

Inadvertent Human Mucosal Exposure to SARS-CoV-2 Recombinant S1 Protein Elicits Immunity

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Research Article

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Abstract

Native immunogenicity of SARS-CoV-2 proteins may be sufficient to elicit protective immunity in humans. Accidental mucosal exposure in a laboratory scientist (P001) to a commercially-obtained, purified SARS-CoV-2 recombinant S1 subunit (S1) protein resulted in S1-reactive antibodies with serum titers up to 1:12,800. A surrogate virus neutralization test (sVNT) demonstrated enduring neutralizing antibodies (nAbs). P001 PBMCs and whole blood exposed to S1 protein resulted in interferon- γ (IFN- γ) release *ex vivo*. Importantly, no systemic adverse events were observed. Potent immunogenicity of full-length S1 protein in a water-in-oil emulsion provides important clues for COVID-19 vaccine development and may serve as the basis of a low-cost, stable, and highly scalable intranasal vaccine candidate to address COVID-19 globally.

Main Text

The global effort to tackle the current COVID-19 pandemic has led to an unprecedented number of novel clinical vaccine candidates from public and private organizations, including several in late stage clinical trials and two receiving FDA Emergency Use Authorization (1). Such rapid vaccine development was enabled by innovative technologies and discoveries made in response to recent outbreaks in the new millennium including SARS, MERS, and the West Africa and Democratic Republic of the Congo Ebola epidemics (2, 3) as well as more recent federal investments in development and manufacturing.

There are no FDA-approved vaccines to any human coronavirus (2). Due to the inherent difficulty of studying viral countermeasures outside of an ongoing infectious disease crisis, the question of what is necessary and sufficient for a safe, effective, and scalable vaccine candidate is rarely addressed (4). Instead—particularly in the setting of an active, deadly pandemic—the rational strategy is to extrapolate from related diseases and err for maximization of efficacy (3, 5). The trade-offs of this approach may include immunogenicity, manufacturability, stability/supply-chain/last mile, cost, global access, safety, and public trust (3, 5-6). In addition, target selection may be suboptimal due to the accelerated timelines and lack of available early data. Here is a case report of a laboratory scientist (P001) who retrospectively discovered minute, unintentional exposure to commercially-obtained recombinant SARS-CoV-2 S1 subunit (S1) protein resulting in systemic and mucosal immunity including enduring nAbs.

In the course of developing a novel antibody discovery platform beginning early August 2020, one of several proof-of-concept projects included producing novel antibodies against the SARS-CoV-2 full-length S1 protein which contains the receptor binding domain (RBD).

No parenteral exposure (e.g., needle stick) occurred throughout the course of the experiment. During parallel assay development, P001 collected their own saliva and serum for use as negative control matrices beginning at 30 to 60 days (D30 to D60) after study start. P001 self-reported no symptoms of illness or COVID-19 in 2020 and analysis of temperature deviation collected by wearable device (Oura Health Ltd., Oulu, Finland) worn daily for a consecutive 22 months confirmed zero fever events in 2020

(Fig. 1A). Confirming serological status, the result from an-EUA cleared total anti-RBD antibody ELISA (Ortho Clinical Diagnostics, NJ, USA) from dried blood spot collected on D10 was negative (Fig. S1). Further, P001 serum collected throughout the study was tested using two commercial IgG ELISA kits against SARS-CoV-2 nucleocapsid protein (N protein) confirming seronegativity (Fig. S2). However, in exploratory studies, P001 serum at D60 was found to be highly positive against recombinant S1 protein in a pilot ELISA suggesting potential systemic exposure to the S1 immunogen (Fig. 1B).

