Two doses of SARS-CoV-2 vaccination induce more robust immune responses to emerging SARS-CoV-2 variants of concern than does natural infection.

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Research Article

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Abstract

Both natural infection with SARS-CoV-2 and immunization with vaccines induce protective immunity. However, the extent to which such immune responses protect against emerging variants is of increasing importance. Such variants of concern (VOC) include isolates of lineage B.1.1.7, first identified in the UK, and B.1.351, first identified in South Africa. Our data confirm that VOC, particularly those with substitutions at residues 484 and 417, escape neutralization by antibodies directed to the ACE2-binding Class 1 and the adjacent Class 2 epitopes but are susceptible to neutralization by the generally less potent antibodies directed to Class 3 and 4 epitopes on the flanks of the receptor-binding domain. To address the potential threat posed by VOC, we sampled a SARS-CoV-2 uninfected UK cohort recently vaccinated with BNT162b2 (Pfizer-BioNTech, two doses delivered 18-28 days apart), alongside a cohort sampled in the early convalescent stages after natural infection in the first wave of the pandemic in Spring 2020. We tested antibody and T cell responses against a reference isolate of the original circulating lineage, B, and the impact of sequence variation in the B.1.1.7 and B.1.351 VOC. Neutralization of the VOC compared to B isolate was reduced, and this was most evident for the B.1.351 isolate. This reduction in antibody neutralization was less marked in post-boost vaccine-induced responses compared to naturally induced immune responses and could be largely explained by the potency of the homotypic antibody response. After a single vaccination, which induced only modestly neutralizing homotypic antibody titres, neutralization against the VOC was completely abrogated in the majority of vaccinees. Importantly, high magnitude T cell responses were generated after two vaccine doses, with the majority of the T cell response directed against epitopes that are conserved between the prototype isolate B and the VOC. These data indicate that VOC may evade protective neutralizing responses induced by prior infection, and to a lesser extent by immunization, particularly after a single vaccine dose, but the impact of the VOC on T cell responses appears less marked. The results emphasize the need to generate high potency immune responses through vaccination in order to provide protection against these and other emergent variants.

Introduction

The emergence of new lineages of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on three continents towards the end of 2020, and their rapid expansion at the expense of the previously dominant lineages, poses significant challenges to public health ([WHO | SARS-CoV-2 Variants](#))¹. In order to address these challenges effectively, there is an urgent need to understand the biological consequences of the mutations found in these lineages, and the consequential impact on their susceptibility to current control measures, including vaccines, drugs and non-pharmaceutical interventions.

Three variants (B.1.1.7, B.1.351 and P1) have been termed variants of concern (VOC). All three variants share the N501Y substitution in the receptor-binding domain (RBD) of spike glycoprotein (S), which increases binding affinity of S with the virus's cellular receptor, angiotensin-converting enzyme 2 (ACE2)² (see Figure 1). As of 1 March 2021, N501Y is present globally in 77% of currently sequenced samples

(data from [GISAID - Initiative](#)). Lineage B.1.1.7, first identified in the UK in September 2020 (0F0F[1]), is characterized by additional mutations in S, such as deletion of residues 69 & 70 and the P681H substitution, for which plausible effects on the virus biology are proposed, as well as five other mutations in S, a premature stop codon in ORF8, three substitutions and a deletion in ORF1 and two amino acid substitutions in nucleoprotein (N), of as-yet unknown significance. Lineage B.1.351³ was first identified in November 2020 in South Africa and is characterized by two additional substitutions of likely significance in RBD, namely, K417N and E484K. The former is predicted to disrupt a salt bridge with D30 of ACE2, a characteristic of SARS-CoV-2 in distinction to severe acute respiratory syndrome coronavirus (SARS-CoV-1), but may not impact on binding, whereas the latter, which might disrupt the interaction of RBD with K31 of human ACE2, may enhance ACE2 binding²⁴. On 1 March 2021, this lineage accounted for 5% of all current sequences globally, and 100% of those identified in South Africa. The third variant of concern, P.1 (formerly B.1.1.28.1) is characterized by K417T, in addition to E484K and N501Y, and accounted for 80% of all viruses sequenced in Brazil on 1 March 2021. In early 2021, E484K had been detected first in lineage B.1.1.7 in the United Kingdom (UK)⁵ and subsequently in lineages A23.1, B.1 and B.1.177, as well as in imported cases of B.1.51 and P.21F1F[2]. Our data confirm that VOC, particularly those such as B.1.351 with substitutions at residues 484 and 417, escape neutralization by antibodies directed to the ACE2-binding Class 1 and the adjacent Class 2 epitopes, but are susceptible to neutralization by the generally less potent antibodies directed to Class 3 and 4 epitopes on the flanks of the RBD

The immune correlates of protection against infection and disease caused by SARS-CoV-2 are imperfectly understood (reviewed by^{6,7}). Classically, neutralization by antibody, measured by reduction in plaque or infectious foci by authentic virus *in vitro* is considered a major component of protection, though indirect effects of antibody, such as complement activation and opsonization may also play a role *in vivo*. Recent studies have demonstrated that symptomatic re-infection within six months after the first wave in the UK was very rare in the presence of anti-S or anti-N IgG antibodies^{8,9}. Virus-specific lymphocytes may play an important direct role in protection, in addition to their indirect effect mediated through help to antibody-producing cells. Robust T cell immune responses (with CD4+ T cells dominating) to S, M, N and some ORF antigens are readily detected after infection, correlate with disease severity and are durable for at least several months¹⁰⁻¹². Furthermore, CD8 depletion studies in non-human primate (NHP) challenge studies suggest T cells also play a protective role especially when antibody levels are low^{13,14 15}. Nevertheless, passive infusion of neutralizing antibody has been shown to be sufficient to mediate effective protection against SARS-CoV-2 in these NHP studies¹⁴. Although studies in NHPs of both adenovirus-26 and DNA-based vaccine candidates found that levels of neutralizing antibodies but not of T cells were significantly correlated with viral clearance^{13,16} recent reports involving subunit vaccine candidates in NHP found not only neutralizing antibodies, but also N-specific CD4+ responses were a statistically significant correlate of protection^{17,13,16}, recent reports involving subunit vaccine candidates in NHP found not only neutralizing antibodies, but also N-specific CD4+ responses were a statistically significant correlate of protection¹⁷.

