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SARSCoV2 vaccines elicit differential Fc effector functions

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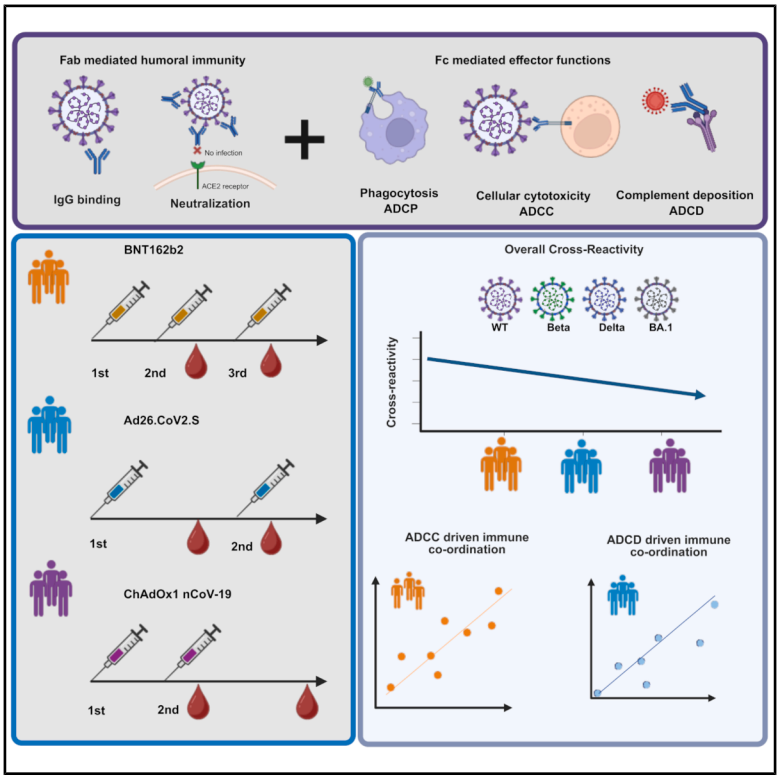
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Article

SARS-CoV-2 vaccines elicit differential Fc effector functions

Graphical abstract



Highlights

- More robust neutralization and Fc effector functions in BNT162b2 than adenoviral regimens
- BNT162b2 triggers more cross-reactive Fab and Fc mediated functions
- Co-ordination between immune functions varies by vaccine modality
- Immune co-ordination is driven by ADCC in BNT162b2, but ADCD in Ad26.CoV2.S

Authors

Nelia P. Manamela, Boitumelo M. Motsoeneng, Holly Spencer, ..., Shabir A. Madhi, Penny L. Moore, Simone I. Richardson

Correspondence

simoner@nicd.ac.za

In brief

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SARS-CoV-2 vaccines elicit differential Fc effector functions

Nelia P. Manamela,[1,2] Boitumelo M. Motsoeneng,[1,2] Holly Spencer,[1,2] Tandile Hermanus,[1,2] Nonkululeko Mzindle,[1,2] Frances Ayres,[1,2] Zanele Makhado,[1,2] Rudolph Serage,[1,2] Glenda G. Gray,[3] Linda-Gail Bekker,[4,5] Shabir A. Madhi,[6] Penny L. Moore,[1,2,7] and Simone I. Richardson[1,2,8,*]

[1]South African Medical Research Council Antibody Immunity Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2]Centre for HIV and STIs, National Institute for Communicable Diseases of the National Health Laboratory Services, Johannesburg, South Africa
[3]The South African Medical Research Council, Tygerberg, South Africa
[4]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa
[5]The Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa
[6]South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[7]Centre for the AIDS Programme of Research in South Africa, Durban, South Africa
[8]Lead contact
*Correspondence: simoner@nicd.ac.za
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SUMMARY

In addition to neutralization, Fc effector function contributes to vaccine-mediated protection against severe SARS-CoV-2 infection. However, differential coordination and cross-reactivity of Fc effector functions across vaccines is not clearly defined. A secondary analysis of three vaccines with BNT162b2, Ad26.CoV2.S (NCT04838795), and ChAdOx1 nCoV-19 (NCT04444674) reveals that BNT162b2 elicits the highest levels of binding, neutralization, and Fc effector function with antibody-dependent cellular cytotoxicity (ADCC), phagocytosis (ADCP), and complement deposition (ADCD) significantly differing by vaccine modality. Ad26.CoV2.S showed low ADCD activity before a homologous boost, which significantly increased this function. BNT162b2 vaccination triggered higher levels of cross-reactive neutralization, ADCC and ADCP than the adenoviral vaccines, while ChAdOx1 nCoV-19 exhibited higher ADCD cross-reactivity. Coordination between functions all varied by modality, with vaccination eliciting neutralization and binding correlations with ADCC for BNT162b2, but with ADCD following Ad26.CoV2.S vaccination. This supports the optimization of Fc effector functions for improved polyfunctional vaccine design.

INTRODUCTION

The emergence of variants of concern (VOC) during the SARS-CoV-2 pandemic resulted in significant evasion of neutralizing antibody responses.[1–6] Despite this, and the fact that neutralization is a known correlate of vaccine elicited protection,[7–11] vaccines still prevent severe disease.[12–14] This can be attributed, in part, to the persistence of T cell induced immunity[15] and humoral factors beyond neutralization despite continued viral evolution. Binding antibodies correlate with protection from severe disease even when neutralizing titers are reduced.[10,16] Moreover, while unable to protect through blocking mechanisms, non-neutralizing binding antibodies can elicit protective cytotoxic functions that are mediated by the engagement of antibody fragment crystallizable (Fc) regions with complement proteins or Fc receptors on the surface of effector cells.

Fc effector functions including antibody-dependent cellular phagocytosis (ADCP) or engulfment, antibody-dependent cellular cytotoxicity (ADCC) or lysis of target cells and complement deposition (ADCD) are largely retained against VOCs.[17–19] These functions are associated with reduced COVID-19 disease severity and mortality and are more durable compared to neutralization following infection.[20–23] Fc effector function is also required for monoclonal antibodies to optimally protect from infection.[11,24–28] Furthermore, vaccine elicited Fc effector functions contribute to protection from SARS-CoV-2 infection in animal models.[29–36]

During the COVID-19 pandemic, several vaccines including ChAdOx1 nCoV-19, BNT162b2,[37,38] and Ad26.COV2.S[39] were deployed worldwide to prevent infection with protection against D614G SARS-CoV-2 infection ranging between 57 and 95%.[40–43] ChAdOx1 nCoV-19 is a chimpanzee adenovirus vectored vaccine, Ad26.COV2.S, a human adenovirus type 26 vectored vaccine and BNT162b2 an mRNA vaccine which all encode a full-length, membrane-bound SARS-CoV-2 spike protein in a prefusion conformation.[39,44–46] Despite utilizing different

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platforms, all three vaccines elicited potent and durable Fc effector functions.[17,47–50] However, several studies have shown subtle differences in Fc effector and isotype elicitation across vaccines, even between BNT162b2 and mRNA-1273, for which neutralizing titers were similar.[51] These data further suggested that these platforms elicit antibodies preferentially targeting different sites; with RBD-specific antibody depletion resulting in a loss of ADCP activity for BNT162b2 vaccinees but not mRNA-1273–immunized individuals.[52] In addition, similar to neutralization capacity, ADCC activity was significantly lower in the whole inactivated virus vaccine BBIBP-CorV and ChAdOx1-nCoV-19 vaccination compared to BNT162b2.[53] While several studies have attributed these differences to modality and dosing schedule, impact on co-ordination and Fc effector breadth across VOCs have not been investigated.

In this study, we show that different vaccine modalities and doses trigger unique Fc effector function levels, breadth, and coordination. Both Fc effector function and breadth was highest in BNT162b2 for neutralization, ADCC, and ADCP, while ChAdOx1 nCoV-19 showed the greatest degree of cross reactivity for ADCD against VOCs. BNT162b2 antibody binding and neutralization was coordinated with ADCC, while in Ad26.CoV2.S vaccinees these correlated with ADCD. These data suggest that mode of delivery of vaccination and dosage influence Fc effector responses. This has important implications in the design of future vaccines that can be modified to elicit a potent Fc effector response.