To verify these findings, an indirect ELISA was developed against S1 protein and interrogated with commercially obtained pre-COVID (PRECOVID; $n = 5$; Table S1) and test-confirmed (i.e., PCR or antigen test) COVID-19 patient (COVID19; $n = 20$; Table S2) serum samples (Fig. 2A). The earliest P001 serum sample (D60) demonstrated the highest S1 protein reactivity of all samples tested including the 20 COVID19 samples (Fig. 2A). P001 serum S1 IgG titers were determined revealing 1:12,800, 1:1,600, and 1:400 for D60, D75, and D90, respectively (Fig. 2B). Finally, immunoglobulins were purified from the D90 P001 serum samples yielding 12.89 mg/ml bulk IgG and a S1 IgG titer of 1:320 (Fig. 2C). To demonstrate specificity, the purified serum IgG was examined competitively in the indirect ELISA with 2 μ g/ml of either recombinant S1 or S2 protein. The S1 condition resulted in nearly 60% inhibition while S2 was not inhibitory (Fig. 2C').

To potentially identify neutralizing antibodies (nAbs) from P001 serum, an indirect surrogate virus neutralization test (sVNT) was developed based on a modified EUA-cleared sVNT (7). nAbs were identified in 80% (16 of 20) of COVID19 samples as 30% inhibition (IC30)(Fig. 3A)(7). Neutralization did not appear to correlate with levels of S1 IgG, RBD IgG, or RBD IgM in this small series (Fig. S3). P001 serum was neutralizing at all timepoints to D90 and maximal nAb titer was 1:1,1600 at D60 (Fig. 3B).

To investigate putative cellular immunity, a cytokine release assay (CRA) from P001 whole blood (D90)(8) was performed which demonstrated 1.8633 ng/ml of IFN- γ after 2 d exposure to 2 μ g/ml S1 protein by indirect ELISA but no release in response to either ACE2 protein or overlapping RBD peptides (Fig. 3C). PBMCs isolated from P001 whole blood by Ficoll-Paque gradient were characterized in a parallel CRA assay. IFN- γ from culture supernatants in S1-exposed cells was undetectable at 2 d and ~15.41 pg/ml at 6 d (Fig. S4).

A second exposure on approximately D110 was suspected after P001 handled S1 protein during ELISA plate coating (working solution of 2 μ g/ml in carbonate buffer). This was manifested as mild irritation of the right eye (i.e., conjunctivitis) for <48 hr, resolving spontaneously with no treatment, visual impairment, or systemic symptoms. An immune response after re-exposure was supported by the finding of a 227.5% increase in anti-RBD IgG in a sandwich ELISA within 3 weeks (Fig. 4A, Fig. S5). This was associated with 143.1% and 325.2% increases in neutralization in the sVNT from prior baseline in serum and saliva, respectively (Fig. 4B).

These results from multiple independent assays demonstrated systemic and mucosal immunity in a laboratory scientist unintentionally exposed to minute quantities of full-length recombinant S1 protein. Functionally-meaningful titers of bAbs and nAbs in serum and saliva were observed for several months

and, importantly, overt toxicity was absent. Although the neutralization assays employed were not equivalent, the P001 titers of nAbs after the first exposure to S1 protein were in a similar range as two mRNA vaccine candidates after two doses (9, 10).

Possible mechanisms of exposure to S1 may have included aerosolization during the emulsification process utilizing the dual syringe technique or—more likely—gloved contact to the S1 protein solutions with subsequent contamination of surrounding surfaces and personal items. In either case, the total amount of mucosal S1 protein exposure would likely be orders of magnitude less than a typical subunit vaccine given the working solutions at primary and secondary exposure were 100 µg/ml and 2 µg/ml, respectively.

Anecdotal mucoconversion of laboratory personnel working with recombinant RBD was described recently, however, the authors speculated this may be due to subinfectious community exposure to the SARS-CoV-2 virus rather than occupational exposure to the recombinant proteins (11). Interestingly, exposure to spike proteins of SARS-CoV has been shown to be sufficient to drive IL-8 expression in PBMCs via a TLR2 mechanism (12) and a truncated S protein of SARS-CoV was also shown to be highly immunogenic in mice without adjuvant (13). Both the N-terminal domain (NTD) and RBD of SARS-CoV-2 have demonstrated to be immunogenic in humans (14) and, indeed, the NTD can modulate neutralization (14, 15) and even serves as the RBD in related β-coronaviruses (15). Accordingly, vaccination with S1 and/or the full-length spike (S1 + S2) may be preferable to RBD-only candidates due to the anticipated emergence of RBD-escape mutants (16, 17).

