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Coronavirus Pandemic

Heterologous immunization with Covishield and Pfizer vaccines against SARS-CoV-2 elicits a robust humoral immune response

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Abstract

Understanding the efficacy and durability of heterologous immunization schedules against SARS-CoV-2 is critical, as supply demands and vaccine choices become significant issues in the global vaccination strategy. Here we characterize the neutralizing antibodies produced in two subjects who received combination immunizations against SARS-CoV-2, first with Covishield (Oxford–AstraZeneca) vaccine, followed 33 days later with a second dose (booster) shot of the Pfizer-BioNTech vaccine. Serum samples were collected 25 days following the primary vaccination and 13 days after the secondary Pfizer vaccination. Both subjects exhibited increased levels of isotype IgG and IgM antibodies directed against the entire spike protein following immunizations. These antibodies also exhibited increased reactivity with the receptor binding domain (RBD) in the spike protein and neutralized the infectivity of replicating vesicular stomatitis virus (VSV) that contains the COVID-19 coronavirus S protein gene in place of its normal G glycoprotein. This VSV pseudovirus also contains the reporter gene for enhanced green fluorescent protein (eGFP). Antibody titers against the spike protein and serum neutralization titers against the reporter virus are reported for the 2 heterologous vaccinated individuals and compared to a positive control derived from a convalescent patient and a negative control from an unexposed individual. The Pfizer-BioNTech vaccine increased antibody binding to the spike protein and RBD, and approached levels found in the convalescent positive control. Neutralizing antibodies against the VSV-S pseudovirus in the 2 subjects also approached levels in the convalescent sera. These results firmly validate the value of the Pfizer-BioNTech vaccine in boosting immunity following initial Covishield inoculation.

Key words: SARS-CoV-2; COVID-19; heterologous immunization; humoral immunity; vaccines; neutralizing antibodies; serum titers; VSV-S reporter pseudovirus.

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Case Report

The outbreak of SARS-CoV-2 was declared a global pandemic in March of 2020 [1]. Since then, the international scientific community has mobilized to produce vaccines in an unprecedented effort to slow viral transmission and prevent severe disease and death [1]. Pooled interim analysis of four clinical trials investigating the efficacy of the Oxford–AstraZeneca's adenovirus-based vaccine ChAdOx1 nCoV-19 (AZD1222) demonstrated 70.4% efficacy in mitigating symptomatic Covid-19 after the administration of two doses [2]. Controlled trials of the Pfizer-BioNTech mRNA vaccine (BNT162b1) demonstrated 95.0% efficacy in preventing symptomatic SARS-CoV-2 infection at least 7 days post second dose [3]. Understanding the efficacy, durability, and safety of heterologous immunization against SARS-CoV-2 is paramount, as the world struggles to supply primary and second doses of these agents. Here we report the isotype specificity of antibodies against the complete spike protein (S) and reactivity of neutralizing antibodies to the receptor binding domain (RBD) of viral spike protein in two subjects who received a combination of vaccines against SARS-CoV-2. Both these individuals received a first dose of Covishield (Oxford–AstraZeneca) vaccine followed by a second dose (booster) shot 33 days later of the Pfizer-BioNTech vaccine. The patients exhibited increased

IgG and IgM anibodies against S, increased levels of antibodies against the RBD, and produced neutralizing antibodies against a replicating VSV pseudovirus. This virus contains the coronavirus S protein (Wuhan strain) gene in place of its normal G glycoprotein, along with the gene encoding enhanced green fluorescent protein (eGFP). This reporter virus, designated as VSV-eGFP-SARS-CoV2-S, was similar to another that was previously reported [4].

Two subjects with no known history of SARS-CoV-2 infection and no known contact with acutely infected individuals were given a first dose of Covishield vaccine (subject 1, age 66, male; subject 2, age 66, female). At 25 days post-immunization with Covishield, blood was collected and plasma was separated from erythrocytes and lymphocytes using Ficoll-Paque density centrifugation. The 2 subjects were subsequently immunized with Pfizer-BioNTech vaccine at 33 days and plasma was again collected 13 days later. In addition, plasma was prepared from one convalescent subject (positive control) with a known history of natural infection from SARS-Cov-2 and one subject (negative control) with no known history of infection. The plasma samples were diluted between 1:50 and 1:10,000 and titers of human anti-viral spike protein IgG and IgM were quantified using an ELISA kit from Invitrogen designed to detect the whole S protein and antibody isotype. An ELISA assay was developed to measure human IgG antibodies to SARS-CoV-2 sT1.1 RBD antigen. The results are summarized in Table 1. Viral neutralizing antibodies were measured by the fluorescence produced in Vero cells that were infected with the VSV-eGFP-SARS-Cov-2-S reporter virus. Prior to infection the reporter virus was incubated with serum dilutions (1/8 to 1/512) prepared from the plasma of Subject 1, Subject 2, the Positive Control (convalescent patient) and the Negative Control (unexposed individual). Fluorescence was measured with a Biotek Cyation 1 Cell Imaging Multi-Mode Reader and results are presented in Figure 1.