RESULTS

The BNT162b2 vaccine elicits higher binding antibody titers and neutralization activity compared to Ad26.CoV2.S and ChAdOx1 nCoV-19 vaccines

To assess the differences in humoral responses elicited by mRNA or adenoviral vector based SARS-CoV-2 vaccines, we assayed plasma from individuals vaccinated with either two doses of BNT162b2 (n = 13), a single dose of Ad26.CoV2.S (n = 13) or two doses of ChAdOx1 nCoV-19 vaccine (n = 23) as primary immunizations as per the vaccine dose recommendations, by the South African government during that period (Figure 1; Table S1).

Using ELISA, we compared the capacity of vaccine-elicited plasma IgG antibodies to bind ancestral (D614G) spike protein. In line with previous studies,[54–57] one dose of Ad26.CoV2.S elicited significantly lower D614G-binding antibody levels (geometric mean titer (GMT) 1.4) compared to two doses of BNT162b2 (GMT 2.9) and ChAdOx1 nCoV-19 (GMT 2.0) two months post vaccination (Figure 2A). This is likely because the primary vaccination schedule for Ad26.CoV2.S includes only a single dose, unlike the two-dose regimens of BNT162b2 and ChAdOx1 nCoV-19. Following the third homologous dose of BNT162b2 vaccine, binding antibody titers significantly increased (p value = 0.02) (Figure 2B). Spike-specific IgG induced by one dose of the Ad26.CoV2.S vaccine was most significantly boosted, compared to all other vaccines, by an additional homologous dose. In contrast, a second ChAdOx-1 nCoV-19 dose did not significantly increase binding antibody titers (Figure 2B). Furthermore, four months following the second dose, ChAdOx-1 nCoV-19- elicited IgG binding antibodies waned significantly (Figure 2B).

Two months following BNT162b2 primary vaccination, neutralization titers against the ancestral (D614G) variant, as measured using a lentiviral pseudovirus assay, were 19 and 28-fold higher compared to Ad26.CoV2.S and ChAdOx1 nCoV-19 vaccinees (Figure 2C), in line with previous studies.[55–59] Despite significantly higher binding antibodies following ChAdOx-1 nCoV-19 compared to Ad26.CoV2.S vaccination (Figure 2A), neutralization was comparable between these modalities (GMT 143 compared to 99) (Figure 2C). For all vaccines, neutralizing antibody titers increased following additional homologous doses with the greatest fold increase (6.2) following a second dose of Ad26.CoV2.S (Figure 2D). Similar to binding antibodies, neutralizing titers elicited by ChAdOx-1 nCoV-19 significantly waned over 6 months to titers that were1.4 times lower than those following the first dose (Figure 2D). Overall, these data confirm the mRNA BNT162b2 vaccine elicits significantly better binding and neutralizing antibody titers than the adenoviral platforms tested.

Fc effector functions vary significantly by SARS-CoV-2 vaccine modality and are boosted in response to additional vaccine doses

As Fc effector functions are associated with vaccine elicited protection in animal models,[17,35,49,52] we investigated how these varied by vaccine modality. Two months after primary vaccination, all Fc effector functions were highest in BNT162b2 vaccinees compared to Ad26.CoV2.S and ChAdOx1 nCoV-19 vaccinees (Figures 3A–3C). The ADCC response was similar in Ad26.CoV2.S (Median RLU 1718) and BNT162b2 vaccinees (Median RLU 2218) with significantly lower function elicited by ChAdOx-1 nCoV-19 (Median RLU 932) compared to BNT162b2 (Figure 3A). This was in contrast to ADCP where Ad26.CoV2.S and ChAdOx-1 nCoV-19 elicited antibodies showed similar activity (Median ADCP score 19.6 and 18.4 respectively), and both were significantly lower than BNT162b2 (Median ADCP score 34.9) (Figure 3B). BNT162b2 induced significantly higher ADCD activity than all adenoviral vaccines (Figure 3C), with Ad26.CoV2.S vaccination showing low activity following one dose. In addition, each of these functions showed distinct patterns between the modalities, which did not match overall SARS-CoV-2 spike binding titers. This illustrates that measurements of binding antibody titer alone does not always mirror Fc effector activity.

We next explored the effect of an additional homologous dose on Fc effector function for each vaccine. All Fc effector functions were significantly enhanced 2– to 3-fold by a third dose of BNT162b2 (Figures 3D–3F). ADCC and ADCP was preserved, with ADCD activity increased 31-fold following a second Ad26.CoV2.S dose (Figure 3F). This ADCD was similar to activity elicited after two doses of BNT162b2 and ChAdOx-1 nCoV-19, indicating that two doses of this vaccine are required to elicit detectable ADCD. ChAdOx-1 nCoV-19 vaccinees also showed significantly boosted levels of ADCC responses following an additional homologous dose, with non-significant increases in ADCP and ADCD (Figures 3D–3F). However, overall the ADCC and ADCD activity waned substantially after 6 months while

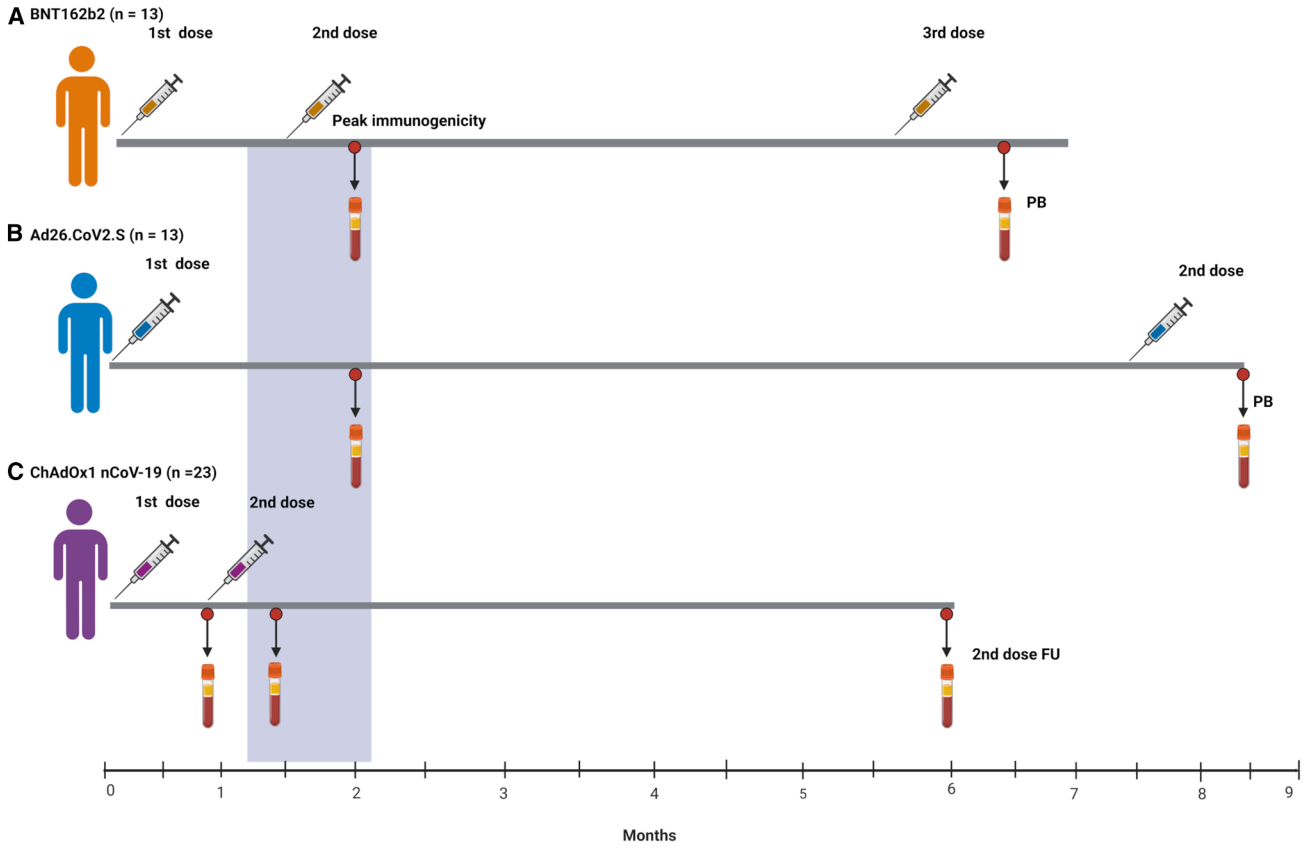


Figure 1. Dosing and sampling strategy of individuals vaccinated with BNT162b2, Ad26.COV2.S and ChAdOx1 nCoV-19

(A) BNT162b2 participants (orange, *n* = 13) were vaccinated using a two-dose strategy with first dose at day 0, second dose after 42 days, and boosted with an additional third dose after 5.5 months. Participants who received the BNT162b2 vaccine were sampled after receiving a full primary vaccination i.e., 2 doses, and 1 month post boost (PB). The post full primary vaccination blood samples were taken 2 months after the initial dose, which translated to a month following their second dose. Although the primary vaccination sampling period was a month less than the other vaccine groups, these samples were regarded as peak immunogenicity because they were taken following the full 2 dose primary vaccination strategy recommended for BNT162b2.