Prior exposure to human coronaviruses (hCoVs) may result in the development of cross-reactive immunological memory to other hCoV strains through common antigens. However, in a recent small study only 1 of 302 (0.3%) of a control cohort demonstrated “weak” seropositivity for the SARS-CoV-2 S1 subunit (18). Seropositivity for N protein has been demonstrated for up to a year after SARS-CoV infection in one study (7).

The immunogenicity of recombinant S1 protein in water-in-oil vehicle with no additional adjuvants in a human is consistent with the high clinical success of the leading spike-targeted vaccine candidates and provides important data for the development of an effective and globally deployable COVID-19 vaccine, particularly subunit vaccines. Advantages of subunit vaccines include prior track record (e.g., influenza, hepatitis B), high immunogenicity, low-cost, scalability, and stability (4). They may also represent a viable option for those subgroups likely to be excluded from use of the mRNA-based technologies. Finally, this approach appears to promote mucosal immunity which may attenuate or prevent asymptomatic spread of the virus (4).

These results from a single subject are provocative and should be interpreted with caution. Further, the occupational safety implications are non-trivial. However, given the concerning ongoing infection rates internationally and emergence of mutant strains, additional investigation is warranted.

Declarations

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Competing interests: A patent application has been filed for the technology disclosed in this study.

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Figures

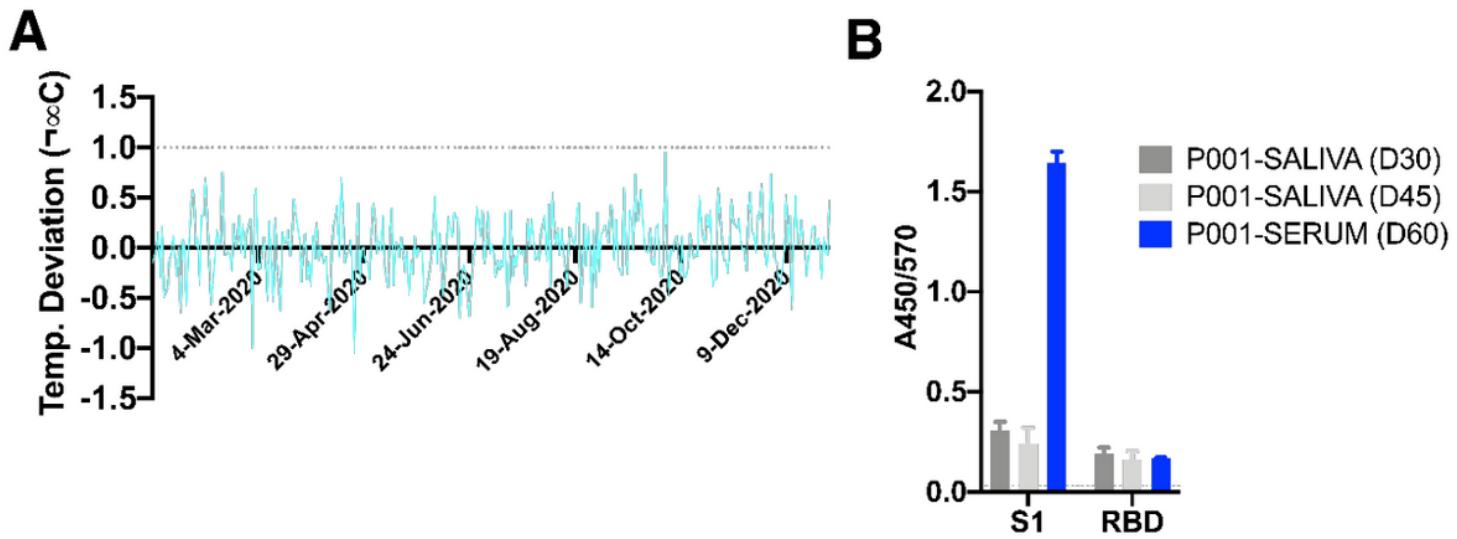


Figure 1

P001 subject characteristics. (A) Fever was assessed with a wearable sleep/activity tracking device worn nightly throughout 2020. Temperature was expressed as deviation from an empirically established baseline based on skin temperature recorded every minute. No fever events, defined by temperature deviation greater than $+1.0^{\circ}\text{C}$, were observed. (B) P001 saliva and serum were screened in a pilot ELISA for IgG against S1 and RBD proteins. P001 serum from D60 was unusually positive for S1 prompting the

suspicion that P001 was inadvertently exposed to the S1 immunogen during the course of the main study.