Multiple vaccines have been reported to have efficacy against COVID-19 (coronavirus disease 2019) in phase III clinical trials. Of these, three – Pfizer/BNT162b2, Moderna/mRNA-1273 and Sputnik V – that were reported to have efficacies against symptomatic infection in the mid-90% range, had also induced classical neutralizing antibody titres substantially higher than those found on average in convalescent patients ^{18–20}. In contrast, one – CoronaVac – that showed approximately 50% efficacy, had been reported to induce neutralizing titres several-fold lower than those found in convalescent patients ²¹. The two remaining vaccines, Sinopharm/BBIBP-CorV and AstraZeneca/AZD1222 (ChAdOx-1 nCoV-19), had intermediate values of both clinical efficacy against symptomatic infection and relative potency in generating neutralizing antibody responses ^{22,23}. mRNA and adenovirus-vectored vaccines generate high magnitude SARS-CoV-2 multispecific CD4+ and CD8+ T cells responses. Reports of vaccines assessed in South Africa where B.1.351 dominates are currently emerging and include Ad26.COV2.S (single dose Ad26 vectored vaccine) ²⁴, Novavax (recombinant spike/adjuvant) ²⁵ and AZD1222 ²⁶. Each of the studies report reduced efficacy in South African populations. Vaccine correlates of protection, and the relative contribution of T cell and humoral immunity, are yet to be precisely defined since detailed immune analysis in people with vaccine breakthrough infections is lacking.

In pseudotype virus neutralization assays, it appears that convalescent sera from patients exposed to prototype strain of SARS-CoV-2, in distinction to vaccine-elicited responses, may not be effective in neutralizing lineage B.1.351 ^{27,28}. As the lineage-defining substitutions include changes in previously identified antibody epitopes and regions of S associated with its processing and rearrangement during cellular infection, this is a very plausible observation. In order to test whether convalescent sera and sera from vaccine recipients were similarly affected in their ability to neutralize authentic virions, we have undertaken classical neutralization assays against reference isolates of both B.1.1.7 and B.1.351 compared to the early pandemic B isolate. We find that, while cross-neutralization of B.1.1.7 is only modestly reduced compared to that of the prototype B lineage, cross-neutralization of B.1.351 may be markedly reduced in convalescent sera, and after a single vaccine dose. However, both the neutralization of VOC and the generation of viral specific T cells, is significantly enhanced by a boost vaccination. In addition, vaccination not only induces enhanced reactivity to S from endemic human coronaviruses, but also results in significant cross-reactivity to both SARS-CoV-1 and Middle East respiratory syndrome-related coronavirus (MERS-CoV).

Since viral mutations may also affect T cell recognition, we also evaluate the contribution of T cells that target epitopes located at sites of amino acid substitution in the spike glycoproteins of VOC. We show that the majority of T cell responses in recipients of two doses of the BNT162b2 vaccine are generated by epitopes that are invariant between the prototype B lineage virus and VOC.

Whilst the T cell data is encouraging, the loss of neutralizing antibody recognition against VOC suggest that reformulation of vaccines to address new variant lineages ought to be considered and indicates that seasonal re-vaccination might be required for this virus.

Footnotes:

[1] Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of <http://filogeneti.ca/covizu/> spike mutations - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology - Virological

[2] Updated regularly at [Nextstrain / groups / neherlab / ncov / S.E484](#)

Methods

Volunteer samples

Volunteers were recruited at Oxford University Hospitals NHS Foundation Trust in ethically approved studies. Healthcare Workers (HCWs) with asymptomatic SARS-CoV-2, defined as being SARS-CoV-2 PCR positive on screening without symptoms (mean 28 days post-PCR testing, range 24 – 34 days) and mild symptomatic COVID-19, defined as being SARS-CoV-2 PCR positive and having symptoms not requiring O2 support/hospitalization (mean 28 days post-symptom onset, range 24 – 37 days) were recruited under the OPTIC Study: Oxford Translational Gastrointestinal Unit GI Biobank Study 16/YH/0247 [[REC at Yorkshire & The Humber – Sheffield]. HCWs not known to be previously infected with SARS-CoV-2, were recruited after vaccination with the COVID-19 mRNA Vaccine BNT162b2 (Pfizer). 11 participants were recruited post-prime (mean 29 days after a single dose, range 18-41). 25 participants were recruited post-boost (mean 8 days after the second dose, range 7-17 days) and assessed again for T cell reactivity 28 days boost. An additional 13 unvaccinated, non-SARS-CoV-2 exposed HCW were recruited and assessed for T cell reactivity. Four unvaccinated participants were recruited under the Observational Biobanking study approvals SthObs (18/YH/0441) and assessed for neutralizing antibodies. Pre-pandemic negative control sera, used for binding assays, were obtained from a prior vaccine study of the National Vaccine Evaluation Consortium performed in 2017. Ethics approval from NHS Heath Research Authority – NRES committee London City and East 2017. A summary table in supplementary materials shows the details for each sample in terms of days since vaccination or infection and the assays that each sample were run on. The study was conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. Written informed consent was obtained for all patients enrolled in the study.

