CORRESPONDENCE

Omicron SARS-CoV-2 Neutralization from Inactivated and ZF2001 Vaccines

TO THE EDITOR: In the third year of the coronavirus disease 2019 (Covid-19) pandemic, the omicron variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has swept the globe and yielded several subvariants.[1,2] Currently, BA.2 is overtaking BA.1 in frequency. In addition, BA.2.12.1 infection is increasing quickly and already accounts for more than 50% of new infections in the United States. Therefore, the protection of current vaccines and the need to develop future vaccination strategies are of great concern.

In this study, we used a pseudovirus assay to assess the neutralizing antibody titers in serum samples obtained from vaccinees against the SARS-CoV-2 prototype (PT) isolate and against omicron subvariants BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3, BA.4, and BA.5. The vaccinees had received three doses of one of two inactivated virus vaccines (CoronaVac and BBIBP-CorV) that are widely used in China, three doses of the protein-subunit vaccine ZF2001 (which uses a dimeric receptor-binding domain [RBD] as the antigen), or two doses of CoronaVac boosted by ZF2001 (Fig. 1 and Figs. S2–S6 and Tables S1–S3 in the Supplementary Appendix, available with the full text of this letter at NEJM.org).

In each vaccine group, the neutralizing antibody titers against all the tested omicron subvariants were significantly lower than corresponding titers against the PT isolate, findings that indicate substantial immune escape for the omicron subvariants. Decreases in neutralizing titers were associated with mutations in the spike proteins.[3,4] BA.1.1 and BA.2 had neutralization similar to that of BA.1 (within a factor of 1.5). BA.2.12.1 (which has an extra L452Q mutation in its RBD, as compared with BA.2) had lower neutralization by a factor of 1.4 to 1.7. In each of the vaccinated groups, the neutralizing antibody titers against BA.4 and BA.5, the subvariants that are currently dominant in South Africa and that have the potential to be the next pandemic subvariants worldwide, were lower by a factor of 2.1 to 2.6 than titers against the BA.2 subvariant. This finding indicated that two mutations (L452R and F486V) in the RBD, as compared with the RBD in the BA.2 subvariant, resulted in lower antibody neutralization efficiency elicited by current vaccines designed on the basis of the PT sequence.

To determine a better strategy for administering ZF2001 vaccines, we collected samples from the vaccinees 1 month after the third dose of vaccine. We then subdivided this group into three subgroups, according to the interval between the second dose and the third dose: 1 month, 2 months, and 4 to 6 months (prolonged-interval subgroup). Moreover, to test the persistence of neutralizing antibodies in the prolonged-interval subgroup after ZF2001 vaccination, we obtained serum samples 4 to 7 months after the third dose.

We found that neutralizing antibody titers increased with the increasing interval between the second and third doses, especially against the omicron subvariants. In vaccinees who had an interval of 4 to 6 months between the second and third doses, neutralizing antibody titers were higher by nearly a factor of 10 against the PT isolate and by a factor of approximately 30 against all omicron subvariants, as compared with vaccinees who had a 1-month interval between doses ($P<0.001$) (Table S3). Vaccinees in the prolonged-interval subgroup were 100% seropositive against all the omicron subvariants that were tested (Fig. S5). In the samples obtained 6 months after the last dose of vaccine in the prolonged-interval subgroup, neutralizing antibody titers against all omicron subvariants and seropositive rates were higher than those in samples obtained 1 month after the last dose in the short-interval subgroup. The heterologous-boosted group had higher neutralizing antibody titers against the PT isolate and all omicron subvariants than the