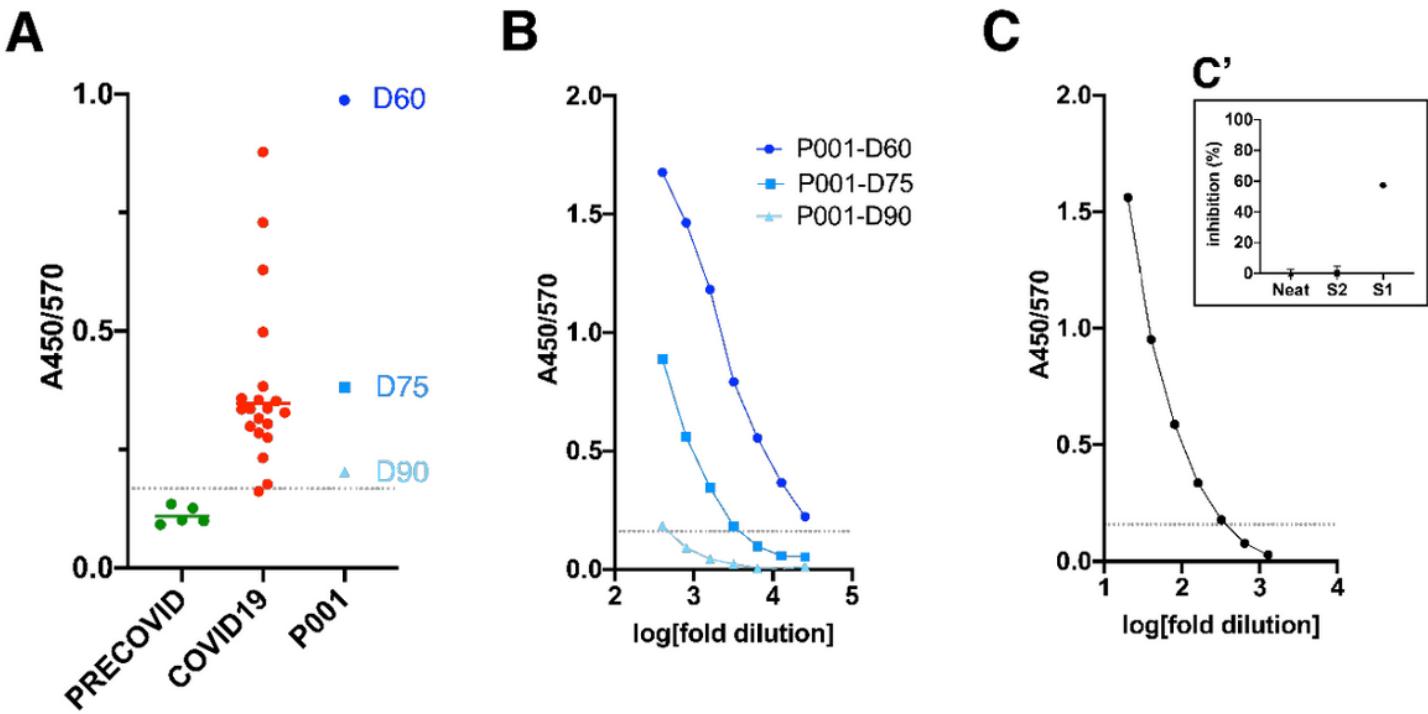


Figure 2

Indirect S1 protein ELISA confirmed P001 immunization with S1 protein. (A) PRECOVID ($n = 5$) and COVID19 ($n = 20$) serum samples (1:3,000) were used to validate the indirect ELISA. S1 IgG was detected from P001 sera at D60, D75, and D90. (B) P001 titers were determined at D60, D75, and D90 with a starting dilution of 1:400 and titers up to 1:12,800 at D60. (C) IgG was purified from P001-D90 serum and titer determined with a starting dilution of 1:20. When 2 μ g/ml S1 protein was added to the purified IgG, there was a 60% inhibition in signal in the indirect ELISA, whereas the addition of 2 μ g/ml S2 protein had no effect.