(B) Ad26.CoV2.S (blue, *n* = 13) was administered as one primary dose received at month 0 and individuals were subsequently boosted 7.5 months after the initial dose. The participants were sampled 2 months after the first dose and 1 month after the second dose.

(C) ChAdOx1 nCoV-19 trial participants (purple, *n* = 23) were vaccinated with a two-dose primary strategy at month 0 and then 1 month thereafter. The individuals were sampled 1 month post first dose, 2 weeks after the second dose regarded as a full primary vaccination sample. and 6 months after they received the first dose defined as a second dose follow-up (2nd dose FU). Samples highlighted with gray shading were used to compare the vaccine modalities throughout the study, including samples from after a full primary dose for each vaccine, approximately 2 months post 2nd dose for BNT162b2, 2 months post 1st dose for Ad26.COV.2.S, and 2 months post the initial dose for ChAdOx1 nCoV-19. Additional characteristics of the different vaccine trial participants have been included in Table S1.

ADCP was mostly preserved. Interestingly, a few participants showed some increases in ADCP activity (Figures 3D and 3E), despite no detectable nucleocapsid titers that would indicate infection. Overall, these data show that vaccine modalities and doses elicit distinct Fc effector responses and that ADCP, ADCC, and ADCD elicitation following a specific vaccine is uniquely modulated.

Neutralization and Fc effector function cross-reactivity differ by vaccine

The ability of a vaccine to trigger antibody responses against SARS-CoV-2 VOCs is critical in the context of ongoing evolution of the virus. We compared the capacity of antibodies triggered by the three vaccine platforms to mediate neutralization, ADCC, ADCP, and ADCD against VOCs including Beta, Delta, and BA.1 2 months following vaccination (Figures 4A–4H).

Neutralization was most significantly compromised against BA.1 across all vaccine platforms, as reported previously,[60–62] with the highest neutralization activity detected against D614G, the closest variant match to the immunogens (Figures 4A and Figure S1A). BNT162b2 triggered substantially higher overall cross-reactive neutralization breadth than the Ad26.CoV2.S (12.7-fold) and ChAdOx1 nCoV-19 (6.5-fold) vaccines (Figures 4A and 4B), with the highest median neutralization titers against all VOCs observed for BNT162b2 (Figure S1A). BNT162b2-triggered ADCC activity was less sensitive to mutations in Beta and Delta compared ADCC triggered by other vaccines (Figure 4C), while all three vaccines elicited antibodies with

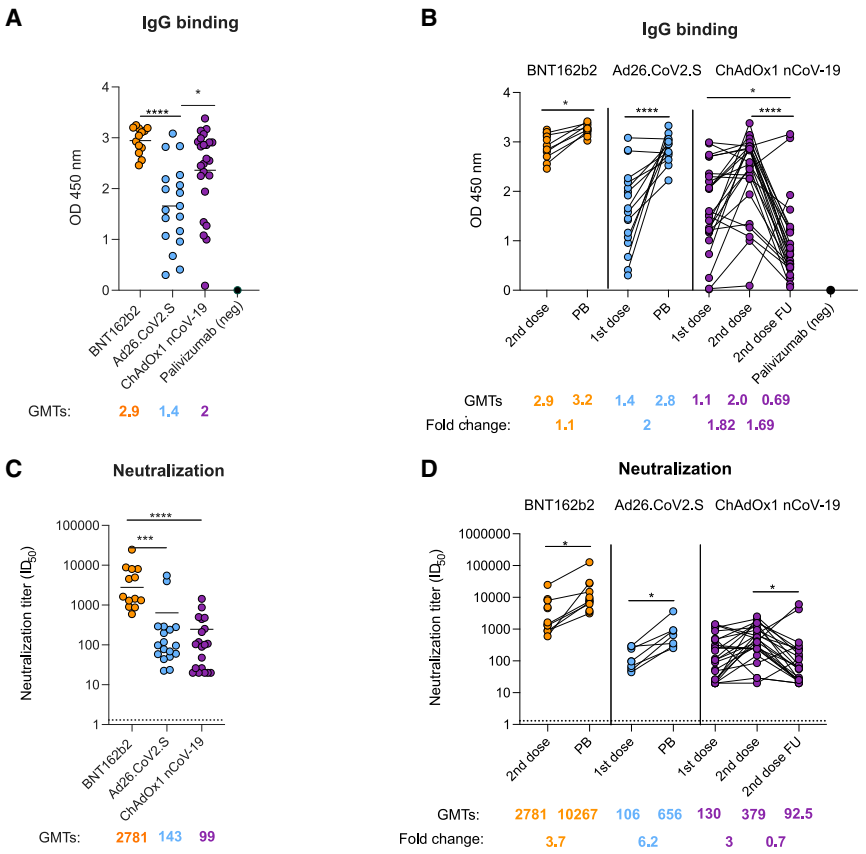


Figure 2. Binding and neutralizing antibody responses across vaccine modalities

Binding antibody titers against ancestral D614G tested by ELISA (A) approximately 2 months following the first vaccine dose and (B) longitudinally over time (1 month post boost (PB) and 6 months 2nd dose follow-up (2nd dose FU). Neutralization of ancestral (D614G) pseudovirus (C) approximately 2 months following the first dose and (D) over time (1 month post boost (PB) and 6 months 2nd dose follow-up (2nd dose FU). BNT162b2 vaccinees are shown in orange, Ad26.CoV2.S in blue and ChAdOx1 nCoV-19 in purple. Ad26.CoV2.S vaccinees with nucleocapsid ELISA confirmed breakthrough infections 2 months before they acquired a booster dose, were excluded from further analysis in Figures 2B and 2D, to prevent the influence of hybrid immunity. Horizontal lines indicate geometric mean binding or neutralization titers (GMT), with change in vaccine elicited neutralization before and after boost represented as a fold change between post boost and pre-boost GMT. Palivizumab (black), the negative control is included, with a dotted line on the y axis indicating the positivity cut-off for binding. The detection limit of ID50 of 20, shown as a dotted line, marks the limit of detection for neutralization. The HIV-specific antibody VRC01 was routinely included as a negative control for neutralization assays. Statistical analyses were performed using the Kruskal-Wallis test with Dunn's multiple comparisons correction across vaccine platforms. Matched comparisons within BNT162b2 and Ad26.CoV2.S vaccine groups over time was analyzed using a Wilcoxon T test while ChAdOx1 nCoV-19 analysis was done using Friedman's test with Dunns's multiple comparisons correction where $^{*}p < 0.05$,$^{**}p < 0.01$ $^{***}p < 0.001$ and $^{****}p < 0.0001$.

similarly compromised ADCC against BA.1 with only significant losses following BNT162b2 and Ad26.CoV2.S vaccination relative to D614G (Figures 4C and Figure S1B). However, compared to neutralization, ADCC breadth was more comparable across vaccines less than 3-fold reduced in the adenoviral vaccines relative to BNT162b2 but between 6 and 12-fold reduced in neutralization breadth. Unlike ADCC and neutralization, BNT162b2-elicited ADCP breadth was similar across all variants tested indicating the resilience of this function in the face of VOCs (Figure 4E) but did show significant decreases in ADCP score against Beta and Delta, similar to Ad26.CoV2.S with ChAdOx nCoV-19 showing only comprised activity against BA.1 (Figure S1C). This further reflects slightly different targeting elicited by different vaccine platforms. Similar to ADCC and neutralization, Ad26.CoV2.S and ChAdOx nCoV-19 vaccines showed similar levels of ADCP breadth (Figures 4E and 4F). BNT162b2 vaccinees showed comparable levels of ADCD activity compared to ChAdOx nCoV-19 against all variants except Beta (4-fold lower) (Figure 4G). Further, BNT162b2 and ChAdOx1 nCoV-19 vaccinees had higher ADCD against all variants tested (ranging between 6 and 95-fold) compared to Ad26.CoV2.S, translating to the lowest ADCD cross reactivity following Ad26.CoV2.S vaccination (9.5-fold lower than BNT162b2) and the lowest ADCD level across all variants tested (Figures 4G, 4H, and S1D). These data show that BNT162b2 vaccination triggers polyfunctional antibodies with more cross-reactive neutralization, ADCC and ADCP responses than the two adenoviral vaccines tested. Overall, the type of vaccine influences not only levels but cross reactivity of both neutralization and Fc effector function.

