Driving potent neutralization of a SARS-CoV-2 Variant of Concern with a heterotypic boost.

Daniel J. Sheward, Marco Mandolesi, Changil Kim, Leo Hanke, Laura Perez Vidakovics, Gerald McInerney, Gunilla B. Karlsson Hedestam, and Ben Murrell

Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden

These authors contributed equally

The emergence of SARS-CoV-2 Variants of Concern (VOCs) with mutations in key neutralizing antibody epitopes threatens to undermine vaccines developed against the pandemic founder variant (Wu-Hu-1). Widespread vaccine rollout and continued transmission are creating a population that has antibody responses of varying potency to Wu-Hu-1. Against this background, it is critical to assess the outcomes of subsequent booster vaccination with variant antigens. It is not yet known whether such heterotypic vaccine boosts would be compromised by original antigenic sin, where pre-existing responses to a prior variant dampen responses to a new one, or whether the primed memory B cell repertoire would bridge the gap between Wu-Hu-1 and VOCs. Here, we show that a single adjuvanted dose of receptor binding domain (RBD) protein from VOC 501Y.V2 (B.1.351) drives an extremely potent neutralizing antibody response capable of cross-neutralizing both Wu-Hu-1 and 501Y.V2 in rhesus macaques previously immunized with Wu-Hu-1 spike protein.

Correspondence: daniel.sheward@ki.se, gunilla.karlsson.hedestam@ki.se, benjamin.murrell@ki.se

At least 20 candidate SARS-CoV-2 vaccines have already entered phase 3 clinical trials. A number of these demonstrated high efficacy, significantly reducing morbidity and mortality, and are being rolled-out globally. This first generation of vaccines all encode or deliver a spike glycoprotein. However, mutations that confer resistance to prior immunity. Of particular concern is the surge of variant 501Y.V2, with multiple mutations in dominant neutralizing antibody epitopes making it several fold more resistant to antibodies elicited by current vaccines. This underpins the substantially reduced vaccine efficacies in South Africa, where this variant is circulating at high frequency. Updated vaccines are likely required to protect against current and future mutated variants. Importantly, by the time these are rolled out, a significant proportion of the global population are likely to be seropositive as a result of either infection, or immunization with Wu-Hu-1-based vaccines.

The first exposure to a pathogen can shape future responses to mutated variants. This immunological imprinting or original antigenic sin is well-described for influenza A virus where protection is highest against the first strain encountered, and diminished against those encountered later in life. It is crucial for the design of updated vaccines and regimens to determine if existing immunity dampens antibody responses to new VOCs, or if a heterotypic boost can efficiently recruit cross-protective memory responses.

To address this, we immunized three rhesus macaques with two doses of soluble prefusion-stabilized Wu-Hu-1 spike protein (2 μg), adjuvanted with 50 μg of saponin-based Matrix-M™ (Novavax AB, Uppsala, Sweden), with a one-month interval between doses, mimicking an immunization schedule for approved SARS-CoV-2 vaccines. After a single dose, neutralizing antibodies were detectable against Wu-Hu-1 but not 501Y.V2 (Fig. 2). Neutralizing antibody responses against Wu-Hu-1 were substantially boosted by the second immunization (GMT = 3980), and then waned over the following months (Fig. 2), as also reported in immunized humans. Notably, the circulating VOC 501Y.V2 was on average 9-fold (range: 5.6 - 12.2 fold) less potently neutralized (GMT = 451 at peak), with this difference less pronounced in one of the animals (H05), consistent with the responses observed in humans following vaccination.
Six months after their first immunization, macaques were boosted with soluble 501Y.V2 RBD, with either a 2 µg (H05), 10 µg (H06), or 50 µg (H07) dose in 50 µg Matrix-M™ adjuvant. One macaque (H05) was terminated 5 days after immunization, due to an unrelated illness that had begun prior to the third immunization, and was sampled for detailed follow-up studies of antibody specificities. The other two (H06 and H07) were followed for 2 weeks. In all three animals, 501Y.V2 RBD efficiently boosted responses that potently cross-neutralized both Wu-Hu-1 and 501Y.V2, with similar titers (Fig. 2a-c; Wu-Hu-1 GMT = 11795, 501Y.V2 GMT = 12595). In contrast, for macaques previously immunized with three doses of Wu-Hu-1 spike, the reduced neutralization of 501Y.V2 compared to Wu-Hu-1 remained after the third homotypic spike immunization (Supp. Fig. 1). Despite weak immunogenicity as a priming antigen, soluble monomeric heterotypic RBD elicited a potent recall response. This was robust to the boosting dose, and effective as low as 2 µg, possibly aided by a dose-sparing effect of Matrix-M™. This is particularly promising as RBD is a small, stable protein that can be rapidly synthesized and efficiently expressed.