IgG-S, IgM-S, and RBD antibody titers were detectable after the first dose of Covishield, and drastically increased following the second dose (boost) with the heterologous Pfizer-BioNTech vaccine (Table 1). Subject 1 had IgM-S, IgG-S, and RBD titers of 3.69, 394.78, and 46 respectively, while Subject 2 had IgM-S, IgG-S, and RBD titers of 28.85, 3547.22, and 163, respectively, following vaccination with Covishield. After the Pfizer-BioNTech boost Subject 1 had IgM-S, IgG-S, and RBD titers of 2107.08, 62364.37, and 1641 respectively while Subject 2 had IgM-S, IgG-S, and RBD titers of 2265.27, 51468.69, and 2000, respectively. These values approached those of the convalescent positive control while the negative control represented background levels of detection. Clearly, the Pfizer-BioNTech boost produced a dramatic increase in S and RBD antibodies 13 days after the secondary heterologous vaccination.

The VSV-eGFP-S pseudovirus neutralization assay (PvNA) was performed to determine the titer of neutralizing antibodies (NAbs) directed against spike protein (Table 1, Figure 1). Pseudovirus neutralization assay values represent the lowest dilution of plasma that yields a 50% reduction in eGFP signal intensity; larger values correspond to a higher serum dilution and indicate that there are higher concentrations of NAbs in the plasma. There were no detectable NAbs in serum collected from the negative control. Levels of NAbs were positive but low after the first dose of Covishield vaccine (Subject 1, 50% dilution 8; Subject 2, 50% dilution 8) but greatly increased levels of NAbs were found 13 days following the second heterologous Pfizer-BioNTech vaccination (Subject 1, 50% dilution 64; Subject 2, 50% dilution 512). These values

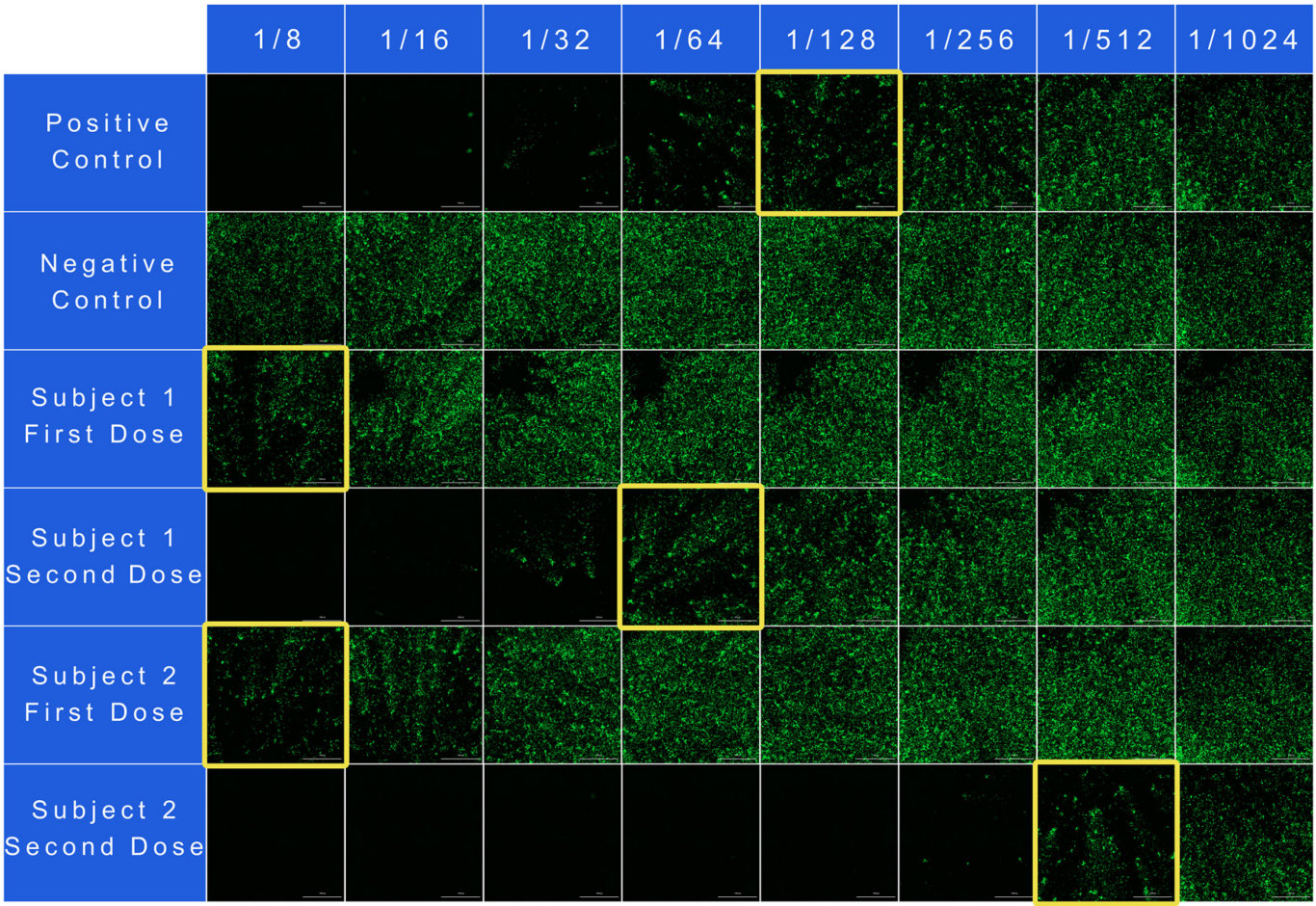
Table 1. Spike antigen and reporter virus neutralization titers (PvNA) from heterologous vaccinated individuals and controls. Comparison of antibody titers between subjects with a history of SARS-CoV-2 natural infection (positive control), no known natural infection (negative control), or heterologous immunization schedules (subjects 1 and 2). Anti-viral spike (S) protein IgM and IgG antibodies, and anti-receptor binding domain (RBD) antibodies were measured using ELISA assays. Pseudovirus neutralization assay values represent the lowest dilution of plasma that yields a 50% reduction in eGFP signal intensity; larger values correspond to a higher serum dilution and indicate that there are higher concentrations of Nabs in the plasma.