Different vaccine modalities induce distinct correlations between antibody functions

A well-coordinated Fc effector response is associated with control and vaccine efficacy against several viruses including SARS-CoV-2 and HIV.[63–69] We therefore examined the association between D614G-specific Fc effector function, binding and neutralization across vaccine platforms 2 months following primary vaccine dosage. BNT162b2-elicited plasma showed significant positive correlations between neutralization, ADCC and binding ($r > 0.7$) (Table S2), with weak but negative correlations between these factors and ADCD ($r < -0.3$) (Figure 5A; Table S2). In contrast, vaccination with one dose of Ad26.CoV2.S elicited significant and strong correlations between neutralization, binding and ADCD ($r > 0.7$) (Figure 5B) Thus, despite ADCD being of low magnitude compared to other vaccine modalities, this activity is well coordinated with both the binding and neutralizing capacity of these antibodies. ChAdOx1 nCoV-19 vaccination showed a

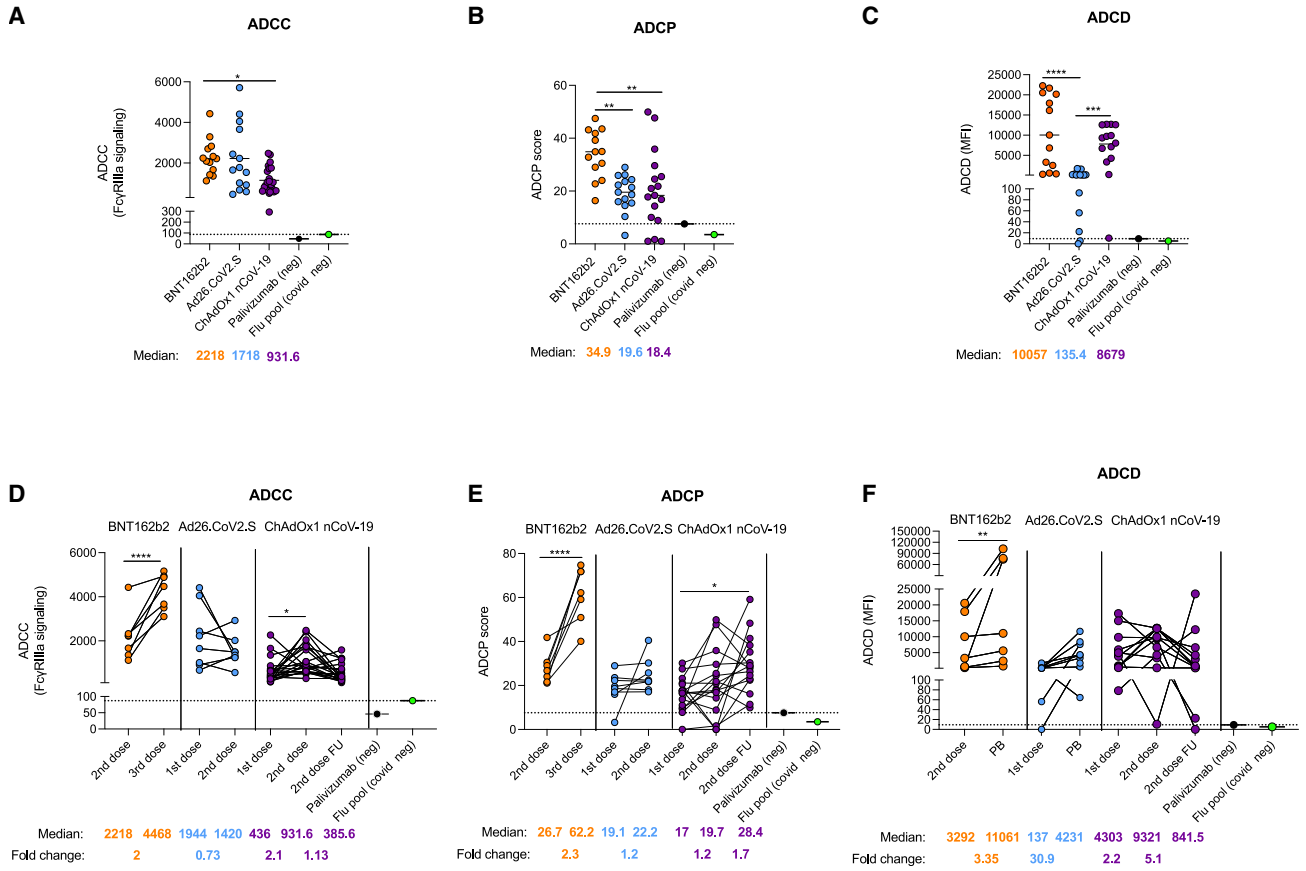


Figure 3. BNT162b vaccination triggers Fc effector responses with enhanced magnitude compared to Ad26.CoV2.S and ChAdOx1 nCoV-19

Fc effector functions against ancestral D614G were measured approximately 2 months following vaccination with BNT162b2 (orange), Ad26.CoV2.S (blue) or ChAdOx1 nCoV-19 (purple).

(A) Antibody-mediated cellular cytotoxicity (ADCC) potential against the ancestral D614G variant is represented as relative light units (RLUs) triggered through activation of FcγRIIIa receptor on CD16 Jurkat cells, (B) Antibody dependent cellular phagocytosis (ADCP) is shown as the percentage of THP-1 cells that take up spike-coated beads multiplied by their geometric mean fluorescence intensity (MFI) (C) Antibody dependent complement deposition (ADCD) is illustrated as the Median Fluorescence Intensity (MFI) of the C3 protein deposited on spike coated beads. The horizontal lines represent the medians.

(D) ADCC, (E) ADCP and (F) ADCD against ancestral D614G were also measured over time, 1 month post boost (PB) and/or 6 months post first dose (2nd dose follow-up FU). Median ADCC, ADCP and ADCD per vaccine, are shown below the graphs with fold changes relative to the first dose. Negative controls, palivizumab (black) and a SARS-CoV-2 naive sera (referred to as the "flu pool", green) — are included, with a dotted line on the *y* axis showing the positivity cut-off for each assay. The statistical analysis between vaccine groups per function, was performed using a Kruskal-Wallis test with Dunn's multiple comparisons test, while over time comparisons within a BNT162b2 and Ad26.CoV2.S were done using a Wilcoxon test and a Friedman's test with Dunn's multiple comparisons was used to determine statistical significance over time for the ChAdOx1 nCoV-19 vaccinees. $^{*}p < 0.05$,$^{**}p < 0.01$ $^{***}p < 0.001$.

poorly coordinated vaccine response with no significant associations between any antibody functions (Figure 5C). Despite not being significant, ChAdOx1 nCoV-19 vaccination induced a negative correlation between ADCD and neutralization similar to BNT162b2 (Figures 5A and 5C; Table S2) and a positive correlation between binding and ADCD (spearman r = 0.5) similar to Ad26.CoV2.S. Overall, Ad26.CoV2.S showed the most positive correlations between antibody functions, despite lower levels of antibody responses compared to BNT162b2.