Taken together, these data indicate that potent, cross-neutralizing antibody responses can be recruited with heterotypic SARS-CoV-2 immunogens following a primary exposure, and that soluble RBD booster immunizations represent an attractive strategy to broaden vaccine protection from new SARS-CoV-2 variants.

ACKNOWLEDGEMENTS

We thank Dr. Bengt Eriksson and all personnel at Astrid Fagraeus laboratory for expert assistance with rhesus macaques. We also thank Novavax, AB, Uppsala, Sweden, for generously making the Matrix-M™ adjuvant available. We gratefully acknowledge Penny Moore and the NICD (South Africa) for providing the 501Y.V2 spike plasmid (used here for PSV neutralization assays) which was generated using funding from the South African Medical Research Council. We acknowledge GISAID for metadata curation, used here for variant frequency estimation, and we gratefully acknowledge all Submitting and Originating laboratories who contributed sequence data to GISAID. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 101003653 (CoroNAb), to GM, GKH, and BM, from the Swedish Research Council to GM, GKH, and BM, and from Karolinska Institutet Development Office and Karolinska Institutet President’s Fund to GM, GKH, and BM.

AUTHOR CONTRIBUTIONS

Conceptualization: DJS, MM, GKH, BM; Formal Analysis: DJS, BM; Funding acquisition: GMM, GKH, BM; Investigation: DJS, MM, GKH, BM; Resources: CK, LH, LPV; Software: BM; Supervision: LH, GMM, GKH, BM; Methodology: DJS, MM, GKH, BM; Writing – original draft: DJS; BM; Writing – review editing: all authors.

METHODS

Non-linear Multinomial Regression for VOC frequency estimation: SARS-CoV-2 lineage metadata was obtained from GISAID (gisaid.org - 2021-03-24 metadata release) comprising 850203 genomes. For each of the lineages in Figure 1, we aggregated daily counts of genomes at the country level, requiring at least 30 samples in the 30 days before 15th Feb, 2020, which was chosen because sequence data diminished rapidly beyond this point. Using a Generalized Linear Model, we model the daily variant counts with a multinomial distribution (and a log-link function), with underlying frequencies parameterized by a linear combination of 400 randomly drawn Fourier basis features (aka. a “Random Kitchen Sink”22) to allow frequencies to vary non-linearly as a function of time. We estimate the model parameters with an L2 norm on the random feature coefficients, using the GLMNet.jl Julia package, plotting the map with Cartopy (https://github.com/SciTools/cartopy). Code available at https://github.com/MurrellGroup/VOCfreq.

Ethics statement: The animal work was conducted with the approval of the regional Ethical Committee on Animal Experiments (Stockholms Norra Djurförsöksnämnden). All animal procedures were performed according to approved guidelines.