ID	Anti-S IgM (KU/ml)	Anti-S IgG (KU/ml)	RBD (IU/mL)	PvNA (50% reduction GFP)
Positive Control	2,983.31	143,866.27	1,988.00	128
Negative Control	17.7	59.52	Not Detectable	<8
Subject 1 First Dose	3.69	394.78	46	8
Subject 1 Second Dose	2107.08	62,364.37	1641	64
Subject 2 First Dose	28.85	3547.22	163	8
Subject 2 Second Dose	2,265.27	51,468.69	2,000	512

approached the level of neutralizing antibodies found in plasma from the positive convalescent control (50% dilution 128). Both heterologously immunized subjects experienced minimal side effects following the first immunization with Covishield. However, both subjects 1 and 2 reported headache, chills, lethargy, fatigue, and induration at the site of infection following the second dose of Pfizer-BioNTech BNT162b1 vaccine. Subject 1's symptoms persisted for 24 hours while subject 2 experienced symptoms that lasted for 36 hours.

Overall, this single case report suggests that individuals receiving an initial dose of Oxford-AstraZeneca Covishield followed by a second dose of Pfizer-BioNTech BNT1621b vaccine against SARS-CoV-2 elicit a robust humoral immune response. Anti-spike IgG titers in subjects 1 and 2 at 13 days post boost exceeded the findings reported by Mulligan et al., who reported a mean IgG-S titer range of 5,880–16,166 U /ml at 14 days following a second dose with BNT1621b [5]. Neutralizing antibody titers were comparable to or exceeded values reported in clinical trials of the Covishield and Pfizer-BioNTech vaccines, which reported a range of 254-424 U/ml and 180-437 U/ml at 14 d post boost, respectively [5,6]. This discovery, although limited in statistical power by its patient sample size, offers insight into the efficacy of heterologous immunization against SARS-CoV-2. Presently, large-scale trials are underway at our Institute and are expected to validate these findings. The significance of this study will extend to larger populations and will allow local jurisdictions to expand their vaccine campaigns by administering combination immunization to maximize vaccine distribution and coverage.

Figure 1. Reporter pseudovirus neutralization assays (PvNA) using diluted serum samples from heterologous vaccinated individuals and controls. Decreased levels of eGFP (enhanced green fluorescent protein) signal intensity measured with a Biotek Cytation 1 Cell Imaging Multi-Mode Reader are present in Vero cells infected with VSV-eGFP-SARS-CoV-S reporter virus that had been incubated with dilutions of plasma following primary and secondary heterologous vaccination against SARS-CoV-2. Grids outlined in yellow represent the dilution of plasma at which a 50% reduction in eGFP signal intensity was observed. Post first dose (Covishield), subjects 1 and 2 exhibited a 50% reduction in eGFP intensity at 1/8 dilution. Post second dose (Pfizer-BioNTech), subjects 1 and 2 exhibited a 50% reduction in eGFP intensity at 1/64 and 1/512, respectively.



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