DISCUSSION

Neutralization and Fc effector functions have been associated with SARS-CoV-2 vaccine protection.[18,30,32,35,70–72] Furthermore, various studies have shown the magnitude and cross-reactivity of vaccine-elicited neutralization activity varies by vaccine platform.[54–57] However, it is unclear how these different vaccine modalities affect the elicitation of Fc effector functions. We demonstrate that different vaccines elicit significantly different Fc effector function levels with BNT162b2 triggering the highest and broadest ADCC, ADCD, and ADCD responses. Against VOCs, each platform triggered unique Fc effector response profiles, showing the resilience of some functions (particularly ADCP and ADCC) over others against viral mutation. Additional dosages of homologous vaccines boosted binding, neutralization and Fc effector function with one dose of Ad26.CoV-2 being insufficient to elicit measurable levels of ADCD. Despite eliciting lower Fc effector levels, Ad26.CoV2.S showed

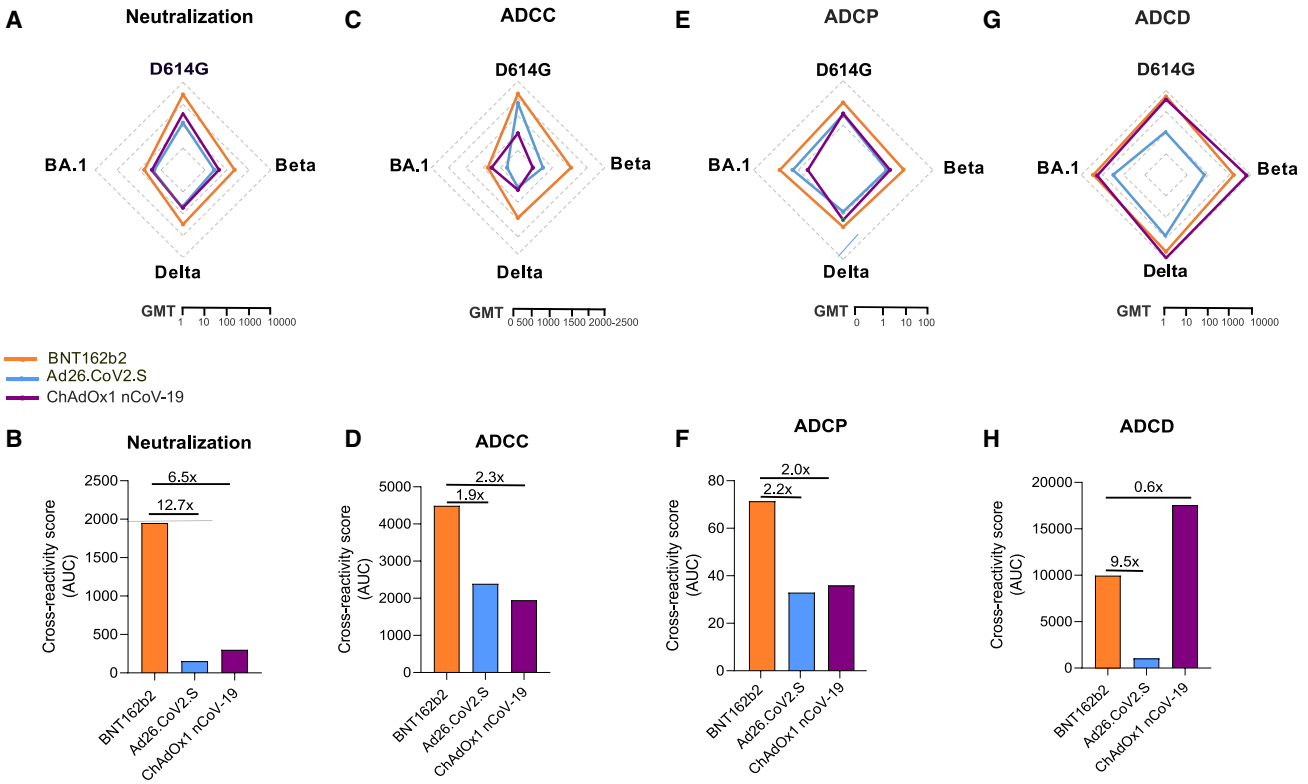


Figure 4. Vaccines elicit unique patterns of neutralization and Fc effector cross-reactivity against VOCs

(A) Spider plots demonstrating vaccine elicited neutralization of D614G, Beta, Delta, BA.1 pseudovirus shown as a geometric mean titers (GMT) following 2 doses of BNT162b2 (orange), a single dose of Ad26.CoV2.S (blue), and two doses of ChAdOx1 nCoV-19 (purple).

(B) Neutralization cross-reactivity scores calculated as a cumulative area under the curve or spiderplot (AUC) are shown.

(C) Antibody-mediated cellular cytotoxicity (ADCC) potential against the ancestral D614G, Beta, Delta, and BA.1 variants is represented as a geometric mean (GM) relative light units (RLUs), (D) overall ADCC cross-reactivity as AUC generated from C.

(E) Antibody dependent cellular phagocytosis (ADCP) represented as a GM ADCP score and (F) overall ADCP cross-reactivity as AUC generated from E.

(G) Antibody dependent complement deposition (ADCD) against D614G, Beta, Delta, and BA.1 is illustrated as GMT of the median Fluorescence Intensity (MFI) of the C3 protein deposited on beads and (H) overall ADCD cross-reactivity as AUC generated from G. Fold changes in cross-reactivity are indicated relative to BNT162b2 vaccination. Scale bars have been added underneath the spider plots (A, C, E, and G) for each antibody function to indicate the intensity of cross-reactivity. The level of cross-reactivity shown as a geometric mean for each variant, increases from the inner dotted grid lines to the outer grid lines of the spider plots for each antibody function. The level of cross-reactivity shown as a geometric mean for each variant, increases from the inner dotted grid lines to the outer grid lines of the spider plots for each antibody function. The cross-reactivity score shown in Figure (B, D, F, and H) is a single cumulative measure showing the total area under curve (AUC) for each vaccine group as a single measure of breadth or cross-reactivity per function. Figure S1 containing additional information on cross-reactivity of neutralization and Fc effector functions against multiple SARS-CoV-2 variants has been included in support of Figure 4.

the most coordinated humoral response of all the platform, while BNT162b2 elicited an ADCC centric co-ordination. Therefore, vaccine modalities elicit quantitatively different responses and degree of co-ordination. Ultimately, varying number of doses and type of vaccine platform (even with identical immunogen sequence) can tune Fc effector responses in addition to neutralization and binding.

Previous studies have similarly reported mRNA vaccines trigger higher antibody titers, Fc receptor binding profiles and ultimately higher Fc effector functions than vectored vaccines, levels which are influenced by intervals post primary vaccination and doses.[73,74] Here, we observed varied levels of Fc effector functions across vaccine platforms in addition to binding and neutralizing responses. In line with previous studies,[54–57] the mRNA-based BNT162b2 vaccine elicited significantly more potent binding and neutralization responses than the adenoviral vaccines Ad26.CoV2.S and ChAdOx1 nCoV-19. This has been widely attributed to the mechanism of immunogen delivery with mRNA vaccines able to induce stronger antigenic stimulation than vector based platforms, in part because they are easily recognized by cell surface, endosomal, and innate immune receptors.[75] This observation extended to Fc effector function where BNT162b2 elicited the highest levels of ADCC, ADCD, and ADCP. While ADCC significantly correlated with IgG titer in BNT162b2 vaccinees, none of the other functions were correlated, suggesting that higher antibody titer alone did not explain elevated Fc effector function.

