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LETTER TO THE EDITOR

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A third booster dose may be necessary to mitigate neutralizing antibody fading after inoculation with two doses of an inactivated SARS-CoV-2 vaccine

To The Editor,

Since the outbreak at the end of 2019, coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, has evolved into a global pandemic, seriously endangering human health.[1,2] At present, inactivated vaccines,[3] messenger RNA (mRNA) vaccines,[4] and adenovirus vaccines[5] have been developed or are undergoing clinical trials. Some vaccines have obtained emergency use authorization (EUA) from the World Health Organization (WHO) or the governments of various countries. Global vaccination will play an important role in the effective control of COVID-19. Classic inactivated COVID-19 vaccines have been developed primarily by companies in developing countries, and clinical trials have indicated that the vaccines have good safety profiles and protect against COVID-19.[3,6] Due to the mature technology and convenience of transportation and storage, inactivated vaccines have been widely used to vaccinate residents in many developing countries.

Currently, inactivated vaccines require two doses at 0 and 14 days (or 0 and 21 days, 0 and 28 days). Inactivated vaccines show an ideal protective effect at 14 days after the second dose.[3] Given that neutralizing titers in the convalescent sera of COVID-19 patients significantly decrease at 6 months,[7] whether a similar phenomenon could occur with inactivated vaccines at a certain period after two doses of vaccination is not clear. In addition, the necessity of improving the effectiveness and durability of inactivated vaccines through booster shots needs to be further explored.

In this study, 355 volunteers participating in the development and production of inactivated vaccines (with informed consent) received two doses (at 0 and 14 days or 0 and 28 days) of inactivated COVID-19 vaccines in 2020 (Figure 1A). At 1 month after the second dose, the positive conversion rate of serum neutralizing antibodies reached 88.5%. However, at 8 months after the second dose, the serum neutralizing antibody titers in this cohort decreased significantly, and the positive conversion rate decreased to 48.5% (Figure 1C). For volunteers of both sexes, those who received vaccines according to different immunization procedures and different age groups, serum neutralizing antibody titers at 8 months after the second dose were significantly lower than those at 1 month after the second dose. Moreover, at the same time point after the second dose, no significant differences in titers were noted regardless of sex, vaccine immunization procedure, and age. These results indicated that the reduction in serum neutralizing antibody titers was not affected by sex, vaccine immunization procedure, or age (Figures 1E, 1G, and 1I).

Due to the need to further explore COVID-19 vaccines, 67 persons in the above cohort voluntarily received a third dose (Figure 1B). One month after the third dose, serum neutralizing antibody titers were tested, and the positive conversion rate of antibodies increased to 95.5% (the positive conversion rates of the 67 patients were 86.6% at 1 month after the second dose and 65.7% at 8 months after the second dose) (Figure 1D). To our surprise, for these volunteers, the titers were not only significantly higher than those at 8 months after the second dose but were also significantly higher than those at 1 month after the second dose (Figure 1D). The titers were also not affected by sex, vaccine immunization procedure, or age (Figures 1F, 1H, and 1J). These results demonstrate that the booster dose of the vaccine (the third dose) can reverse the decrease in neutralizing antibodies after the second dose. Moreover, in terms of neutralizing antibody levels, the effect of a three-dose immunization procedure was significantly better than that of the two-dose immunization procedure. Importantly, these findings were not affected by sex, vaccine immunization procedure, or age. It is good news for a special population who need the third enhancer dose. As if so, it is not necessary to set up different vaccination strategies under emergency use. Of course, these results should be confirmed by large-scale clinical studies.

In addition, although a recent study reported that immune memory was still active at six months after the second dose,[8] more research is needed to confirm this finding and fully elucidate the underlying immunological principles. First, to prove whether this phenomenon is specific to inactivated vaccines, related data on the immune persistence and effectiveness of mRNA vaccines, adenovirus vaccines, and subunit vaccines are needed. Second, some studies have reported the immune memory characteristics of convalescent patients[9], some studies have compared the characteristics of antibody responses in asymptomatic and symptomatic infected people or convalescent patients,[10,11] and some researchers have demonstrated that memory plasma cells in bone marrow may play a key role in immune persistence.[12] The characteristics of the immune system after vaccination are still unclear, and in-depth studies on antibody responses and cellular immunity after two-dose and three-dose vaccination strategies are needed. In addition, this study was based on data from volunteers. Compared with large-scale clinical studies,

