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BNT162b2 mRNA COVID-19 vaccine induces antibodies of broader cross-reactivity than natural infection but recognition of mutant viruses is up to 10-fold reduced

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To the Editor

The receptor binding domain (RBD) of SARS-CoV-2 spike (S) glycoprotein, which is involved in virus attachment and cell entry, is the primary target for neutralizing antibodies¹. Immunization against full-length S or parts of it may result in more potent and longer-lasting antibody responses than natural viral infection². Therefore, global vaccination programs based on induction of neutralizing antibodies are the most promising strategy for controlling the COVID-19 pandemic.

A worrying factor, however, has been the emerging variants capable to escape immunity produced by vaccination or infection. Three variants have attracted attentions due to their abnormally high rates of propagation: B.1.1.7 (N501Y, D614G); B.1.351 (K417N, E484K, N501Y, D614G); and P.1 (K417N/T, E484K, N501Y). Limited knowledge of the presence of cross-neutralizing antibodies induced by natural infection or vaccination is a key gap in current understanding of the spread of SARS-CoV-2. It is imperative to determine the impact of these mutations on the responses induced by currently marketed vaccines.

A study has shown that E484K mutation is associated with reduced neutralization, by SARS-CoV-2 infection or BNT162b2-elicited sera³. Whether reduced neutralization was due to impaired binding was, however, not analyzed. Here we assessed the presence of such cross-reactive antibodies in convalescent sera and sera from individuals immunized with mRNA-based BNT162b2 vaccine.

To this end, we generated four mutant RBDs: K417N (RBD₄₁₇), E484K (RBD₄₈₄), N501Y (RBD₅₀₁) and one triple mutated version with all three mutations (RBD_{trip}) (Fig 1A). Two of these mutations, E484K, N501Y, are localized within the receptor binding motif⁴, directly interacting with ACE2. Our ELISA results show that binding of convalescent sera was strongly reduced for RBD₄₁₇ and RBD₅₀₁ and, however, essentially abolished for RBD₄₈₄ and RBD_{trip} (Fig 1B, left). In contrast, BNT162b2-elicited antibodies exhibited only weakly reduced binding to RBD₄₁₇ and RBD₅₀₁ (2.5-3-fold), but 10-fold reduced

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binding to RBD₄₈₄ and RBD_{trip} (Fig 1B, right). Figure 1C summarizes the antibody titers and Figure 1D quantifies the reduced binding of vaccine induced sera to the mutant RBDs compared to RBD_{wt} (wild type RBD). Interestingly, the E484K mutation was equally potent at reducing antibody binding as in RBD_{trip}, indicating that the mutation E484K is particularly problematic, perhaps because it involves a change from positive to negative charge. This is consistent with recent data showing less neutralization titer against a variant containing E484K mutation, however only with 1.41-fold reduction⁵.

We performed assays to estimate the antibodies' avidity for RBD and mutants. Interestingly, virus-induced antibodies were of limited avidity for RBD_{wt}, and binding to mutant RBDs was essentially abolished with 7M urea wash, indicating that the antibodies binding to mutant RBDs were all of low avidity (Fig 2A shows results for RBD_{wt}). In contrast, BNT162b2 induced antibodies were of significantly higher avidity (Fig 2B). In addition, there was some residual binding to mutant RBDs, indicating, however, overall low avidity as well (Fig 2C). The avidity index allows to quantify the loss in binding caused by the 7M urea wash and therefore reflects the "quality" of the antibodies. Indeed, the avidity index of vaccine-induced antibodies is much higher for RBD_{wt} and mutants than those induced by infection. This reduced affinity of antibodies induced by infection is consistent with the notion that individual RBDs are spaced by 25 nm on SARS-CoV-2, too large for inducing optimal antibodies⁶.

In conclusion, BNT162b2-induced antibodies recognize mutant RBDs better than those by natural infection. Recognition may, however, be 10-fold reduced for the variants B.1.351/P.1, suggesting that development of a new vaccine may be warranted. E484K mutation is shown here to be a key hurdle for immune recognition. Hence, monoclonal antibody therapy and serological assays based on wildtype sequence may therefore be seriously impaired.