The association of mRNA vaccines with higher humoral immune responses suggests that antigen exposure levels are a crucial factor in vaccine-induced immunity. Our data support

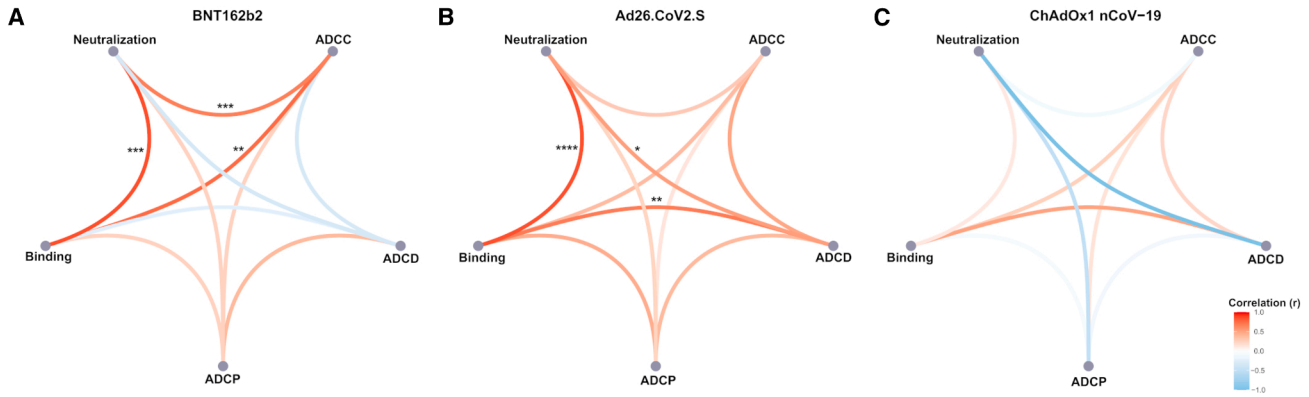


Figure 5. Vaccine platforms induce differential coordination of binding, neutralization and Fc effector functions

(A–C) Edge bundling plots showing Spearman's correlations between neutralization, binding and Fc effector functions against D614G, approximately 2 months post primary vaccination schedule with two doses of BNT162b2 (A), one dose of Ad26.CoV2.S (B) and two doses of ChAdOx1 nCoV-19 (C). Red lines represent positive correlations while blue lines show negative correlations with intensity of the color indicating the strength of the correlation. Statistically significant correlations are indicated with asterisks, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and a comprehensive Table S2 has been included to indicate p-values and confidence intervals for all the correlations in support of Figure 5.

this association even with vectored vaccines, with ADCD poorly induced following one dose of Ad26.CoV2.S, but reaching levels comparable to those seen after two doses of BNT162b2 and ChAdOx1 nCoV-19 after boosting. This illustrates the need for a two dose Ad26.CoV2.S regimen to elicit ADCD, in contrast to ADCP and ADCC which were robustly elicited by one dose. This suggests that different Fc effector functions may require different thresholds of antigen presentation to elicit potent responses.

Modulators of Fc effector function are known to be differentially altered by vaccination. Factors including isotype and Fc glycosylation,[76,77] not examined here, likely play a significant role in the differential imprinting of Fc effector function across vaccine modalities. For example, afucosylation of Fc portions which result in potent ADCC activity[78] has been shown to transiently increase in previously uninfected BNT162b2 vaccinees,[79,80] plausibly contributing to observed differences in Fc functionality between vaccine groups. However, a recent study suggested that this was comparable for both mRNA and adenoviral vectored vaccines,[77] all of which result in host membrane embedding of the antigen, which is thought to be a major determinant of afucosylation.[81] Intriguingly, others have found that decreasing galactosylation appeared more frequent following Ad26.CoV2.S compared to either BNT162b2 or ChAdOx1 nCoV-19.[77] As increased galactosylation elevates the potential of IgG to activate the classical complement pathway via increased binding to C1q, this may also have contributed to the poor ADCD activity elicited by Ad26.CoV.2.S in our study. Isotype and IgG subclass elicitation has not been shown to be significantly different between vaccine platforms,[82,83] with the exception of increased levels of IgG4 noted post a third boost of BNT162b2 likely as a result of high and continuous antigenic stimulation in this platform.[76,84] While previously reported to result in lower Fc effector functions,[76] our study revealed no such effect, with significant boosting following a third dose. Since our mRNA cohort consisted of significantly older participants (average 70 years) it is possible that boosting of these functions differs in this age group.

The differences in Fc effector against VOCs shown in this study suggest that different vaccine modalities elicit antibodies that target spike in varied ways. Indeed, we previously showed in individuals previously infected with D614G, that loss of activity against Beta was not equivalent for all functions, with ADCD most affected.[17] RBD, is a major target for complement binding in both vaccinated and convalescent individuals[85] and RBD depletion greatly decreased antibody-dependent neutrophil phagocytosis (ADNP) activity in contrast to ADCP, which remained unaffected in convalescent sera.[83] Additionally, RBD specific mAbs have limited ability to cross-link FcγRIIa,[11] contributing to poor ADCP activity. Although we have shown that immunogen sequence imprints level and breadth of Fc effector function following infection,[17] the vaccines studied here utilized the same spike sequence. However, the immunogens have different stabilization, with Ad26.CoV2.S and BNT162b2 stabilized with two proline mutations to lock the spike into a prefusion conformation while the spike in ChAdOx1-nCoV-19 was not stabilized.[39,44–46,72] This may contributed to differences in activity between these platforms.

We have previously shown than Fc effector functions are substantially better preserved against VOC compared to neutralization.[83,86,87] Here we extend this to show that ADCP is more resilient to mutations in the spike than ADCC. We previously observed a similar trend in infection, where ADCP was more cross-reactive against Omicron infection than ADCC activity,[88] highlighting the robustness of this Fc effector function in both vaccination and infection. This suggests that ADCP elicitation may be a useful goal of vaccine design.

In addition to differences in magnitude, each vaccine modality drives a unique co-ordination of humoral responses.

Neutralization and binding antibody titers elicited by BNT162b2 significantly correlated with ADCC, while Ad26.CoV2.S triggered neutralization and binding responses strongly correlated with ADCD. This highlights that ADCC may be a crucial mechanism for mRNA vaccine-induced immunity[11,49] while ADCD is important for adenoviral vaccine efficacy,[50] and suggests that these vaccines may induce immunity along unique pathways. The lack of correlation between ADCD and binding IgG titers post BNT162b2 vaccination (Figure 5) may be due to higher IgM specific antibody levels elicited by this vaccine platform. IgM is able to mediate potent ADCD activity and has been associated with enhanced ADCD activity following mRNA vaccination.[74] The strength of the correlation within the Ad26.CoV2.S vaccination, despite the low levels of ADCD, likely further illustrates the critical impact a two dose regimen has on improving vaccine elicited immunity. In contrast, ChAdOx1 nCoV-19 vaccinees showed a poorly coordinated antibody response, suggesting that may depend on other forms of immunity such as T cell responses to elicit protection.[49,89,90] Overall, the polyfunctional co-ordination between antibody functions is influenced by the type of vaccine administered.

Limitations of the study

This study is limited as the comparisons between the three vaccine cohorts were based on different timings and dosing. However, our study compared modalities post primary vaccination, as was initially rolled out to South African participants with the time point as commonly close to peak immunogenicity as possible. For this reason, and owing to only ChAdOx1 nCoV-19 vaccine samples having a durability time point, no conclusions can be drawn about durability or long-term immunity of these platforms. In addition, since BNT162b2 was first rolled out to individuals 60 years and above in South Africa, the BNT162b2 cohort in our study had significantly older participants (70 median years) than Ad26.CoV2.S (41 years) and ChAdOx1 nCoV-19 (35 years), which could have influenced differences in antibody responses observed in the three cohorts, as age typically reduces vaccine elicited antibody functionality.[49] While we acknowledge our sample sizes were small, these are well characterized samples. Additionally, while we only included individuals with no detectable nucleocapsid IgG antibodies as a proxy for lack of infection, it has been shown that only 40% of individuals vaccinated with mRNA-1273 prior to breakthrough infections compared to 93% unvaccinated individuals develop anti-nucleocapsid IgG responses.[91] It is therefore possible that we underestimated the occurrence of breakthrough infections in these vaccinated participants.[92] Further, the role of various modulators of Fc effector functions such as glycosylation and antibody isotypes which may have contributed to the differential Fc effector activity across vaccine groups, have not been investigated in our study.

This study highlights that the levels cross-reactivity and immune co-ordination of Fc effector functions differ by SARS-CoV-2 vaccine modality, and that similar to neutralization, mRNA-based regimens elicit more potent, cross-reactive Fc effector functions. Overall, designing future regimes with improved polyfunctionality requires fine-tuning of these mechanisms.

RESOURCE AVAILABILITY

Lead contact

Further information and reasonable requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Simone Richardson (simoner@nicd.ac.za).

