"Investigation of SARS-CoV-2 proteins as a potential vaccine targets and development of companion diagnostics for use in nonhuman primates."

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Abstract

Non-human primates (NHP) are a top research model for human diseases due to similar immune systems and disease susceptibility. In early 2020 as the SARS-CoV-2 pandemic was unfolding, NHPs became a critical animal model to study infection, disease, treatment, and vaccines against SARS-CoV-2. The susceptibility of NHPs to SARS-CoV-2 raises significant concern for welfare of captive NHP colonies, their use in research, the validity of studies, and for potential transmission to or from humans. Accurate antibody-based diagnostics may prove essential to preclinical studies for development of vaccines for human use and to ensure integrity of research studies where a subclinical preexisting SARS-CoV-2 infection could cause confounding results. PCR based tests would not detect animals recovered from SARS-CoV-2 infection. Finally, a vaccine designed for use in NHPs may help protect animals should SARS-CoV-2 remain in circulation. A collaborative study between 4 organizations assessed the immunization of six male cynomolgus macaques (*M. fascicularis*) with recombinant SARS-CoV-2 Spike proteins that could generate neutralizing antibodies. Three groups of 2 animals each were immunized with recombinant Spike S1, recombinant Spike S2, or a mix of the two proteins. Serum antibodies were tested on the CSA: Simian SARS-CoV-2 assay developed by Intuitive Biosciences, both for antibody titer over the course of the study and to evaluate the generation of neutralizing antibodies. The multiplex CSA: Simian SARS-CoV-2 test was developed to distinguish between infected and immunized individuals with a high sensitivity and specificity by evaluating IgG response to several different SARS-CoV-2 proteins. A robust IgG response to the Spike protein was detected as early as 2 weeks after immunization and maintained for over 12 weeks. Using a neutralizing antibody assay, antibodies from the immunized NHP were shown to prevent binding to the ACE2 receptor. Both the successful immunization and the development of the companion diagnostic to distinguish vaccinated from infected animals may prove helpful to groups seeking to screen and protect the animals in their care, without relying on the supply of human diagnostics or vaccines.

Materials and Methods

Recombinant Proteins

For vaccination, two recombinant proteins representing portions of the spike S1 subunit were provided by Lytic Solutions (Madison, WI). The CTD-Fc protein is a SARS-COV-2 Spike protein C-terminal S1 fragment (aa319-591) fusion to human Fc, expressed in CHO cells and affinity purified. The NTD-Fc protein is a SARS-COV-2 Spike protein N-terminal S1 fragment (aa16-309) fusion to human Fc, expressed in CHO cells and affinity purified.

For the immunoassay, the CSA: SARS-CoV-2 kit was used (Intuitive Biosciences, Madison, WI). This kit contains antigens for Spike S1 subunit (aa16-685), Spike S2 (aa686-1213), and Nucleocapsid (aa1-419). All recombinant proteins used were expressed in mammalian expression systems and should represent native glycosylation.

Animal Vaccination

Six male SARS-CoV-2 seronegative cynomolgus macaques (*M. fascicularis)* were randomized into three groups (n=2) and scheduled for immunization and serial blood draws over the 12-week study. An additional blood draw was obtained from 5 of 6 animals 20 weeks after the initiation of the study.

All animal studies were approved by Covance-Greenfield and Novartis Institutional Animal Care and Use Committee and all procedures performed adhere to and are in compliance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

Test articles were diluted in sterile Phosphate Buffered Saline (PBS) and mixed with TiterMax Gold adjuvant (Sigma Aldrich) at 200 µg/animal, or a total of 250 µg/mL for each dose. Each dose was delivered intramuscularly. Formulations were prepared fresh on the day of dosing and used withing 3 hours of preparation. Animals were dosed at 0.8 mL per animal.

Blood draws were schedule at the beginning of the study and every 2 weeks following. Blood was collected from a femoral vein into serum separator tubes (SST). The collected SST were centrifuged within 1 hour of collection for 10 minutes in a refrigerated centrifuge at 2700 rpm. Serum was aliquoted and stored at -80°C until further use.

Serology Testing

Serum samples were evaluated using the CSA: SARS-CoV-2 kits from Intuitive Biosciences according to the user protocol. Briefly, samples were diluted 1:100 and incubated for 1 hour at room temperature. Following 3 washes, a 1:200 dilution of anti-simian gold conjugate was incubated for 1 hour at room temperature. Following 3 washes, signal was developed by a 3-minute incubation with SilverQuant™ reagents at room temperature. After rinsing each well with water, individual wells were analyzed on the AQ 1000 analysis system (Intuitive Biosciences). Samples with a Relative Intensity Unit above the product cut-off value were considered positive.

Antibody Titer

Serum samples were diluted 1:100 in CSA Buffer provided with the CSA: SARS-CoV-2 kits. A serial 1:2 dilution was then performed with CSA Buffer to a maximum dilution of 1:256,000. Each dilution was

assayed using the CSA: SARS-CoV-2 kit as described above. The dilution at which the sample was still above the cut-off value was considered the antibody titer.

Neutralizing Antibody Titer

In addition to several internal controls, recombinant ACE2 protein was immobilized on the surface of ultra-thin nitrocellulose protein microtiter plates (Intuitive Biosciences). Test serum was diluted 1:100 with CSA buffer incubated for 1 hour with 0.0001-0.1µg/mL CTD-TEV-Fc (Lytic Solutions). Pre-incubated samples were individually added to wells of the ACE2 multiplex plates and incubated for 1 hour at room temperature. After washing, a 1:200 dilution of anti-simian gold conjugate was added to each well and incubated for 1 hour at room temperature. After washing signal was developed with a 3 min incubation with SilverQuant reagents. After rinsing with water, plates were analyzed on the AQ 1000 analysis system. Results were calculated as percent inhibition of three no serum wells.

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