Virus isolates

Prototype isolate (PANGO lineage B) was Victoria/01/2020²⁹, received at P3 from Public Health England (PHE) Porton Down (after being supplied by the Doherty Centre Melbourne) in April 2020, passaged in VeroE6/TMPRSS2 cells, used here at P5, and confirmed identical to GenBank MT007544.1, B hCoV-19_Australia_VIC01_2020_ EPI_ ISL_ 406844_ 2020-01-25.

B.1.1.72F2F[3] (20I/501Y.V1.HMPP1) isolate, H204820430, 2/UK/VUI/1/2020, received in Oxford at P1 from PHE Porton Down in December 2020, passaged in VeroE6/TMPRSS2 cells (NIBSC reference 100978), used here at P4.

B.1.351 (20I/501.V2.HV001) isolate³⁰ was received at P3 from the Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, in Oxford in January 2021, passaged in VeroE6/TMPRSS2 cells (NIBSC reference 100978), used here at P4.

For all isolates, identity was confirmed by deep sequencing at the Wellcome Trust Centre for Human Genetics, University of Oxford.

Microneutralization Assay (MNA)

The study was performed in the containment level 3 facility of the University of Oxford operating under license from the Health and Safety Authority, UK, on the basis of an agreed Code of Practice, Risk Assessments (under the Advisory Committee on Dangerous Pathogens guidance) and Standard Operating Procedures. The microneutralization assay determines the concentration of antibody that produces a 50% reduction in infectious focus-forming units of authentic SARS-CoV-2 in Vero CCL81 cells. Quadruplicate serial dilutions of serum or monoclonal antibody (20 μ L) were preincubated with 100-200 FFU (20 μ L) of SARS-CoV-2 for 30 minutes at room temperature. After pre-incubation, 100 μ L of Vero CCL81 cells (4.5×10^4) were added and incubated at 37°C, 5% CO₂. After 2 hours, 100 μ L of a 1.5% carboxymethyl cellulose-containing overlay was applied to prevent satellite focus formation. Eighteen (B.1.351) or 23 hours (B, B.1.1.7) post-infection, the monolayers were fixed with 4% paraformaldehyde, permeabilized with 2% Triton X-100 and stained for the nucleocapsid (N) antigen or spike (S) antigen using monoclonal antibodies (mAbs) EY 2A and EY 6A, respectively³¹. After development with a peroxidase-conjugated antibody and TrueBlue peroxidase substrate, infectious foci were enumerated by ELISpot reader. Data were analysed using four-parameter logistic regression (Hill equation) in GraphPad Prism 8.3.

Expression and purification of monoclonal antibodies

Monoclonal antibodies FI 3A (Class 1), GR 12C (Class 2), FD 11A (Class 3) and EY 6A (Class 4) used in this study were isolated from convalescent patients as previously described³². In brief, plasmablasts from hospitalised RT-PCR-confirmed SARS-CoV-2 infected patients (day 14 to day 22 post onset of symptoms) were isolated. Freshly separated or thawed PBMCs were stained with fluorescent-labelled antibodies to cell surface markers; Pacific blue anti-CD3 (clone UCHT1, Cat. No. 558117, 420 BD), Fluorescein isothiocyanate anti-CD19 (clone HIB19, Cat. No. 555412, BD), 421 Phycoerythrin-Cy7 anti-CD27 (clone M-T271, Cat. No. 560609, BD), 422 Allophycocyanin-H7 anti-CD20 (clone L27, Cat. No. 641396, BD), Phycoerythrin423 Cy5 anti-CD38 (clone HIT2, Cat. No. 555461, BD) and Phycoerythrin anti-

human IgG (clone G18-145, Cat. No. 555787, BD). The CD3^{neg} CD19^{pos} CD20^{neg} CD27^{hi} CD38^{hi} IgG^{pos} plasmablasts were gated as single cells.

Sorted single cells were used to produce human IgG mAbs, as previously described³³. Briefly, the variable region genes from each single cell were amplified in a reverse transcriptase polymerase chain reaction (RT-PCR: QIAGEN, Germany) using a cocktail of sense primers specific for the leader region and antisense primers to the Cy constant region for heavy chains and Cκ and Cλ for light chains. The RT-PCR products were amplified in separate PCR for the individual heavy and light chain gene families using nested primers to incorporate unique restriction sites at the ends of the variable gene as previously described³³. Monoclonal antibodies C121 (Class 2) and S309 (Class 3) were derived from the published sequences^{34,35} by gene synthesis (GeneArt). These variable genes were then cloned into expression vectors for the heavy and light chains. Plasmids were transfected into the Expi293F cell line for expression of recombinant full-length human IgG mAbs in serum-free transfection medium. The mAbs were then affinity purified using a MabSelectSure column (Cytiva, USA) according to the manufacturer's protocol and buffer exchanged into 1xPBS using a 10k MWCO Amicon Ultracentrifugal Unit.

NIBSC 20/130 reference serum was obtained from the National Institute for Biological Standards and Control, UK. It is human plasma from a donor recovered from COVID-19.