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Conflict of interests

M. F. Bachmann is a board member of Saiba AG, involved in the development of RBD-CuMV a vaccine against COVID-19. All other authors declare no conflict of interest.

Figures Legends

Fig 1. Strongly reduced recognition of mutant RBDs by convalescent and BNT162b2 vaccinated human sera. (A) Structure of RBD and location of the individual mutations used in this study (E484K, K417N, N501Y, or all 3 mutations combined). (B) Titration on RBDs of sera from convalescent patients (left panel) or from BNT162b2 vaccinated individuals (right panel). (C) Antibody titers (OD₅₀) of sera from 6 BNT162b2 vaccinated individuals on RBDs. (D) Fold reduction of mutant RBDs compared to wild type RBD-recognition by sera of BNT162b2 vaccinated individuals.

Fig 2. Increased avidity of BNT162b2 induced antibodies compared to antibodies induced by infection. (A,B) Average recognition of RBD_{wt} by convalescent (A) or vaccine-induced sera (B) by avidity ELISA. Samples washed with PBS-Tween (black) and 7M urea (red). (C) Avidity indexes are shown for recognition of mutant RBDs by vaccine induced sera. These indexes are calculated by dividing the area under the curve (AUC) of urea washed samples by AUC of PBS-Tween washed samples. Note that recognition of mutant RBDs by convalescent sera was too low to calculate a meaningful avidity index.

