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

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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.

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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 derived from the pandemic founder strain, Wu-Hu-1. Driven by multiple evolutionary forces, SARS-CoV-2 is rapidly evading our response. Globally, a number of VOCs are rising in frequency (see Fig 1), each harbouring spike 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 for 501Y.V2. 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. 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. 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.
Fig. 2. Heterotypic RBD boost drives a potent cross-neutralizing antibody response. (a) Depiction of the RBD immunogen (PDB:6MOJ) used as a heterobivalent boost in this study, that incorporates the three RBD mutations (located in red) defining lineage 20H/501Y.V2. The cellular receptor, ACE2, is shown in green. (b-d) Neutralizing antibody responses over time to Wu-Hu-1 (blue) and 501Y.V2 (red) pseudotyped viruses (PSV) are shown for three immunized macaques: (b) H06 and H07 (left) and H05 (right), plotted separately as they exhibit different trajectories prior to the heterotypic boost. Syringes indicate the timing of immunizations (blue: Wu-Hu-1 spike at 0 and 4-weeks, red: 501Y.V2 RBD at 30-weeks). Titers from 27-30 weeks (shown with dashed lines) have been extrapolated for clarity. Error bars depict the geometric SD. (c) While neutralization of 501Y.V2 was significantly reduced at 6 weeks, corresponding to peak responses 2 weeks following the second spike dose (left), neutralization was restored following subsequent heterotypic RBD boost (right), such that 501Y.V2 (red) and Wu-Hu-1 (blue) were potently neutralized at similar titers (d) in all three animals.

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 spike20, 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 antigen20, 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.

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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”)25 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/MurrellGroup/VOCfreq). 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 Djurförsöksnämd). 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 elfbols), and cloned into a mammalian expression vector (pcDNA3.1), using a Gibson Assembly Mastermix (New England Biolabs). Spike ectodomain (prenctstabilized with 6 prolines27) and RBD were produced by the transient transfection of Freestyle 293-F cells using FreeStyle MAX reagent (Thermo Fisher) or polyethyleneimine (PEI), re-
The effectiveness of the COVID-19 vaccines has been studied extensively. For instance, a study by the Oxford COVID Vaccine Trial Group demonstrated that the B.1.351 variant was not neutralized by antibodies raised against the original SARS-CoV-2 strain [1]. Another study by the Regeneron Pharmaceuticals showed that their REGN-COV2 antibody cocktail could neutralize the B.1.1.7 variant [2]. These findings highlight the importance of ongoing research to understand the evolution of SARS-CoV-2 and its potential impact on vaccine efficacy.

Additionally, a meta-analysis by the COVID-19 Genomics UK (COG-UK) consortium found that the B.1.1.7 variant was associated with an increased risk of hospitalization and death compared to other variants [3]. This reinforces the need for continued surveillance and vaccination campaigns to prevent the spread of variants with increased transmissibility and pathogenicity.

In conclusion, while the current vaccines are highly effective against the original SARS-CoV-2 strain and its major variants, there is a need to remain vigilant and adapt vaccination strategies as new variants emerge. Further research is essential to understand the evolution of SARS-CoV-2 and to develop effective interventions to control the pandemic.


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.