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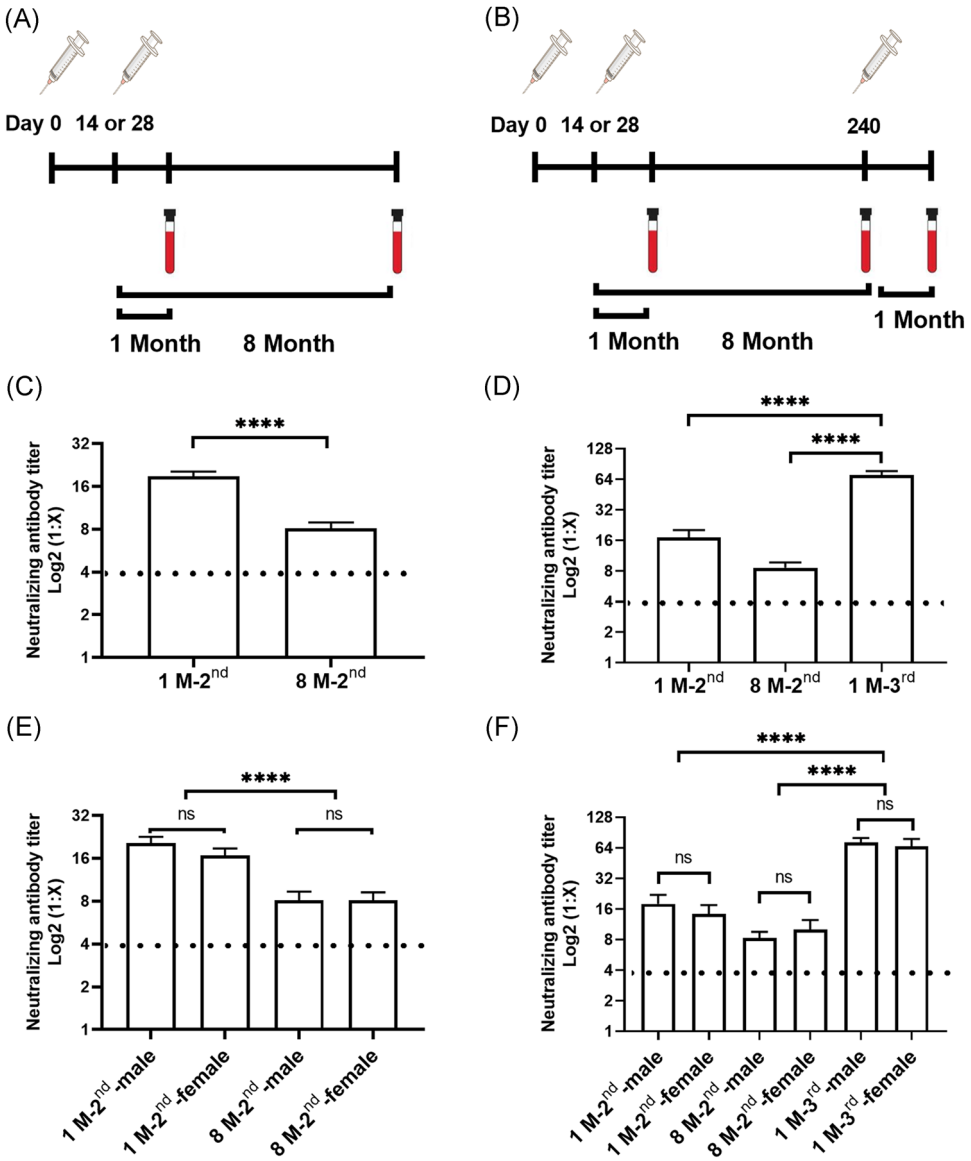

