain despite the lack of cross-reactive neutralizing antibodies may be attributable to mucosal immunity or T-cell responses in ancestral strain–infected and Delta-infected mice (8).

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Serologic Evidence of Human Exposure to Ehrlichiosis Agents in Japan

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In retrospective analyses, we report 3 febrile patients in Japan who had seroconversion to antibodies against *Ehrlichia chaffeensis* antigens detected by using an immunofluorescence and Western blot. Our results provide evidence of autochthonous human ehrlichiosis cases and indicate ehrlichiosis should be considered a potential cause of febrile illness in Japan.

Human ehrlichiosis is a tickborne infectious disease caused by *Ehrlichia* sp. that has primarily been detected in the United States. Common clinical manifestations of human ehrlichiosis are fever, headache, myalgia, and malaise. Leukopenia and thrombocytopenia often occur. Symptoms range from mild fever to severe illness with multiple organ dysfunction, which is occasionally fatal (*1*). In a retrospective analysis, we show serologic evidence for human ehrlichiosis in 3 febrile patients in Japan.

In case 1, a male patient, who was 48 years of age and worked in the manufacturing industry, sought care at a primary care clinic in 2015 for high fever (>40°C) and headache ≈1 month after hiking in the mountains. The clinic physician prescribed levofloxacin and acetaminophen, but the treatment was not effective. Therefore, the patient was seen at the Japanese Red Cross Wakayama Medical Center. The day before onset of high fever, the patient found a small rash on the left side of his abdomen. This date was considered day 0, although there might have been symptoms that the patient was unaware of before that time. The rash was an erythema migrans–like lesion that expanded on day 5. The patient was hospitalized, and borreliosis or tick-associated rash illness, which is similar to Lyme borreliosis–like erythema migrans, was suspected (*2*); however, a tick bite or eschar was not observed. After intravenous administration of minocycline (200 mg/d), the patient's fever abated, but the lesion expanded and was accompanied by puritis. On day 10, the patient was discharged from the hospital, after which the rash gradually disappeared. Diagnostic tests for borreliosis were negative. We retrospectively performed immunofluorescence assays (IFAs) and Western blot (Appendix, https://wwwnc.cdc.gov/EID/article/28/11/21-2566-App1.pdf) using patient serum samples collected on days 2 and 17. We showed seroconversion to antibodies against *Ehrlichia chaffeensis* antigens by IFA and the presence of IgM and IgG against *Ehrlichia* sp. P28 protein by Western blot (Table; Figure). We suspected the patient had ehrlichiosis and tick-associated rash illness.

In case 2, a male patient, who was 66 years of age and worked as a truck driver, sought care at the Ise Red Cross Hospital in 2018 for fever (38°C), annular erythema, and malaise. The patient had renal impairment and jaundice. The principal physician suspected leptospirosis, but diagnostic tests for leptospirosis were negative. The physician suspected other bacterial infections, including Japanese spotted fever (JSF) or anaplasmosis. The patient was treated intravenously with minocycline (200 mg/d) and sulbactam/ampicillin (6 g/d) for 4 days. Subsequently, amoxicillin (1.5 g/d) was administered orally for 14 days, and the patient recovered. Diagnostic tests for JSF were negative. We retrospectively analyzed patient serum samples collected on days 14, 32, and 60 after onset of illness. We showed seroconversion to antibodies against *E. chaffeensis*