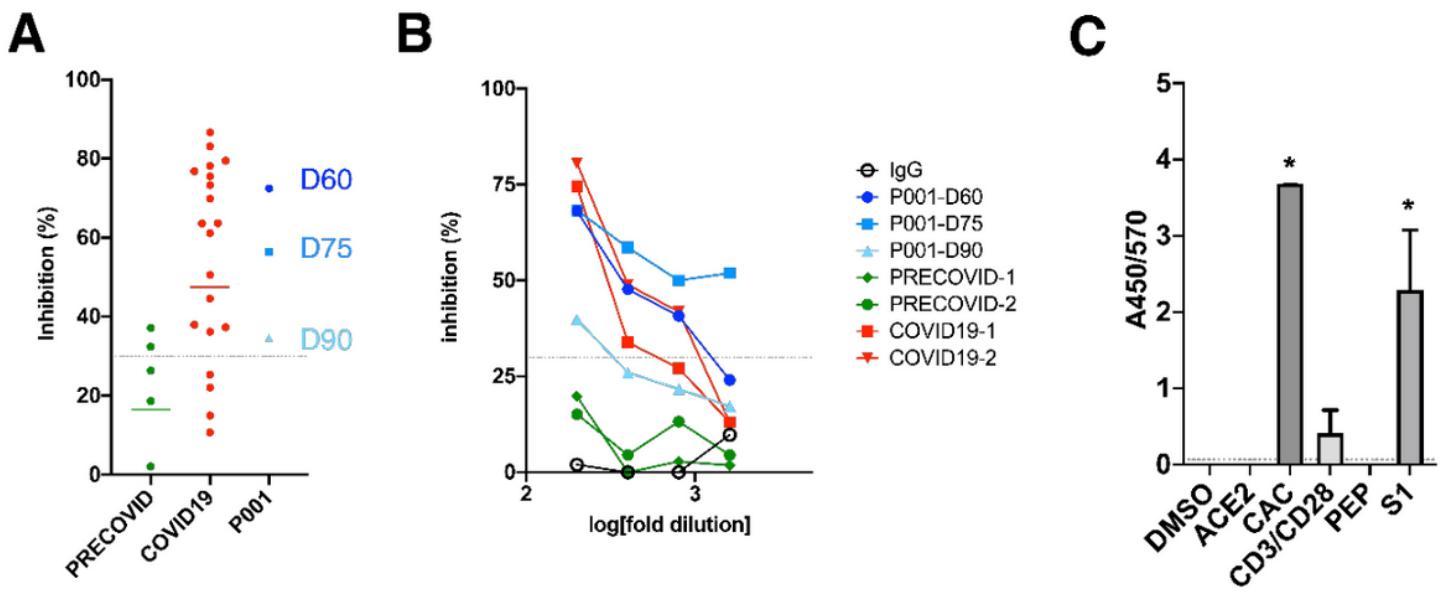


Figure 3

Single exposure to S1 protein promotes neutralizing antibodies (nAbs) and putative cellular immunity. (A) Indirect sVNT was established with the PRECOVID and COVID19 sera. IC30 was established as the cutoff (7). (B) nAb titers were established with P001 sera at D60, D75 and D90 with positive (COVID19) and negative (PRECOVID) controls beginning at 1:200. (C) Cytokine release assay was performed in whole blood diluted 1:2 in complete RPMI media and mixed with the indicated conditions for 48 hours. IFN- γ release was determined by sandwich ELISA from the supernatants. DMSO, dimethyl sulfoxide; ACE2, human recombinant ACE2 protein (negative control, 2 μ g/ml); CAC, cell activation cocktail (positive control, 1:1,000); CD3/CD28 (positive control, 1:1000/1:200, respectively); PEP, RBD peptides; S1 (recombinant S1 protein). Asterisks denote significant differences between each condition compared to DMSO control ($P < 0.001$, ANOVA with Dunnett's multiple comparisons test).