FIGURE 1 Three doses of vaccine elicited a more robust neutralizing antibody response than two doses of the inactivated severe acute respiratory syndrome coronavirus 2 vaccine. (A) and (B) The immunization and blood collection protocol for assigned to the immunization procedure with two-dose injections (A) or three-dose injections (B). (C) The persistence of neutralizing antibodies induced by the inactivated vaccine in individuals assigned to the immunization strategy with two-dose injections ($n = 355$). (D) Neutralizing antibodies boosted by the third dose of the inactivated vaccine ($n = 67$). (E) and (F) The influence of sex on neutralizing antibodies with two-dose (E) or three-dose (F) immunization strategy. (G) and (H) The influence of the immunization procedure at a second dose interval of 14 or 28 days on neutralizing antibodies with the two-dose injections (G) or three-dose (H) immunization strategy. (I) and (J) The influence of age on neutralizing antibodies with two-dose (I) or three-dose (J) immunization strategy. The neutralizing antibody-positive judgment threshold is marked with a dotted line. (****$p < 0.0001$; *ns*, not significant)

this study has many limitations, such as the sample size, age group, sex composition, and physical characteristics. Continuously observing the persistence of the protection provided by vaccines in real cases and the effectiveness of a booster dose (a third dose), conducting long-term clinical trials, and obtaining post-clinical data are essential tasks.

To some extent, this study showed that serum neutralizing antibody levels decreased after the second dose of inactivated vaccines. Importantly, the results suggest that a booster dose (a third dose) is necessary to maintain the effectiveness of inactivated vaccines regardless of sex and two-dose immunization procedure.

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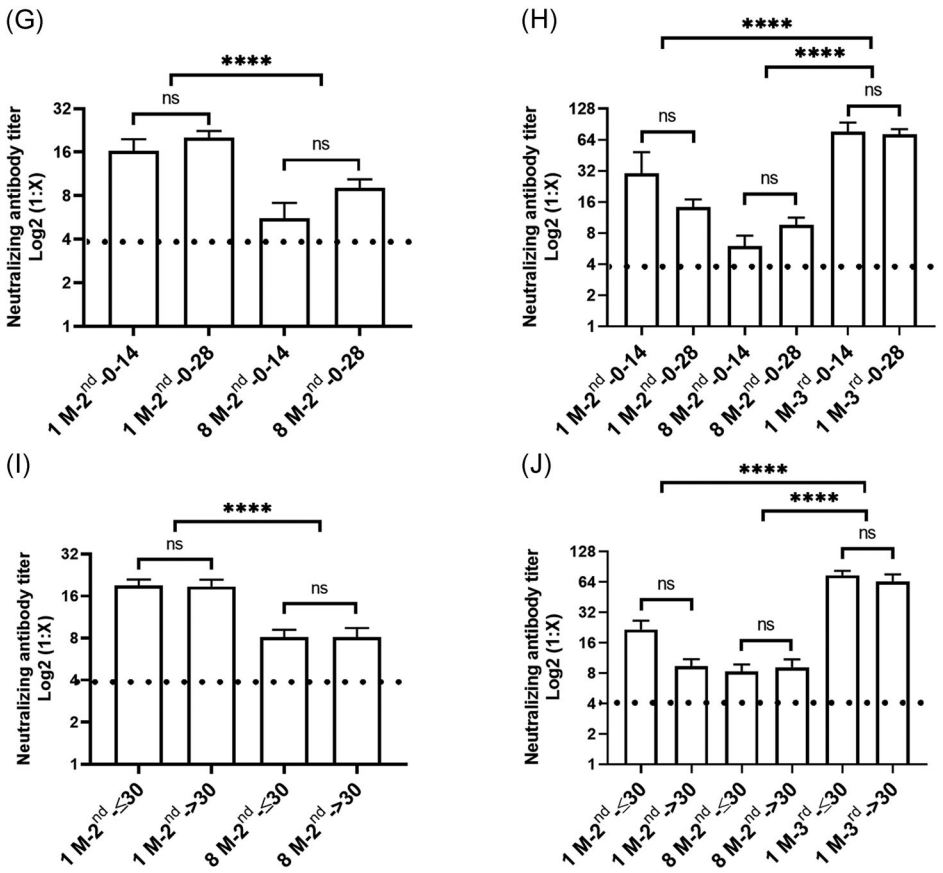

FIGURE 1 Continued

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CONFLICT OF INTERESTS

All the authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

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DATA AVAILABILITY STATEMENT

The results supporting the findings in this study are available upon request from the corresponding authors.

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