Characterization of the immune resistance of SARS-CoV-2 Mu variant and the robust immunity induced by Mu infection

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Summary

Two mutations in the SARS-CoV-2 Mu variant spike, YY144-145TSN and E484K, are responsible for the pronounced resistance to COVID-19 convalescent and vaccine sera. The convalescent sera of SARS-CoV-2 Mu-infected individuals are broadly antiviral against Mu as well as other variants.

Abstract

We have recently revealed that the SARS-CoV-2 Mu variant shows a pronounced resistance to antibodies elicited by natural SARS-CoV-2 infection and vaccination. However, it remains unclear which mutations determine the resistance of SARS-CoV-2 Mu to antiviral sera. Also, it is unclear how SARS-CoV-2 Mu infection induces antiviral immunity. Here we reveal that the two mutations in the SARS-CoV-2 Mu spike protein, YY144-145TSN and E484K, are responsible for the resistance to COVID-19 convalescent sera during early 2020 and vaccine sera. Notably, the convalescent sera of SARS-CoV-2 Mu-infected individuals are broadly antiviral against Mu as well as other SARS-CoV-2 variants of concern/interest.

Keywords: SARS-CoV-2, COVID-19, Mu variant, immune resistance

Introduction/Background

In February 2022, WHO defined five variants of concern and two variants of interest [1]. Mu variant represents a variant of interest and has spread mainly in some South American countries such as Colombia and Ecuador [1]. We have recently revealed that Mu variant shows more pronounced resistance to antibodies elicited by natural severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during the early pandemic (before May 2020) and the BNT162b2 vaccine than Alpha, Beta, Gamma, Delta, Epsilon and Lambda variants [2]. However, it remains unclear which mutations determine the pronounced resistance of Mu variant to antiviral sera. Also, it is unclear how Mu infection induces antiviral immunity.

Methods

Ethics statement

For the use of human specimens, all protocols involving human subjects recruited at Kyoto University and Universidad San Francisco de Quito were reviewed and approved by the Institutional Review Boards of The Institute of Medical Science, The University of Tokyo (approval ID: 2021-1-0416), Kyoto University (approval ID: G0697), Universidad San Francisco de Quito (approval ID: CEISH P2020-022IN), and the Ecuadorian Ministry of Health (approval IDs: MSP-CGDES-2020-0121-O and MSP-CGDES-061-2020). The export of sera from Ecuador to Japan was approved by ARCSA ID: ARCSA-ARCSA-CGTC-DTRSNSOYA-2021-1626-M. All human subjects provided written informed consent.

Human sera

Fifteen serum samples obtained from COVID-19 convalescents who had infected with SARS-CoV-2 in the early pandemic (before May 2020) were purchased from RayBiotech (**Supplementary Table 1**). Peripheral blood was collected four weeks after the second vaccination with BNT162b2 (Pfizer-BioNTech), and the sera of fourteen vaccinated individuals were isolated (**Supplementary Table 2**). Note that the fourteen vaccinated individuals were not naturally infected with SARS-CoV-2 prior to vaccination. Peripheral blood was collected from four COVID-19 convalescents who had infected with SARS-CoV-2 Mu variant and the sera were isolated (**Supplementary Table 3**). At the time of sera collection, the four Mu-infected individuals did not have the histories of SARS-CoV-2 vaccination and COVID-19 symptoms. Sera were inactivated at 56°C for 30 min and stored at –80°C until use.

Protein homology model

All protein structural analyses were performed using Discovery Studio 2021 (Dassault Systèmes BIOVIA). In **Figure 1A** and **Supplementary Figure 1**, the crystal structure of SARS-CoV-2 spike protein (B.1 lineage; PDB: 7KRS) [3] was used as the template, and 40 homology models of the spike protein of Mu variant were generated using Build Homology Model protocol MODELLER v9.24 [4]. Evaluation

of the homology models was performed using PDF total scores and DOPE scores and the best model for the spike protein of Mu variant was selected.