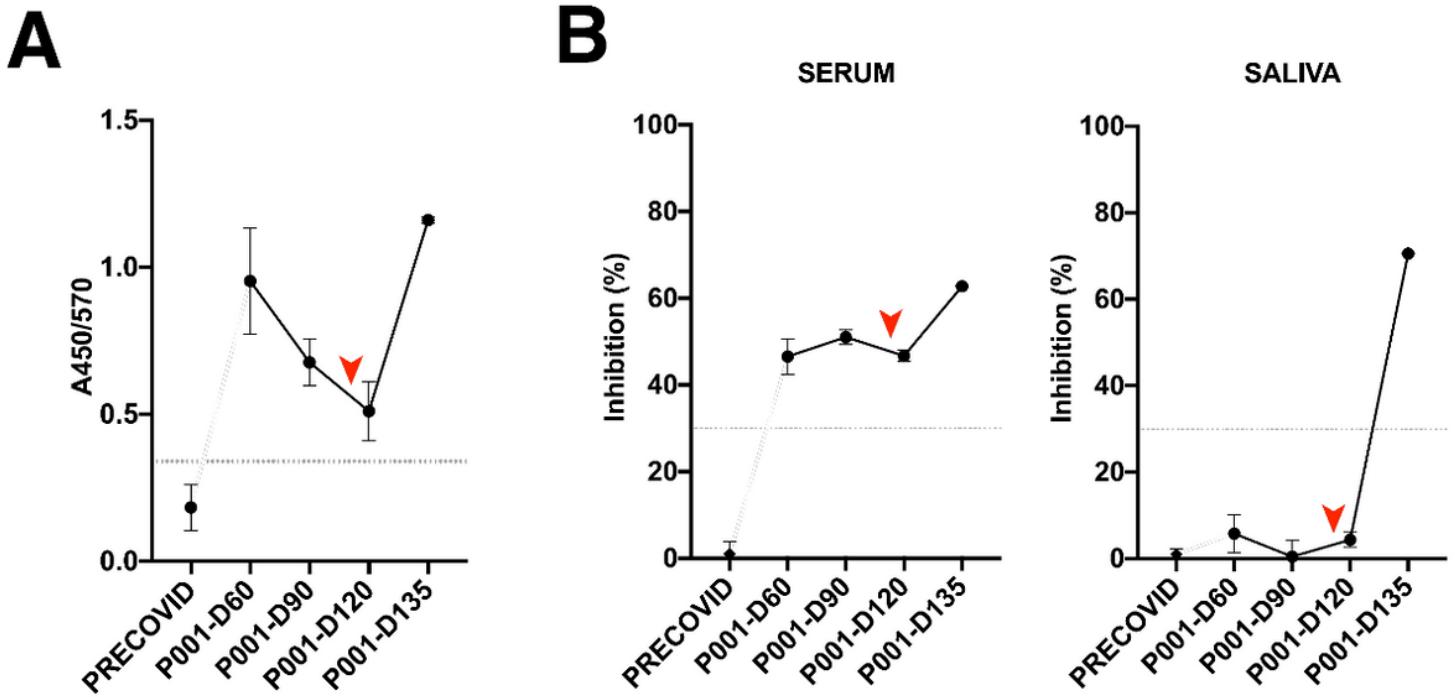


Figure 4

P001 immune responses to a second inadvertent exposure to S1 protein. (A) Serum RBD IgG was assessed in a sandwich ELISA demonstrating a 227.5% post-exposure (red arrowhead) increase from D120 to D135. (B) nAbs were assessed in the indirect sVNT prior to and after the suspected re-exposure in P001 serum and saliva demonstrating increases in neutralization of 143.1% and 325.2% from prior baseline, respectively.

Supplementary Files

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