Mesoscale Discovery (MSD) binding assays

IgG responses to SARS-CoV-2, SARS-CoV-1, MERS-CoV and seasonal coronaviruses were measured using a multiplexed MSD immunoassay: The V-PLEX COVID-19 Coronavirus Panel 3 (IgG) Kit (Cat # K15399U) from Meso Scale Diagnostics, Rockville, MD USA. A MULTI-SPOT® 96-well, 10 Spot Plate was coated with three SARS CoV-2 antigens (S, RBD, N), SARS-CoV-1 and MERS-CoV spike trimers, as well as spike proteins from seasonal human coronaviruses HCoV-OC43, HCoV-HKU1, HCoV-229E, HCoV-NL63 and bovine serum albumin. Antigens were spotted at 200–400 µg/mL (MSD® Coronavirus Plate 3). Multiplex MSD Assays were performed as per the instructions of the manufacturer. To measure IgG antibodies, 96-well plates were blocked with MSD Blocker A for 30 minutes. Following washing with washing buffer, samples diluted 1:500 in diluent buffer, as well as the reference standard and internal controls, were added to the wells. After 2-hour incubation and a washing step, detection antibody (MSD SULFO-TAG™ Anti-Human IgG Antibody, 1/200) was added. Following washing, MSD GOLD™ Read Buffer B was added and plates were read using a MESO® SECTOR S 600 Reader. Statistical analysis was performed using Kruskal-Wallis one-way ANOVA.

A multiplexed MSD immunoassay (MSD, Rockville, MD) was also used to measure the ability of human sera to inhibit ACE2 binding to SRAS-CoV-2 spike (B, B.1, B.1.1.7, B.1.351 or P.1). A MULTI-SPOT® 96-well, 10 Spot Plate was coated with five SARS-CoV-2 spike antigens (B, B.1, B.1.1.7, B.1.351 or P.1). Multiplex MSD Assays were performed as per manufacturer's instructions. To measure ACE2 inhibition, 96-well plates were blocked with MSD Blocker for 30 minutes. Plates were then washed in MSD washing buffer,

and samples were diluted 1:10 – 1:100 in diluent buffer. Importantly, an ACE2 calibration curve which consists of a monoclonal antibody with equivalent activity against spike variants was used to interpolate results as arbitrary units. Furthermore, internal controls and the WHO international standard were added to each plate. After 1-hour incubation recombinant human ACE2-SULFO-TAG™ was added to all wells. After a further 1-hour plates were washed and MSD GOLD™ Read Buffer B was added, plates were then immediately read using a MESO® SECTOR S 600 Reader.

Peptides used in IFN-γ ELISpot assays

Peptides corresponded to SARS-CoV2 prototype lineage B isolate, VIC01., 15-18 amino-acids overlapping by 10 amino-acids and spanning the entire spike region, were used in IFN-γ ELISpot assays. Spike peptides were used in two pools (S1 and S2) (Mimotopes). CMV, EBV, influenza and tetanus antigens (CEFT) were used in single pools as positive control antigens (2 µg/mL: Proimmune, Oxford, UK). Single peptides (Mimotopes, Victoria Australia) that mapped to sites containing substitutions in lineages B.1.1.7 (n=17), B.1.351 (n=21) and P.1 (n=22) with reference to B were used in single peptides or pooled by individual VOC. T cell responses to original B strain peptides covering the areas of known sequence/amino acid mutations/deletions in the VOC (B.1.1.7, B.1.351 and P.1) relative to B are assessed. Three peptides, each of which span a single mutational site/region, were used in these assays: i) firstly in pools to cover all mutation regions within each VOC and then ii) mapped to single mutational regions. T cell responses to the peptide pools that span the mutational regions are also assessed alone and in relation to the total T cell response against the entire spike antigen.

IFN-γ T cell ELISpot assays

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Lymphoprep™ (ρ=1.077 g/mL, Stem Cell Technologies), washed twice with RPMI (Roswell Park Memorial Institute)-1640 (Sigma, St. Louis, MO, USA) containing 10% heat-inactivated FCS (fetal calf serum) (Sigma), 1 mM Pen (100U/ml)/Strep (100 ug/ml) and 2 mM L-glutamine (Sigma) and resuspended in R10 and counted using the Guava® ViaCount™ assay on the Muse Cell Analyzer (Luminex Cooperation). PBMCs were frozen and stored in liquid nitrogen before use.

96-well Multiscreen-I plates (Millipore, UK) were coated for 3 hours with 10 µg/mL GZ-4 anti-human IFN-γ (Mabtech, AB, Sweden) at room temperature. PBMC were added at 2×10^5 cells in 50 µL per well and stimulated with 50 µL of SARS-CoV-2 peptide pools (2 ug/mL per peptide) in duplicate. R10 with dimethyl sulfoxide (DMSO) (final concentration 0.4%, Sigma) was used as negative control and CEFT ((2 µg/mL, Proimmune)/ Concanavalin A (5 µg/mL final concentration, Sigma) were used as positive control antigens. After 16-18 hours at 37°C PBMC were removed and secreted IFN-γ detected using anti-IFN-γ biotinylated mAbs at 1 µg/mL (7-B6-1-biotin, Mabtech) for 2-3 hours, followed by streptavidin alkaline phosphatase at 1 µg/mL for 1-2 hours (SP-3020, Vector Labs). The plates were developed using

BCIP/NBT (5-bromo-4-chloro-3-phosphatase/ nitro blue tetrazolium) substrate (Thermo Scientific/Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. ELISpot plates were read using an AID ELISpot Reader (v.4.0). Results were reported as spot-forming units (SFU)/10⁶ PBMC. Background (mean SFU in negative control wells) was subtracted from antigen stimulated wells to give the final result.

Footnotes:

[3] <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563>

Results

Spike protein sequence differences in SARS-CoV-2 lineages.