Materials availability

Materials will be made by request to Simone Richardson (simoner@nicd.ac.za).

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

N.P.M. performed the Fc effector function experiments, analyzed the data, produced the figures, and wrote the manuscript. B.M.M. performed Fc effector function experiments. P.L.M. and S.I.R. interpreted the data, reviewed and edited the manuscript, as well as supervised the research. R.S. assisted in producing the figures while H.S. processed samples. T.H. and N.M. performed neutralization assays while F.A. and Z.M. performed ELISA assays. S.A.M. established the ChAdOx1 nCoV-19 cohort and had access to the clinical trial data of the participants. G.G.G. and L.-G.B. established the Ad26.CoV2.S cohort as part of the Sisonke trial and had access to clinical trial data from this cohort. S.I.R. conceptualized and designed the study and established the BNT162b2 cohort. N.P.M. and S.I.R. had access to all the data generated and analyzed from the study samples. N.P.M. and S.I.R. conducted statistical analyses of the generated data in the study. N.P.M., S.I.R., and P.L.M. prepared the first draft of the manuscript while all authors have reviewed, edited, and approved the final version of this manuscript and confirmed their agreement to submit for publication. All authors take full responsibility for the manuscript content, including the accuracy of the data and the statistical analyses presented herein.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Palivizumab	Medimmune	Synagis; RRID: AB_2459638
CR3022	Genscript (https://www.genscript.com)	N/A
AIRU946-A6	Richardson et al.[88]	N/A
Bacterial and virus strains		
SARS-COV-2 pseudovirus for D614G, Beta, Delta and Omicron BA.1	Richardson et al.[88]; Richardson et al.[17]; Wibmer et al.[3]	N/A
Biological samples		
Pooled SARS-CoV-2 negative sera samples	National Institute for Communicable Diseases	https://www.nicd.ac.za
Pooled SARS-CoV-2 positive sera samples	National Institute for Communicable Diseases	https://www.nicd.ac.za
Ad26.CoV2.S vaccinee sera samples	Open label Sisonke phase 3b trial	ClinicalTrials.gov number NCT04838795; Pan-African Clinical Trials registry number PACTR202102855526180
BNT162b2 vaccinee sera samples	South African National Vaccine Program	N/A
ChAdOx1 nCoV-19 vaccinee sera samples	South African ChAdOx1 nCoV-19 trial	ClinicalTrials.gov number NCT04444674; Pan-African Clinical Trials registry number PACTR202006922165132
Chemicals, peptides, and recombinant proteins		
Original SARS-CoV-2 spike protein variant	Dr Jason McKellan, Department of Molecular Biosciences at the University of Texas	N/A
Critical commercial assays		
FITC conjugated neutravidin fluorospheres, 1μm	Thermo Fisher scientific	Cat #F8776
PEI Max 40 000	Polysciences	Cat # 24765-1
QUANTI-Luc luciferase	Davies Diagnostics	Cat # rep-qlc2
500× cell stimulation cocktail	Thermo Fisher scientific	Cat # 00-4970-93
PE conjugated neutravidin fluorospheres, 1μm	Thermo Fisher scientific	Cat #F8775
Experimental models: Cell lines		
Jurkat-Lucia™ NFAT-CD16 cells	InvivoGen	Cat # jktl-nfat-cd16
THP-1 monocyte cell line	NIH HIV Reagent program	Cat # ARP-9942
Human embryo kidney (HEK) 293T	Dr. Nicole Doria-Rose, VRC, USA	N/A
Human embryo kidney (HEK) 293T/ACE2.MF	Dr. Michael Farzan, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation and Technology, USA	N/A
Human embryo kidney (HEK) 293F	Dr. Nicole Doria-Rose, VRC, USA	N/A
Recombinant DNA		
SARS-CoV-2 D614G	Wibmer et al.[3]	N/A
Beta (L18F, D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G and A701V)	Wibmer et al.[3]	N/A
Delta (T19R, Δ156–157, R158G, L452R, T478K, D614G, P681R, D950N)	Richardson et al.[17]	N/A

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
BA.1 (A67V, Δ69–70, T95I, G142D, Δ143–145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, L981F, N969K)	Richardson et al.[17]	N/A
Software and algorithms		
FlowJo 10	FlowJo, LLC	https://www.flowjo.com
Biorender scientific image and illustration software	Biorender	https://www.biorender.com
GraphPad prism 9	GraphPad, San Diego, CA, USA	https://www.graphpad.com

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human subjects

Health care workers (n = 16) that received one dose of Ad26.CoV2.S intramuscularly were recruited in South Africa between February and March 2021 as part of the open-label Sisonke phase 3b trial[93] (ClinicalTrials.gov number NCT04838795; Pan-African Clinical Trials registry number PACTR202102855526180) that aimed to aid in rapid vaccination rollout in the country. As national guidelines recommended a Ad26.CoV2.S two-dose regimen, trial participants transitioned into the national vaccination program where several elected to receive their second dose an average of seven months after initial dose. Samples were collected two months after the second dose and one month following the additional dose (Figure 1). BNT162b2 vaccinees (n = 13, all over 60 years old) received two doses as part of the South African national vaccine program and were not part of a clinical trial. The group of individuals included in this study is representative of the first age group to be allowed access to BNT162b2 in South Africa. The BNT162b2 was administered as an intramuscular two-dose regimen where participants received their second dose a median of 42 days after the initial dose. A third booster dose was administered an average of 5.5 months following the second. Samples were collected after receiving a full primary vaccination i.e., 2 doses, and 1 month post boost (PB) (Figure 1). The post full primary vaccination blood samples were taken 2 months after the initial dose, which translated to a month following their second dose. Although the primary vaccination sampling period was a month less than the other vaccine groups, these samples were regarded as peak immunogenicity because they were taken following the full 2 dose primary vaccination strategy recommended for BNT162b2 (Figure 1). Ethics approval to sample both BNT162b2 and Ad26.CoV2.S vaccinees was obtained from the University of the Witwatersrand (M210465). The collection of plasma from South African ChAdOx1 nCoV-19 trial (ClinicalTrials.gov number NCT04444674; Pan-African Clinical Trials registry number PACTR202006922165132) participants (n = 15) was approved by the University of the Witwatersrand (M200501).[40,94] ChAdOx1 nCoV-19 was administered as an intramuscular two-dose regimen where vaccinees received their second dose one month after the first dose. ChAdOx1 nCoV-19 vaccinees were sampled one month post initial dose and subsequently two weeks and then six months following a second dose (Figure 1). All participants were screened for nucleocapsid-specific antibodies by ELISA as a proxy for prior infection and only nucleocapsid antibody negative samples included in the study. Participants with breakthrough infections prior or after receiving their primary and booster dose were excluded in analysis for all assays. This includes Ad26.CoV2.S vaccinees with nucleocapsid ELISA confirmed breakthrough infections 2 months before they acquired a booster dose. Written informed consent was received from all the study participants.

Cell lines

Jurkat-Lucia™ NFAT-CD16 cells were cultured in IMDM media supplemented with 10% heat-inactivated fetal bovine serum (Gibco, Gaithersburg, MD) and 1% Penicillin Streptomycin (Gibco, Gaithersburg, MD) with 10 μg/mL of Blasticidin and 100 μg/mL of Zeocin added during alternating passages. THP-1 cells were obtained from the AIDS Reagent Program, Division of AIDS, NIAID, NIH. These cells were maintained at 37°C, 5% CO_2 in RPMI with added 10% heat-inactivated fetal bovine serum (Gibco, Gaithersburg, MD), 1% Penicillin Streptomycin (Gibco, Gaithersburg, MD) and 0.05 mM 2-mercaptoethanol. Human embryo kidney HEK293T cells were cultured at 37°C, 5% CO_2, in DMEM supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL Life Technologies) and 50 μg/mL gentamicin (Sigma). HEK293T/ACE2.MF cells were obtained from Dr Michael Farzan (Scripps) and maintained as for HEK293T cells but additionally supplemented with 3 μg/mL puromycin. HEK293F suspension cells were cultured in 293 Freestyle media (Gibco BRL Life Technologies) within a 125 rpm shaking incubator at 37°C, 5% CO_2 and 70% humidity. Jurkat-Lucia™ NFAT-CD16 and THP-1 cell lines were routinely evaluated for CD16 and CD32 FcR receptor expression using fluorescently labelled CD16/CD32 specific antibodies, respectively. Additionally, all cell lines used in this study were tested for mycosplama contamination, routinely.