Viral genome sequences

RNA was extracted from the nasopharyngeal swabs of COVID-19 patients using SV total RNA isolation system (Cat# Z3101, Promega). Reverse transcription was performed following the ARTIC protocol SARS-CoV-2 primer scheme v3 (https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w). The reverse transcription products were purified using AMPure XP magnetic beads (Cat# A63880, Beckman Coulter) according to the manufacturer instructions and then quantified using Qubit RNA assay kit (Cat# Q32852, Thermo Fisher Scientific). Genomic library was generated using the native barcoding expansion 96 kit (Cat# EXP-NBD196, Oxford Nanopore Technologies) with ligation sequencing kit (Cat# LSK-109, Oxford Nanopore Technologies) and loaded into the MinION flow cell (Cat# FLO-MIN 106, Oxford Nanopore Technologies). RAMPART software v1.0.5 from the ARTIC Network (https://github.com/artic-network/rampart) was used to monitor the sequencing in real-time. Porechop v0.2.4 (https://github.com/rrwick/Porechop) was used to carry out demultiplexing and adapter removal. The ARTIC Network bioinformatics pipeline was employed to create consensus sequences and variant calls (https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w). To generate the consensus genomes, the reads were mapped on the reference genome of SARS-CoV-2 strain Wuhan-Hu-1 (GenBank accession number MN908947). The sequences were uploaded to the online tool to determine genome clades and identify mutations. Pangolin COVID-19 lineage assigner v3.1.11 [5] and NextClade v1.6.0 [6] were used for the lineage classification**.** The viral sequences were deposited in the GISAID database (https://www.gisaid.org), and the GISAID IDs are listed in **Supplementary Table 3**.

Cell culture

HEK293T cells (a human embryonic kidney cell line; ATCC CRL-3216) and HOS-ACE2/TMPRSS2 cells [2, 7-9], a derivative of HOS cells (a human osteosarcoma cell line; ATCC CRL-1543) stably expressing human ACE2 and TMPRSS2, were maintained in Dulbecco's modified Eagle's medium (high glucose) (Wako, Cat# 044-29765) containing 10% fetal calf serum, 100 units penicillin and 100 ug/ml streptomycin.

Plasmid construction

Plasmids expressing the SARS-CoV-2 spike proteins of the parental D614G (B.1), Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Lambda (C.37) and Mu (B.1.621) variants were prepared in our previous studies [2, 7-9]. Plasmids expressing the SARS-CoV-2 spike protein derivatives of parental D614G (B.1) and Mu (B.1.621) variant were generated by site-directed overlap extension PCR using the expression plasmid of either parental D614G (B.1) [7] and Mu (B.1.621) variant [2] as the template and the primers listed in **Supplementary Table 4**. The resulting PCR fragment was

digested with KpnI and NotI and inserted into the corresponding site of the pCAGGS vector. Nucleotide sequences were determined by DNA sequencing services (Eurofins), and the sequence data were analyzed by Sequencher v5.1 software (Gene Codes Corporation).

Neutralization assay

Pseudoviruses were prepared as previously described [2, 7-9]. The pseudoviruses were stored at –80°C until use. Neutralization assays were performed as previously described [2, 8, 9]. Briefly, the SARS-CoV-2 spike pseudoviruses (counting ~20,000 relative light units) were incubated with serially diluted (40-fold to 29,160-fold dilution at the final concentration) heat-inactivated human sera at 37°C for 1 h. Pseudoviruses without sera were included as controls. Then, an 80 µl mixture of pseudovirus and serum was added to HOS-ACE2/TMPRSS2 cells (10,000 cells/50 µl) in a 96-well white plate. Two days post infection, the infected cells were lysed with a One-Glo luciferase assay system (Promega, Cat# E6130), and the luminescent signal was measured using a GloMax explorer multimode microplate reader 3500 (Promega). The assay of each serum was performed in triplicate, and the 50% neutralization titer was calculated using Prism 9 (GraphPad Software).

Results

The majority of Mu variants harbor the T95I and YY144-145TSN mutations in the N-terminal domain; the R346K, E484K, and N501Y mutations in the receptor-binding domain; and the D614G, P681H, and D950N mutations in other regions of the spike protein (**Figure 1A**) [2]. To screen the mutation(s) that attribute to the robust immune resistance of Mu variant, we generated a series of pseudoviruses that harbors the spike protein of the D614G-bearing B.1 lineage virus (parental virus) bearing with each mutation in the Mu variant. Virus neutralization assay was performed with the use of serum samples obtained from 15 coronavirus disease 19 convalescents who were infected early in the pandemic (before May 2020) (**Supplementary Table 1**) and 14 persons who had received the BNT162b2 vaccine (**Supplementary Table 2**). As shown in **Figure 1B** (top), two mutations, YY144-145TSN and E484K, conferred the resistance to antibodies induced by natural SARS-CoV-2 infection and vaccination. The immune resistance by the E484K mutation corresponds to previous reports [10, 11]. To verify the effect of these two mutations on the neutralization resistance, we next generated a series of Mu-based pseudoviruses that lose respective mutations. Consistent with the gain-of-function experiments based on the parental virus (**Figure 1B**, top), the loss-of-function experiments showed that the spike proteins of Mu variant reverting YY144-145TSN or E484K mutations loses the neutralization resistance (**Figure 1B**, bottom). Since the Mu pseudovirus derivative that loses both YY144-145TSN and E484K mutations almost completely lost the neutralization resistance (**Figure 1B**, bottom), our data suggest that the pronounced resistance of Mu variant against neutralizing antibodies is attributed to these two mutations.