The primary structure of the spike glycoprotein (S), and the characteristic sequence variants of the current three lineages of concern are illustrated in Figure 1. In this study, we analysed the homotypic neutralization of the prototypic, PANGO lineage B isolate, VIC001 (hereafter referred to simply as “B”), by mAbs, sera from convalescent individuals following SARS-CoV-2 infection, and recipients of the BNT162b2 (Pfizer) vaccine, which are each induced by prototypic S antigen. We then assessed heterotypic neutralization of two new VOC (B.1.1.7 and B.1.351). In Figure 1, we indicate the residues of S at which the respective lineage – as well as a third lineage of concern, P.1 – differ from lineage B.

Binding of antibodies to coronavirus proteins, and inhibition of ACE2-spike binding

We probed the antibody-binding properties of sera from vaccinated, convalescent and pre-pandemic control sera using a customised MSD coronavirus antigen immunoassay (Figure 2). We observed that sera from individuals receiving two doses of the Pfizer vaccine showed a non-significant increase in binding to SARS-CoV-2 spike and RBD compared to those receiving single dose and a significant difference from sera of convalescent individuals one month after infection (Figures 2A, and 2B, respectively, $p < 0.0001$ in all cases). The absence of N binding in vaccinees (Figure 2C) supports the designation of these individuals as SARS-CoV-2 unexposed, although it does not prove absence of previous infection.

There was significant antibody binding to both SARS-CoV-1 and MERS-CoV spike protein in vaccinated and COVID-19 convalescent individuals compared to the pre-pandemic control sera (Figures 2D & 2E, respectively). This was particularly marked for SARS-CoV-1 reactivity in fully vaccinated individuals,

suggesting that the vaccine can induce a broad response to widely shared epitopes, such as those exemplified by EY 6A³² and CR3022³⁶.

We also screened for antibody binding to the spike antigen of the four common circulating coronaviruses (Figure 2 F-I). There is a significant increase in binding to the *Betacoronavirus* clade A isolates, HCoV-HKU1 and HCoV-OC43, in vaccinated and COVID-19 convalescent sera ($p < 0.0001$) compared to unvaccinated naïve sera. Binding to the *Alphacoronavirus* isolates, HCoV-229E and, to a lesser extent, HCoV-NL63S was also greater in the vaccinees, but not in convalescent sera.

As a surrogate to neutralization, we assessed the ability of sera to inhibit ACE2-spike binding using MSD plates printed with spike proteins representing the prior circulating B lineage, and the more recently evolved VOC (B.1, B.1.1.7, B.1.351 and P.1). Figure 2J indicates that serum from vaccinated individuals receiving either single or double vaccination was able to inhibit ACE2 binding of SARS-CoV-2 spike. The inhibitory effect was significantly higher (>30 -fold, $p < 0.001$ by Mann Whitney comparison) in those sera derived from individuals receiving the boost vaccination compared to prime. Furthermore, sera from boosted individuals had a >3 -fold and 10-fold lower mean inhibitory activity for B.1.351 and B.1.1.7 respectively compared to the heterosubtypic B lineage spike. Following vaccine boost, the mean inhibitory activity of B differs significantly from B.1.351 and P.1 but not B.1.1.7 (Friedman test, $p < 0.0001$).

Neutralization by mAbs to the four epitopes of RBD and by reference serum

We made use of a panel of six, epitope-mapped neutralizing monoclonal antibodies (NmAbs, Figure 3A,) ^{34,35,37,38} in order to map the neutralization sensitivity of VOC to changes in RBD epitopes. We have devised a “squirrel” diagram to help visualise the binding sites of the various mAbs on the RBD (Figure 3A). One NmAb, FI 3A, a Class 1 RBD monoclonal antibody (binds to the left side of the head of the squirrel), whose homotypic IC₅₀ is of the order of 1 nM, is largely unaffected by the changes in B.1.1.7 (IC₅₀ = 1.365 nM) but does not neutralize B.1.351. Two other NmAbs, GR 12C and C121, that are Class 2 RBD binding mAbs (binding to the right side of the head of the squirrel), and which have homotypic IC₅₀ ~ 0.1 nM, show some reduced effectiveness in neutralizing B.1.1.7 and have lost almost all potency against B.1.351. This might be expected, as class 2 antibodies bind to an epitope that includes residue 484 (reviewed by Barnes et al and ³⁹). In contrast, NmAb FD 11A and S309, which are Class 3 RBD mAbs, that bind to the right haunch of the squirrel, and EY 6A, Class 4 monoclonal antibody, that binds to the left haunch of the squirrel, appears to be unaffected by the mutations in the VOC.

Polyclonal responses generated by different individuals to natural infection or in response to vaccination may include a varying proportion of antibodies to these and other neutralization epitopes. We also noted significant deviations in heterotypic neutralization potency against a currently approved reference serum 20/130 (NIBSC, Figure 3B). While homotypic NT₅₀ was 918.2 (95% CL 729.6 – 1,165), close to the result

of 1,280 quoted on the 20/130 data sheet, neutralization of B.1.1.7 was decreased to 125 (86 – 164), and of B.1.351 to 14 (0.1 – 51).

Neutralization by sera from convalescent COVID-19 individuals

Sera from convalescent individuals neutralized prototype B virus with highly variable potency (NT50 range <5 to 1,140, Figures 3C and 3E), though sera from those with mild symptoms were significantly more potent on average than those with asymptomatic infection (NT50 438.4 and 38.5, respectively, $P=0.002$).

Neutralization titres against B.1.1.7 were below the limit of detection in 9/12 asymptomatic convalescent individuals but were detectable in all those with mild symptoms. The neutralizing potency of mild convalescent sera against B.1.1.7 was significantly greater than that of asymptomatic sera (NT50 133 and 9.3, respectively; Kolmogorov-Smirnov test, $P = 0.0005$).