Protein production: 501Y.V2 RBD (encoding amino acid mutations K417N, E484K, and N501Y, and a C-terminal His-tag) was synthesized (IDT oligos), and cloned into a mammalian expression vector (pcDNA3.1), using a Gibson Assembly Mastermix (New England Biolabs). Spike ectodomain (preliminary stabilized with 6 prolines23) and RBD were produced by the transient transfection of Freestyle 293-F cells using FreeStyle MAX reagent (Thermo Fisher) or polyethylenimine (PEI), re-
The his-tagged Spike ectodomain and RBD were purified from filtered supernatant using nickel IMAC resin (HisPur Ni-NTA, Thermo Fisher Scientific) followed by size-exclusion chromatography on a Superdex 200 (Cibula) in PBS. On the day of immunization, indicated doses were mixed with 50 μg Matrix-M adjuvant (Novavax, Uxbridge, Sweden) in a final inoculum volume of 50 μl.

**Animal Model** Rhesus macaques (Macaca mulatta) of Chinese origin, 5-6 years old, were housed at the Astrid Fagraeus Laboratory at Karolinska Institutet. Housing and care procedures complied with the provisions and general guidelines of the Swedish Board of Agriculture. The facility has been assigned an Animal Welfare Number by the Animal Welfare Board (OLAW) at the National Institutes of Health (NIH). The macaques were housed in groups in enriched 14 m3 cages. They were habituated to the housing conditions for more than six weeks before the start of the experiment and subjected to positive reinforcement training in order to reduce the stress associated with experimental procedures. The macaques were weighed at each sampling. All animals were confirmed negative for simian immunodeficiency virus, simian T cell lymphotropic virus, simian retrovirus type D and simian herpes B virus. Macaques were immunized intramuscularly (i.m.) with half of each dose administered in each quadricep. All immunizations and blood samplings were performed under sedation with 10-15 mg/kg ketamine (Ketaminol, Intervet, Sweden) administered i.m. Blood plasma was isolated by centrifugation, and heat inactivated at 56°C for 60 min.

**Pseudotyped neutralization assays:** HEK293T and HEK293T/ACE2 (human/female) cells were cultured in a humidified 37°C incubator (5% CO2) in Dulbecco’s Modified Eagle Medium (Gibco) supplemented with 10% Fetal Bovine Serum and 1% PenStrep/Gentamicin, and were passaged when reaching confluency using 1X Trypsin-EDTA. Pseudotyped lentiviruses displaying either the SARS-CoV-2 pandemic founder variant (Wu-Hu-1) or 501Y.V2 variant spike and packaging a luciferase reporter gene were generated by the co-transfection of HEK293T cells with pCMV-MCS-EcoR1 (Invitrogen) and pMDLg/pRRE (RetroNect) per the manufacturer’s protocol, on a GM-2000 lumi-plates. Plates were incubated at 37°C for 48 hours, and luminescence was then measured was changed 12-16 hours after transfection, and pseudotyped viruses were harvested at 48- and 72-hours post-transfection, clarified by centrifugation, and stored at -80°C until use. Pseudotyped viruses sufficient to generate 50,000 relative light units (RLU) were used in duplicate with serial dilutions of plasma for plates that contained a 96-well plate, and then 15,000 HEK293T/ACE2 cells were added to each well. Plates were incubated at 37°C for 48 hours, and luminescence was then measured using Bright-Glo (Promega) per the manufacturer’s protocol, on a GM-2000 luminescence plate reader. IFUs were interpolated as the reciprocal of the dilution where RLU’s were reduced by 50% relative to control wells in the absence of serum, fitting a four-parameter logistic curve in Prism (GraphPad Software).

**Bibliography**


Fig. SI1. (left) Longitudinal neutralizing antibody responses against Wu-Hu-1 (blue) and 501Y.V2 (red) for plasma samples from Mandolesi et al. 20, where three rhesus macaques (NHP1-NHP3) were immunized with three doses of Wu-Hu-1 spike (100 µg) in Matrix-M™ adjuvant. Vertical blue lines indicate the timing of immunizations (at 0, 4, and 9 weeks). (right) Comparison of the titers at 6 weeks (post 2) and 11 weeks (post 3) illustrating that reduced titers to 501Y.V2 (red) compared to Wu-Hu-1 (blue) were maintained after a third homotypic spike boost.