METHOD DETAILS

SARS-CoV-2 antigens and pseudovirus

SARS-CoV-2 D614G, Beta (L18F, D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G and A701V), Delta (T19R, Δ156–157, R158G, L452R, T478K, D614G, P681R, D950N) and BA.1 (A67V, Δ69–70, T95I, G142D, Δ143–145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, L981F, N969K) full spike proteins used for ELISA and Fc effector function assays were expressed in HEK293F suspension cells by transfecting the cells with the relevant expression plasmid using PEIMAX (Polysciences). The cells were incubated for 6 days at 37°C, 70% humidity and 10% CO_2. Proteins were purified via nickel affinity chromatography and size-exclusion chromatography and stored at −80°C until use.

SARS-CoV-2 variants were also cloned into pCDNA3.1 and the resulting plasmids were used for transfections in the ADCC assay or pseudovirus production. For pseudovirus, HEK293T/17 were co-transfected with a lentiviral backbone (HIV-1 pNL4.luc encoding the firefly luciferase gene) and either of the produced spike plasmids using PEI-MAX (Polysciences) and incubated at 37°C for 3 days. Following the 3 days incubation, culture supernatants containing SARS-CoV-2 pseudoviruses were harvested, filtered (0.45-μM filter) and stored at −70°C.

SARS-CoV-2 spike enzyme-linked immunosorbent assay (ELISA)

High binding 96 well plates (Sigma), were coated with 2 μg/mL of the spike protein (original, Beta, Delta and BA.1) and incubated at 4°C overnight. Plates were washed and further incubated in a blocking buffer containing 5% skimmed milk powder, 0.05% Tween 20 and 1× PBS (Sigma) for up to 2 h. Vaccine plasma samples were diluted by 1:100 in blocking buffer, added to the plates and incubated for 1 hour. IgG specific secondary antibody was diluted to 1:3000 in blocking buffer and added to the plates for 1 h followed by TMB substrate (Thermofisher Scientific). To stop the reaction, 1 M H_2SO_4 was added and absorbance was measured at a 450 nm wavelength. CR3022 and AIRU946-A6 (In house SARS-CoV-2 control), monoclonal antibodies (mAbs) were used as positive controls and Palivizumab (RSV mAb; MedImmune, LLC) as a negative control.

Antibody-dependent cellular phagocytosis (ADCP) assay

The BirA biotin-protein ligase bulk lyophilized reaction kit was used to biotinylate avi-tagged SARS-CoV-2 spike proteins to ensure correct orientation of the antigen and coated on fluorescent neutravidin beads as previously described.[95] Spike coated beads were incubated for two hours at 37°C, 5% CO_2 with a single 1 in 100 dilution of vaccine plasma or monoclonal antibodies at 10 μg/mL and titrated five-fold. Beads were washed and further incubated with THP-1 cells overnight, fixed with Paraformaldehyde (PFA) (Sigma) and interrogated on the FACSAria II (BD Biosciences). ADCP score was calculated as a proportion of THP-1 cells that internalized beads multiplied by the geometric mean fluorescence intensity of the population. A "no antibody" negative control was subtracted to remove background signal. Pooled plasma from 5 individuals who had PCR-confirmed SARS-CoV-2 infection with the original variant (D614G) and the CR3022 were used as positive controls, while plasma from 5 pre-pandemic controls and a Palivizumab were used as negative controls for this and all other Fc effector assays. The pooled SARS-CoV-2 positive and negative samples were only used to distinguish between positive and negative Fc functionality. The samples were acquired on the BD FACSAria™ II Cell Sorter and analysed using FlowJo software.

Antibody-dependent cellular cytotoxicity (ADCC) assay

Plasma antibodies from the vaccine groups were screened for their capacity to cross-link and activate the FcγRIIIa (CD16) receptor on the surface of Jurkat cells and SARS-CoV-2 spike expressing cells as a proxy for ADCC. HEK293T cells were transfected with 5μg of SARS-CoV-2 ancestral variant spike (D614G), Beta, Delta or BA.1 spike plasmids using 1 mg/mL PEI-MAX 40,000 (Polysciences) and incubated for 2 days at 37°C. To confirm spike expression from the HEK293T cells, differential binding of CR3022, 946-A6 and P2B-2F6 was detected by anti-IgG APC staining and measured by flow cytometry. Spike transfected cells (1×10^5 per well) were incubated with heat inactivated plasma (1:100 final dilution) or monoclonal antibodies (final concentration of 100 μg/mL) in RPMI 1640 media supplemented with 10% FBS 1% Pen/Strep (Gibco, Gaithersburg, MD) for 1 h at 37°C. Jurkat-Lucia™ NFAT-CD16 cells (Invivogen) were added at concentrations of 2×10^5 cells/well and incubated for 24 h at 37°C, 5% CO_2. Twenty μL of supernatant was transferred to a white 96-well plate with 50 μL of reconstituted QUANTI-Luc secreted luciferase and read immediately on a Victor 3 luminometer with 1s integration time. Additionally, 1× cell stimulation cocktail (Thermofisher Scientific, Oslo, Norway) and 2 μg/mL ionomycin in R10 were added as controls to induce the transgene and confirm sufficient expression of the Fc receptor. Relative Light Units (RLUs) for D614G, Beta, Delta or Omicron BA.1 variants were normalized to each other and between runs using CR3022 and 946-A6.

Antibody-dependent complement deposition (ADCD) assay

ADCD was measured as previously described.[96] Briefly, biotinylated spike protein was coated at a 1:1 ratio onto red fluorescent 1 μM neutravidin beads (Molecular Probes Inc.) for 2 h at 37°C. Subsequently, 10 μL of the coated beads per well were incubated with a 1:10 plasma sample dilution or 5-fold titration of mAb at a starting concentration of 100 μg/mL for 2 h at 37°C. Guinea pig complement was then diluted 1 in 50 with gelatin/veronal buffer, 200 μl of this was added to the plate per well and incubated for 15 min at 37°C.

Beads were washed in PBS, stained with anti-guinea pig C3-FITC antibody, fixed with 4% Paraformaldehyde (PFA) (Sigma-Aldrich) and their fluorescence measured on a FACS Aria II. The deposition of complement onto the beads is measured as a proportion of beads bound to an anti-C3 FITC antibody and represented as the median fluorescence intensity (MFI) of FITC with the no antibody or heat inactivated controls subtracted.

Pseudovirus based neutralization assay

To determine the neutralizing activity of plasma antibodies, the plasma samples were heat-inactivated and clarified by centrifugation. Subsequently, the heat-inactivated plasma samples were incubated with the SARS-CoV-2 pseudotyped viruses i.e., D614G, Beta, Delta and BA.1 for 1 h at 37°C, 5% CO_2. Thereafter, 1×10^4 HEK293T cells engineered to over-express ACE-2 were added and incubated at 37°C, 5% CO_2 for 72 h. Luciferase gene expression/activity was then measured as luminescence (RLUs). Neutralization was defined as a reduction in expression of the luciferase gene and the SARS-CoV-2 neutralization titers calculated as the reciprocal plasma dilution (ID_{50}) causing 50% reduction of RLUs. CB6 and CA1 were used as positive controls, while VRC01, an HIV specific monoclonal, antibody was included as a negative control for the assay.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed in Prism (v9; GraphPad Software Inc, San Diego, CA, USA) and data from at least two independent experiments per assay were used for analyses. Non-parametric tests were used for all comparisons. The Kruskal Wallis test with multiple comparisons was used to compare unmatched and paired samples, respectively. p values less than 0.05 considered to be significant.

ADDITIONAL RESOURCES

- Information on Open-label Sisonke phase 3b trial[93]: (ClinicalTrials.gov number NCT04838795; Pan-African Clinical Trials registry number PACTR202102855526180). https://doi.org/10.1101/2021.12.20.21267967.
- Information on South African ChAdOx1 nCoV-19 trial: (ClinicalTrials.gov number NCT04444674; Pan-African Clinical Trials registry number PACTR202006922165132). https://clinicaltrials.gov/study/NCT04444674.