The decline in neutralization potency was more marked against the B.1.351 isolate, with convalescent sera from 12/12 asymptomatic and 7/12 mild having undetectably low neutralizing potency. Although there was no significant difference between the mean NT50 of mild versus asymptomatic sera against B.1.351 (119 and <5 respectively, $P = 0.25$), the reduction in potency overall in relation to prototype B virus was very significant ($P = 0.000003$)

Neutralization by sera from vaccine recipients.

After a single dose of BNT162b2 vaccine, homotypic neutralization potency was on average comparable to that of an asymptomatically infected cohort (NT50 53.8 and 38.5, respectively, $P=0.36$), but lower than sera from those who had recovered from mild infection (NT50 438.3, $P=.003$; see Figures 3D and 3E). Neutralization after one dose was undetectable against B.1.1.7 in 7/11 samples, and in all 11 sera tested against B.1.351.

Sera drawn between 7 and 17 days after a second dose of BNT162b2 vaccine - administered 18 to 28 days after the first - neutralized lineage B virus with high potency (average NT50 = 768) and 23/25 individuals had NT50 $>> 1/100$, Figure 3D), whereas 2/25 individuals showed more modest titres ($10 < \text{NT50} < 100$). These sera neutralized the B.1.1.7 isolate with a significantly lower potency (average NT50 = 320; $p < 0.0001$, Kolmogorov-Smirnov test); the same 23/25 had NT50 titres > 100 and 2/25 NT50 titres 10-100. The decline in neutralization potency against the B.1.351 isolate was further significantly reduced (NT50 = 171; $P = 0.000001$), but 12/25 retained NT50 titres > 100 , 11/25 NT50 10-100 with only the 2/25 with modest homotypic neutralization potency having undetectable heterotypic neutralizing potency.

The relationship of the neutralizing titre of each individual's serum to B to the corresponding titre against each variant apparent in Figure 3D is significant. Spearman correlation coefficients (r) are: 0.76 (B to B.1.1.7, CL 0.52 – 0.98; $P = 0.0000092$); 0.74 (B to B.1.351, CL 0.48 – 0.88. $P = 0.00002$); and 0.79 (B.1.1.7 to B.1.351, CL 0.57 – 0.91, $P = 0.000002$).

T cell responses to spike antigens in prototypic B strain and VOC

Following two doses of BNT162b2, spike-specific T cells were detected in all individuals against spike antigens covering the prototypic B strain, assessed in IFN- γ ELISpot assays peaking 7 days after the second vaccine (mean magnitude 561, range 110-1717 SFC/ 10^6 PBMC) (Figure 4A and S4). Spike specific T cells could not be detected in unvaccinated SARS-CoV-2 unexposed HCW (S3). T cells

Assessing the contribution of T cells that target epitopes located at the site of B.1.1.7, B.1.351 and P.1 spike mutation sites, we find that T cells target epitopes spanning mutation sites in 18/24 individuals (Figure 4B). In each individual, T cells targeted 0-19 (mean 6) epitopes located at mutation sites (Supplementary Table S2) with a total of 8, 9 and 10 epitopes targeted in lineage B.1.1.7, B.1.351 and P.1 respectively. The overall contribution of T cells targeting mutation regions to the total spike specific response is (mean and range) 13% (0-67%) for B.1.1.7, 14% (0-44%) for B.1. 351 and 10% (0-29%) for P1 (Figure 4C). Although the overall contribution of T cell responses to mutational regions/total spike responses was low, in general multiple individuals had T cells that targeted each of the mutational regions, spanning all spike domains (Figure 4D and Supplementary Table S2). T cell responses to total spike and mutation sites were further assessed in a small number of vaccinees after only a single vaccine; here low magnitude T cell responses were detected (Figure S5A), with T cells targeting mutational regions in 3/5 vaccinees (Figure S5B). Similar to post boost responses, the relative contribution of these to total spike was low (% mean contribution and range; 24% (2-34%) for B.1.1.7, 11% (0-20%) for B.1. 351 and 7% (0-23%) for P1) (Figure S5C).

Prediction of heterotypic neutralization by immunoassay

Authentic virus neutralization assays require specialist staff and facilities that are not widely available, and access to reference isolates of virus that are laborious to distribute. Accordingly, we asked whether high throughput ELISA-style immunoassays could provide a degree of predictive value for heterotypic neutralization following two vaccine doses. We performed Spearman non-parametric correlation analysis between the neutralization results, the spike-binding, and ACE2-spike binding-inhibition results obtained from the same sera, and the degree of T cell response to whole S protein determined by ELISPOT analysis from the same donors, as detailed in the foregoing sections.

The results (summary heatmap in Figures 5A and 5B, in table form in Supplementary Table 3) show that there is a consistently highly significant correlation ($P < 0.0001$) between both spike-binding and ACE2-

spike binding-inhibition activity and authentic virus neutralization. For example, the Spearman r between neutralization by serum of lineage B virus and the binding activity to lineage B RBD is 0.68 (95% CI 0.5 to 0.8, $n = 56$, $P = 1 \times 10^{-10}$), and the r between neutralization of lineage B.1.351 and binding to B RBD is 0.71 (0.5 to 0.8, $n=56$, $P = 8 \times 10^{-10}$). The correlation between neutralization and ACE2-spike binding-inhibition is, if anything, slightly stronger, with $r = 0.71$ (0.5 to 0.9, $n = 35$, $P = 4 \times 10^{-6}$) for lineage B, and $r = 0.79$ (0.6 to 0.9, $n = 35$, $P = 2 \times 10^{-8}$) for lineage B.1.351. (NB in this assay, the spike sequences correspond to the virus lineage in the neutralization assay.)