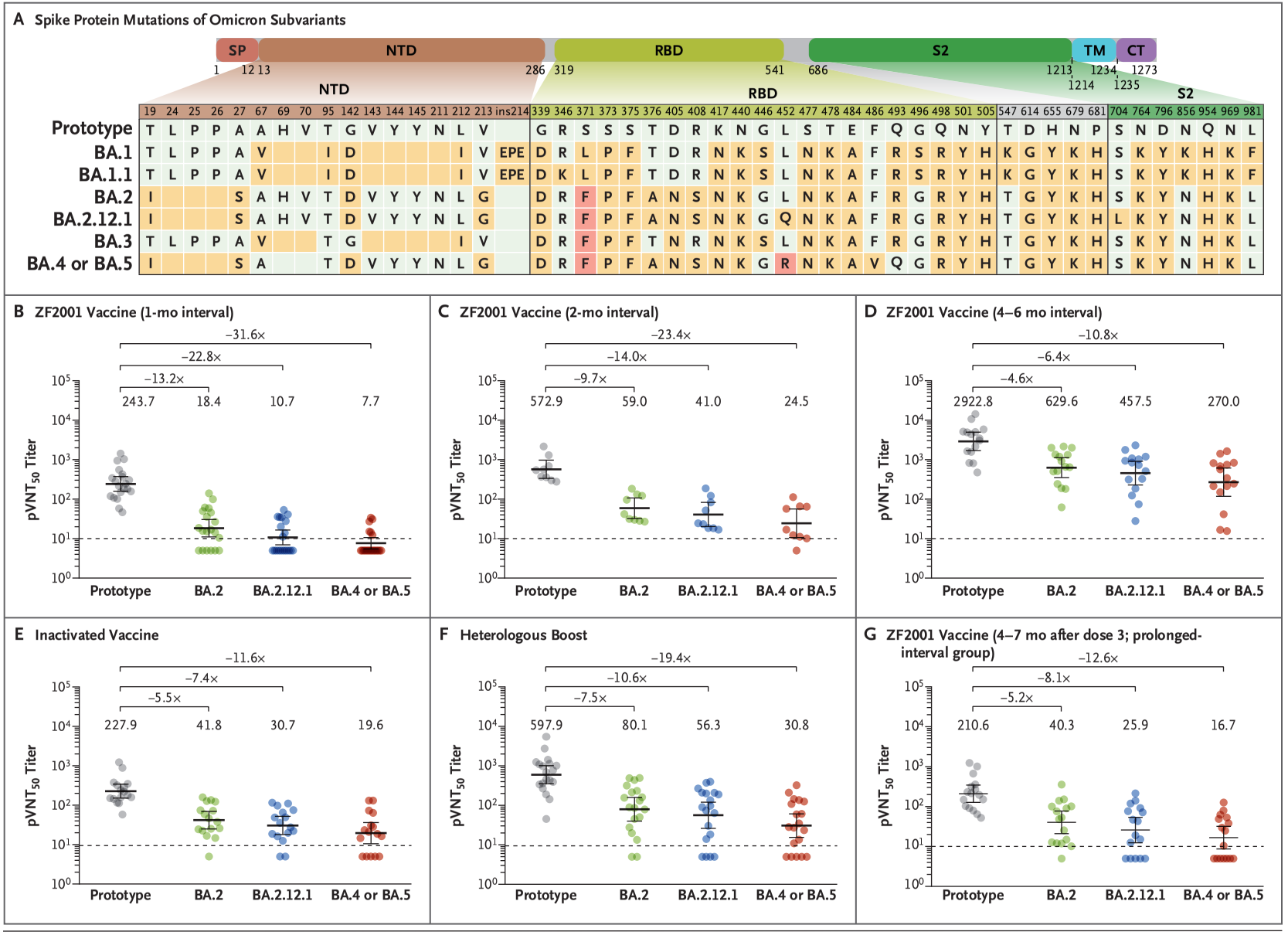
A Spike Protein Mutations of Omicron Subvariants
SP
NTD
RBD
S2
TM
CT
1
12 13
286
319
541
686
1213
1214
1234
1235
1273
NTD
RBD
S2
19 24 25 26 27 67 69 70 95 142 143 144 145 211 212 213 ins214 339 346 371 373 375 376 405 408 417 440 446 452 477 478 484 486 493 496 498 501 505 547 614 655 679 681 704 764 796 856 954 969 981
Prototype T L P P A A H V T G V Y Y N L V G R S S S T D R K N G L S T E F Q G Q N Y T D H N P S N D N Q N L
BA.1 T L P P A V I D I V EPE D R L P F T D R N K S L N K A F R S R Y H K G Y K H S K Y K H K F
BA.1.1 T L P P A V I D I V EPE D K L P F T D R N K S L N K A F R S R Y H K G Y K H S K Y K H K F
BA.2 I S A H V T D V Y Y N L G D R F P F A N S N K G L N K A F R G R Y H T G Y K H S K Y N H K L
BA.2.12.1 I S A H V T D V Y Y N L G D R F P F A N S N K G Q N K A F R G R Y H T G Y K H L K Y N H K L
BA.3 T L P P A V T G I V D R F P F T N R N K S L N K A F R G R Y H T G Y K H S K Y N H K L
BA.4 or BA.5 I S A T D V Y Y N L G D R F P F A N S N K G R N K A V Q G R Y H T G Y K H S K Y N H K L
B ZF2001 Vaccine (1-mo interval)
−31.6×
−22.8×
−13.2×
243.7
18.4
10.7
7.7
pVNT50 Titer
Prototype
BA.2
BA.2.12.1
BA.4 or BA.5
C ZF2001 Vaccine (2-mo interval)
−23.4×
−14.0×
−9.7×
572.9
59.0
41.0
24.5
D ZF2001 Vaccine (4–6 mo interval)
−10.8×
−6.4×
−4.6×
2922.8
629.6
457.5
270.0
E Inactivated Vaccine
−11.6×
−7.4×
−5.5×
227.9
41.8
30.7
19.6
F Heterologous Boost
−19.4×
−10.6×
−7.5×
597.9
80.1
56.3
30.8
G ZF2001 Vaccine (4–7 mo after dose 3; prolonged-interval group)
−12.6×
−8.1×
−5.2×
210.6
40.3
25.9
16.7