We next assessed the immunological spectrum of the serum samples obtained from the convalescents who had infected with Mu variant (**Supplementary Table 3**). Although the Mu variant was more than 9 times resistant to the sera induced by natural SARS-CoV-2 infection during early pandemic and vaccination, which is consistent with our recent report (**Figure 1C**) [2], the Mu variant

did not exhibit resistance to the sera induced by Mu infection (**Figure 1D**). Notably, the sera induced by Mu infection exhibited broad antiviral effect against various variants of concern/interest (**Figure 1D**).

Discussion

In this study, we showed that the pronounced immune resistance of Mu variant is conferred by the two mutations, YY144-145TSN and E484K. Although it is known that the E484K mutation closely associates with the resistance to neutralizing antibodies [10, 11], the association of an insertion in the N-terminal domain, YY144-145TSN, with immune resistance is novel.

It should be noted that the four Mu-infected patients enrolled in this study were selected for having no previously reported histories of SARS-CoV-2 vaccination or COVID-19 symptoms. Although we cannot reject the possibility that these four patients were asymptomatically infected with SARS-CoV-2 prior to the Mu infection, Marchi et al. showed that anti-SARS-CoV-2 antibodies are less efficiently induced by asymptomatic SARS-CoV-2 infection [12]. Therefore, it is suggested that the anti-SARS-CoV-2 immunity was induced by Mu infection in these four patients, and that the antibodies elicited by Mu infection are broadly antiviral against Mu as well as other SARS-CoV-2 variants of concern/interest. Although it remains unclear why the Mu infection can induce broader immunity than other variants, one possibility is that the mutations in Mu spike are commonly identified in other variants: E484K (shared with Beta and Gamma), N501Y (shared with Alpha), P681H (shared with Alpha) and D950N (shared with Delta). Sharing antigenic epitopes with other variants might lead to the induction of broad antiviral immunity by Mu infection.

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Figure legend

Figure 1. Characterization of the immune resistance of the Mu variant.

(**A**) The position of the mutations in Mu variant. Cartoon and surface models are overlayed. The mutations in Mu variant are indicated. The structure of N-terminal domain is shown in **Supplementary Figure 1**.

(**B-D**) Pseudovirus neutralization assays. Neutralization assays were performed with the use of pseudoviruses harboring the SARS-CoV-2 spike proteins of parental virus (the B.1 lineage virus, which harbors the D614G mutation)-based derivatives (**B**, top), the spike proteins of Mu-based derivatives (**B**, bottom), or the spike proteins of the Alpha, Beta, Gamma, Delta, Lambda or Mu variants (**C** and **D**). Serum samples were obtained from 15 convalescent persons who had infected with SARS-CoV-2 in the early pandemic (before May 2020), 14 persons who had received the BNT162b2 vaccine, and 4 convalescent persons who had infected with SARS-CoV-2 Mu variant.

In **B** and **C**, the heights of the bars (serum samples obtained from the convalescent persons who had infected with SARS-CoV-2 in the early pandemic) and the circles (serum samples obtained from the persons who had received the BNT162b2 vaccine) indicate the average difference in neutralization resistance of the indicated variants as compared with that of the parental virus.

In **D**, the heights of the bars (serum samples obtained from the convalescent persons who had infected with Mu variant) indicate the average difference in neutralization resistance of the indicated variants as compared with that of the parental virus. The error bars indicate standard error of the mean. The vertical dashed lines indicate value 1.

The assay of each serum sample was performed in triplicate to determine the 50% neutralization titer. The raw data of the 50% neutralization titer are summarized in **Supplementary Figure 2** and **Supplementary Tables 1-3**. The information regarding the convalescent donors (sex, age, and dates of testing and sampling) and vaccinated donors (sex, age, and dates of second vaccination and sampling) of serum samples are summarized in **Supplementary Tables 1-3**.

Figure 1