Interestingly, binding activity to SARS-CoV-2 S predicted binding to both SARS-CoV-1 S and MERS-CoV S very well ($r = 0.92$ (0.86 to 0.95), $n = 56$, $P = 2 \times 10^{-23}$; and $r = 0.55$ (0.3 to 0.7), $n=56$, $P = 9 \times 10^{-6}$). Moderate correlations (r of the order of 0.5) were seen with binding to the spike of endemic human betacoronaviruses and to the spike of alphacoronavirus OC43, but not to that of alphacoronavirus HCoV-HKU1. No significant correlations were observed between humoral and T cell responses (see Supplementary table 3c).

Discussion

Our results show that both binding and neutralization by antibodies induced by the S protein of prototypic lineage B is diminished to S from recent VOC; B.1.351 to a greater extent than B.1.1.7. This broad trend masks both qualitative and quantitative differences in antibody responses by individuals, whose serum may contain differing proportions of antibodies to neutralizing epitopes that we show here are sometimes conserved between lineages.

Given the cost and difficulty of authentic virus neutralization assays, it is encouraging that in our hands, both a high-throughput spike-binding assay and a spike-ACE2 binding-inhibition assay provide a significant correlation with the neutralizing potency – both homotypic and heterotypic – of human sera.

It is also reassuring to find that the majority of T cell responses in recipients of two doses of the BNT162b2 vaccine are generated by epitopes that are invariant between the prototype and two of the current VOC (B.1.1.7 and B.1.351). This data is compatible with a recent preprint report that the epitope sequences of the vast majority of SARS-CoV-2 T cell epitopes are not affected by the mutations found in the B.1.1.7 or B.1.351 variants⁴⁰, with no significant differences observed in CD4 and CD8 responses to a pool of S peptides corresponding to the ancestral sequence and those corresponding to the different variants. T cell responses to SARS-CoV-2 are known to target a wide range of regions in spike⁴¹. Consistent with this, our data show that neutralization of sera and T cell activity are independent¹⁶. Moreover, in over 90% of the recipients of two vaccine doses, heterotypic neutralizing titres (NT50) remain comfortably above the level associated with immune protection in non-human primate challenge studies^{13,16}. However, in a majority of individuals whose homotypic neutralization titres were more modest – including over 50% of convalescent COVID-19 individuals and recipients of a single dose of vaccine – heterotypic neutralization dropped to negligible levels. This loss of cross-neutralization was particularly notable against B.1.351 with potential implications for vaccine effectiveness in populations

where this VOC dominates and when only moderate levels of S antibodies are generated after vaccination.

It should be noted that neutralization escape, observed in a well of a micro-titre plate, is not direct evidence of vaccine failure. Non-neutralizing antigen-specific antibodies, T cells and innate lymphocytes clearly have the potential to contribute to vaccine efficacy⁴². The acceptance that prior infection with influenza virus results in reduced disease against subsequent infection with heterosubtypic strains, in both human and animal challenge studies, provides further evidence that cellular components and non-neutralizing antibodies make an important contribution to protection^{43–45}. We also note that the recent South African and UK vaccine clinical trials for Novavax reportedly showed 60 and 85.6% protective efficacy against infection for the B.1.351 and B.1.1.7 VOC respectively, with no cases of vaccinated individuals requiring hospitalization due to severe disease⁴⁶. Ongoing analysis of real-world vaccine roll out will illuminate the extent of vaccine breakthrough with VOC, although there is already evidence that two-dose regimen of AZD1222 does not protect against mild-to-moderate COVID-19 caused by B.1.351⁴⁷.

Nevertheless, our results re-emphasize the urgent need to deploy the most effective vaccine strategies as widely and rapidly as possible in order to provide population protection against the emerging lineages of concern of SARS-CoV-2. Our findings show clearly that the weaker responses generated for example by natural infection or single doses of vaccine, do not provide adequate cross-neutralization. The results support the recommendations by Pfizer, the FDA and EMA for a two-dose vaccine regimen.

Declarations

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Competing Interests:

The authors declare no competing interests.

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Data Availability:

Data relating to the findings of this study are available from the corresponding author upon request. Source data are provided with this paper.

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Figures

Figure 1: Sequence variation in spike glycoprotein

Figure 1

Sequence variation in spike glycoprotein. The open reading frame encoding spike is illustrated, with the position of key features of processing and function indicated to approximate scale (residue number indicated above). During co-translational translocation to the endoplasmic reticulum (ER), the short leader peptide (LP) is proteolytically removed. Following folding, trimer assembly and glycosylation in the ER and Golgi, the trans-Golgi localized protease, furin, cleaves the boundary between the S1 and S2

polypeptides. Following binding of the receptor-binding domain (RBD, cyan) to ACE2 on host cells, cell-surface TMPRSS2 proteolytically cleaves the S2' site, facilitating conformational changes to spike that result in fusion of the virus envelope with the plasma membrane. Variant residue positions are indicated below, and their approximate location on the S polypeptide is indicated. Residue identities are shown at each of these positions for a prototype lineage B isolate, and at each position in the three lineages of interest (B.1.1.7, B.1.351, and P.1) at which the respective lineage differs from prototype. Δ indicates deletion of one or more residues. Note, there are lineage-defining substitutions outside RBD, in the N-terminal domain (NTD) and C-terminal domain (CTD) of S1 (dark blue), and in S2 (tan). These may include changes that directly or indirectly affect antibody-mediated neutralization by loss or altered dynamics of epitope, respectively.