Figure 1 (facing page). Neutralizing Antibodies against Omicron Subvariants Induced by ZF2001 and Inactivated Virus Vaccines.

Panel A shows the mutations in the spike protein of omicron subvariants BA.1, BA.1.1, BA.2, BA.2.12.1, BA.3, BA.4, and BA.5. (The latter two subvariants are grouped together because they have the same spike protein.) The amino acid differences in omicron subvariant sequences from those in the SARS-CoV-2 prototype isolate (PT) — including substitutions, deletions, and insertions — are highlighted in gold. The third type of amino acid on the same locus is marked in red. Deletion mutations are shown as blank squares with background color. CT denotes C-terminal cytoplasmic domain, NTD N-terminal domain, RBD receptor-binding domain, SP signal peptide, S2 S2-protein subunit, and TM transmembrane domain. Panels B through G show the 50% pseudovirus neutralization titers ($pVNT_{50}$) against the listed PT isolate and omicron subvariants BA.2, BA.2.12.1, BA.4, and BA.5. Samples are grouped according to the vaccine type and immunization strategy. The groups include 20 vaccinees who received three shots of the ZF2001 vaccine at 1-month intervals (Panel B); 9 vaccinees who had a 2-month interval between the second and third doses (Panel C); 15 vaccinees who had a prolonged interval (4 to 6 months) between the second and third doses (Panel D); 16 vaccinees who received three doses of an inactivated vaccine (Panel E); and 19 vaccinees who received two doses of an inactivated vaccine boosted by the ZF2001 vaccine (heterologous boost) (Panel F). In these five groups, samples were obtained approximately 1 month after the last dose had been administered. In the sixth group, samples from 16 vaccinees who had received three doses of the ZF2001 vaccine with a prolonged-interval strategy were obtained 4 to 7 months after the last dose (Panel G). The $pVNT_{50}$ in each group is shown as the geometric mean titer (GMT) at the top of each panel, along with the factor reduction as compared with the PT isolate; the I bars indicate 95% confidence intervals. The dashed horizontal line indicates the lower limit of detection for the pseudovirus neutralization assay. Values of less than 10 for the $pVNT_{50}$ indicate a negative sample and were counted as 5. Similar data for subvariants BA.1, BA.1.1, and BA.3 are provided in Figure S2 in the Supplementary Appendix.

groups that had received three doses of the same inactivated vaccine. However, the factor reductions in the response against the BA.2, BA.2.12.1, BA.4, and BA.5 subvariants as compared with the response against the PT isolate were greater in the heterologous-boosted group than in the inactivated-vaccine group. This finding indicated that these subvariants had more mutations in the RBD, which resulted in immune escape.

The rapid emergence of new variants makes variant-specific vaccine development difficult. Our findings show that a better immunization strategy for current vaccines could contribute to higher neutralization levels of omicron subvariants. Because the ZF2001 vaccine consists of a protein subunit with a focused antigen on the RBD, its use could induce increased titers of neutralizing antibodies against omicron subvariants through the administration of multiple booster doses and immune-maturation methods. However, the development of updated vaccines as boosters is needed for better protection against immune escape of current subvariants (especially BA.4 and BA.5) and possible future epidemic subvariants.[5]

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