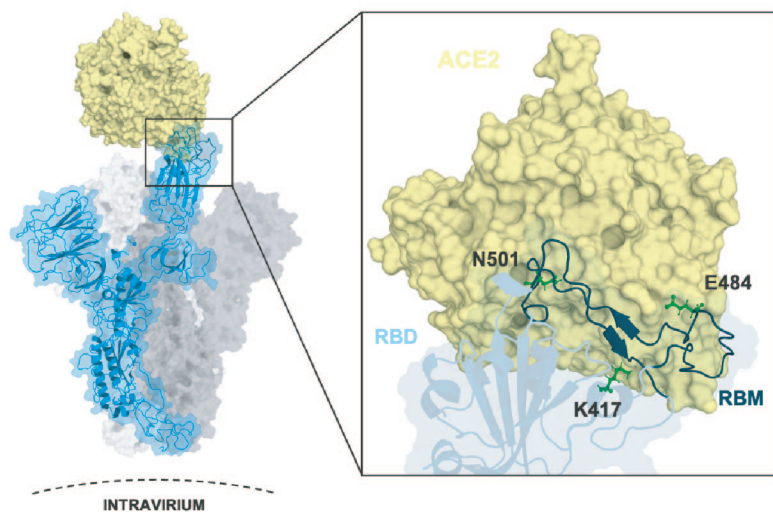
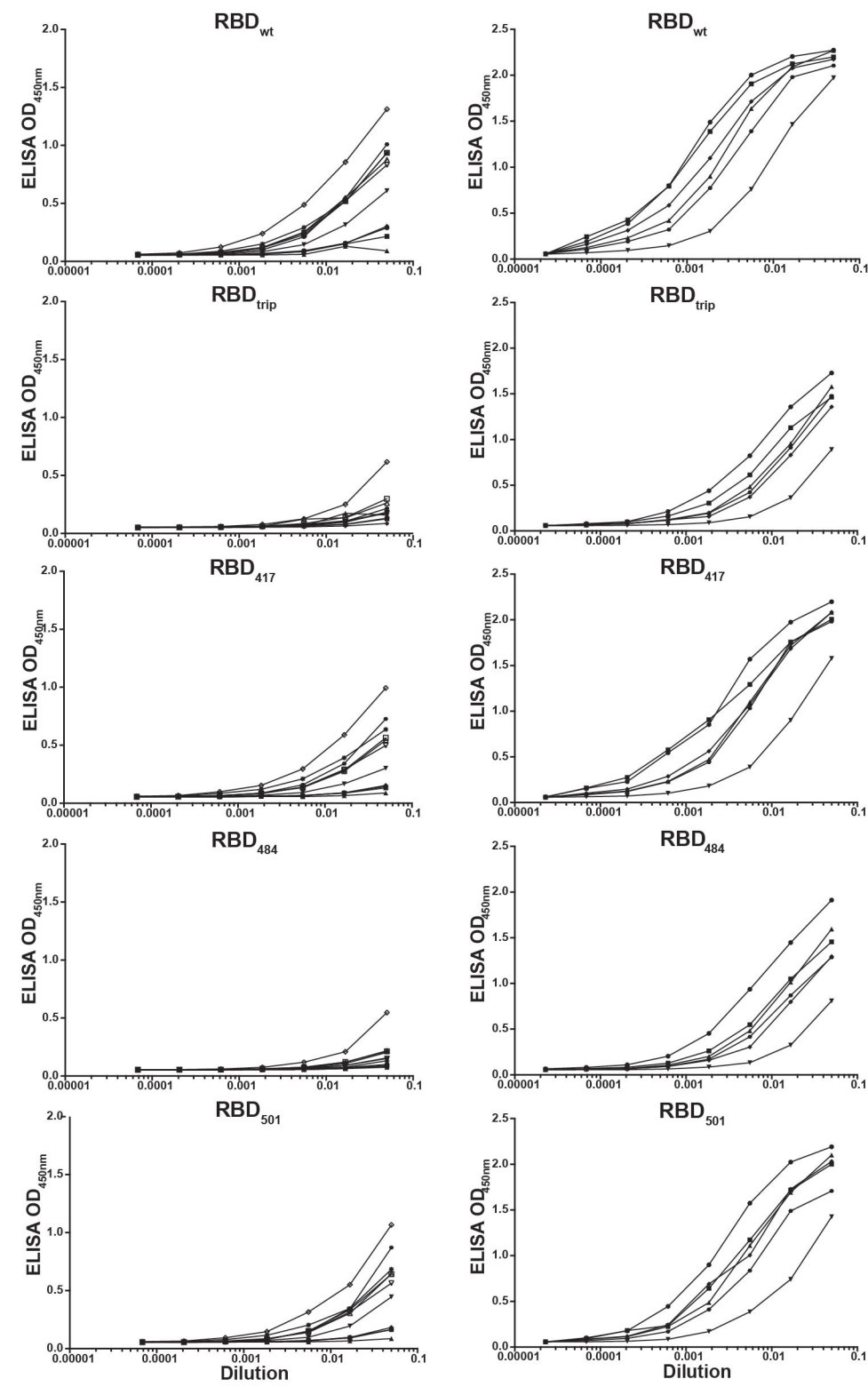
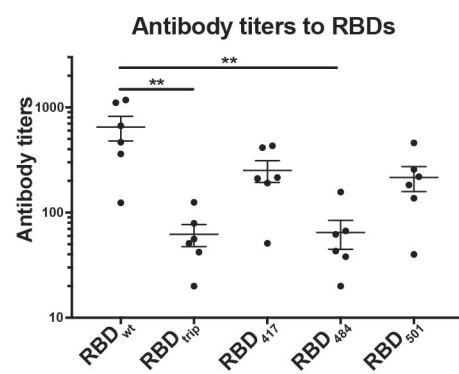
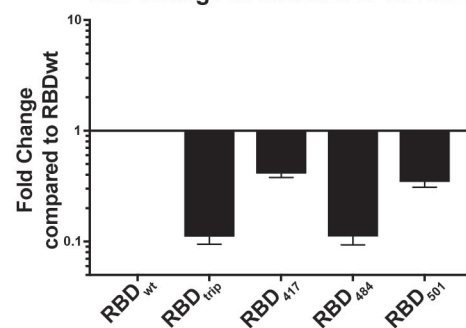
Figure 1**A****B****Convalescent sera****BNT162b2 Vaccinated sera****C****BNT162b2 Vaccinated sera****D****Titer change of mutated to wt RBD**

Figure 2