Figure 2. Binding Assays

Figure 2

Binding assays. IgG antibodies specific to; A-C SARS-CoV-2 (S, RBD, N), D-E SARS-CoV-1 S, MERS-CoV S, F-I HCoV-OC43 S, HCoV-HKU1 S, HCoV-229E S, HCoV-NL63 S, were measured using an MSD technology platform customised array. Sera analysed was from vaccinees (post-prime and post-boost), asymptomatic (mean 27 days post-PCR positive test, range 22-33 days) and mild COVID-19 convalescent sera (mean 29 days post-symptom onset, range 18-40 days) and a cohort of prepandemic sera collected between 2014 and 2018 negative for SARS-CoV-2 (negatives). Data are displayed as calculated

concentrations which use an MSD standard reference curve to interpret Arbitrary Units (AU). J. Inhibition analysis between ACE2 and recombinant spike from the designated homotypic and heterotypic lineages. Sera derived from individuals receiving prime or boost vaccination. Statistical difference between the groups was performed using a Kruskal-Wallis one-way ANOVA. Vaccinees post-prime n=11; vaccinees post-boost n=25; negatives n=103; asymptomatic COVID-19 convalescents n=11; mild COVID-19 convalescents n=62. The dashed lines in A-C show the cut-offs determined as the mean of negatives + 3SD. *p<0.05, **p<0.01, ***P<0.001, ****p<0.0001

Figure 3

Homotypic and heterotypic neutralization of key SARS-CoV-2 lineages by antibody. The potency of neutralization was determined by a focus-forming unit microneutralization assay against authentic virus of prototype B lineage and isolates of lineages B.1.1.7 and B.1.351. Detailed neutralization curves and confidence limits on the estimates of NT50 are given in the supplementary data. sera A. Neutralization by a panel of monoclonal antibodies binding to four distinct epitopes of RBD. (upper left) A space-filling model of prototype RBD (PDB 6YZ5) created in PyMOL, shows the residue of the mutations present in the B.1.1.7 and B.1.351 lineages in blue. (upper middle and upper right; same aspect and reverse aspect as the space-filling model, respectively) A cartoon of a “squirrel” we devised to illustrate the RBD. Markings on the squirrel cartoon show the epitopes of the RBD antibodies used in this study (classes 1 to 4 38). B. Neutralization by the international reference serum NIBSC 20/130 (see WHO/BS.2020.2403 Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody), nominally NT50 = 1/1,000. The mean number of foci ± SD relative to a no-antibody control is plotted against the reciprocal of the respective serum dilution (i.e. 103 corresponds to a 1/1000 dilution of serum). Data were fitted by non-linear regression in GraphPad Prism 9 to the Hill Equation, with TOP and BOTTOM constrained to 100% and 0%, respectively. Where a significant fit was obtained, it is represented

by a trend line on the respective plot, and NT50 values used in Figure S1 and main results. NT50 against B established in our assay indicated by the vertical dashed line with grey bars indicating the 95% confidence interval. C. Neutralization by convalescent sera from asymptomatic patients (left) and those with mild symptoms (right) against B, B.1.1.7 and B.1.351 isolates. D. Neutralization by sera from recipients of a single dose (left) and both prime and boost doses (right) of BNT162b2 vaccine. E. Homotypic and heterotypic neutralization potencies of the three sources of antibody against the three isolates, shown by individual and sub-population mean and SD of NT50 values (upper panel). For each isolate, pairwise comparisons of average NT50 estimates were made between groups of serum using the Kolmogorov-Smirnov non-parametric test. P values for the r statistic are shown (lower panel), both numerically and symbolically. $P > 0.05$ in green and “ns”. No results for $0.05 > P > 0.01$. $0.01 > P > 0.001$ in yellow and **. $P < 0.001$ in red and ****.

Figure 4. IFN- γ ELISpot responses to B strain, Δ B.1.1.7, Δ B.1.351 and Δ P.1 pools after 2nd dose of BNT162b2 vaccine

Figure 4

ELISpot responses to prototype, B.1.1.7, B.1.351 and P.1. T cell responses were measured using IFN- γ ELISpot assays in 24 healthy volunteers, 7-17 days after receiving the 2nd dose of BNT162b2. A. T cell responses to 15-18-mer peptides in B strain overlapping by 10 amino-acids and spanning the entire spike region. B. Summed T cell responses to peptides from B strain that mapped to sites with mutations in B.1.1.7 (n=17 peptides), B.1.351 (n=21 peptides) and P.1 (n=22 peptides). C. Percentage contribution T

cells (using B peptides) that target mutational regions within B.1.1.7, B.1.351 and P.1, relative to the total T cell spike response in each of the 24 volunteers. D. T cell responses to 22 individual peptides in B strain that have corresponding mutations in B.1.1.7, B.1.351 and P.1 variants. Each bar represents one volunteer with a positive response (defined as a response to the peptide minus the background that was greater than twice the background). SFC/106 PBMC = spot forming cells per million peripheral blood mononuclear cells, with background subtracted.

Figure 5 Cross-correlation of immune parameters following two vaccine doses.

Figure 5

Cross-correlation of immune parameters following two vaccine doses. For each serum, pairwise Spearman correlation analyses were undertaken between the value of binding of serum antibody to coronavirus antigens, the ACE2-spike binding-inhibition potency (see Figure 2), and the homotypic and heterotypic neutralizing titre of the same sera (see Figure 3). A. Heatmap of Spearman's r parameter for each comparison in which spike binding data was available ($n = 56$). Colour mapping is dual gradient from Blue ($r = 1.0$) through White ($r = 0.5$) to Red ($r = 0$). Values outside this range are Black. B. Heatmap Spearman's r parameter for each comparison in which ACE2-spike binding-inhibition data were available ($n = 35$). Colour mapping as in A.

Supplementary Files

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