CORRESPONDENCE

Ferritin nanoparticle-based SARS-CoV-2 RBD vaccine induces a persistent antibody response and long-term memory in mice

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To date, the global coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in more than 90 million people infected and over 2 million deaths. A safe and effective vaccine is in high demand. For an effective vaccine, antibody persistence and long-term memory are favorable features. The poor antibody persistence after natural SARS-CoV-2 infection raised concerns about whether a vaccine could induce a long-lasting antibody response and whether a memory recall response would be induced upon reinfection.[1,2] Currently, over 200 vaccine candidates have been documented, some of which have advanced to clinical trials with encouraging results. However, to the best of our knowledge, the extent of antibody persistence and long-term memory post vaccination is still unclear. Here, we report that a ferritin nanoparticle (NP)-based SARS-CoV-2 receptor-binding domain (RBD) vaccine induced an efficient antibody response in mice that lasted for at least 7 months post immunization. A high number of memory B cells (MBCs) were maintained and recalled significantly upon antigen challenge.

The SpyTag/SpyCatcher technique-based click vaccine platform was developed in our laboratory and has been widely used.[3–5] The same strategy was applied for the construction of a ferritin NP-based SARS-CoV-2 RBD vaccine (Fig. 1a). Ferritin-NP-RBD was prepared as described in the supplementary information (Supplemental Fig. S1).

To assess the immunogenicity of ferritin-NP-RBD, naive wild-type (WT) C57BL/6 mice were immunized with a ferritin-NP-RBD vaccine or equimolar RBD-SpyTag as a control in the presence of CpG-1826 adjuvant at days 0, 14, and 28 (Fig. 1b). The ferritin-NP-RBD vaccine induced an approximately 100-fold higher antibody level than soluble RBD-SpyTag at day 28 (Fig. 1c). After the third immunization, the control vaccine group reached antibody titers of ~10^5 on day 35, and the ferritin-NP-RBD group reached antibody titers of ~10^6 (Fig. 1c). Thus, the RBD conjugated to ferritin NPs elicited a dramatically enhanced RBD-specific antibody response.

To test whether the antiserum induced by the ferritin-NP-RBD vaccine could provide protection against live SARS-CoV-2 in vitro, Vero cells were infected with live SARS-CoV-2 (C-Tan-nCoV strain 04) in the presence of day 35 sera from different immunization groups. The results showed that four out of five mice from the RBD-SpyTag group neutralized over 50% of the live virus at serum dilutions ranging from only 1:100 to 1:400, with an average 50% microneutralization (MN_{50}) titer of $10^{3.8}$/ml (Fig. 1d, e). Strikingly, all five mice from the ferritin-NP-RBD vaccine group had neutralizing effects at serum dilutions ranging from 1:1600 to 1:3200, with an average MN_{50} of $10^{4.8}$/ml (Fig. 1d, e). These results confirm that the antiserum to the RBD elicited by the ferritin-NP-RBD vaccine can prevent in vitro SARS-CoV-2 infection much more effectively than antiserum elicited by the RBD-SpyTag vaccine.

To determine the antibody persistence induced by the ferritin-NP-RBD vaccine in mice, we continued to monitor the antibody responses. The anti-RBD level at 5 months was comparable to that at day 35 (Fig. 1c). At 6 and 7 months, while the antibody endpoint titers of both groups gradually dropped, the ferritin-NP-RBD vaccine group still maintained significantly higher anti-RBD levels than the RBD-SpyTag control vaccine group (Fig. 1c), confirming the benefit of ferritin NPs for maintaining the antibody response.

To further determine whether ferritin NPs promote a better memory response, we first examined RBD-specific MBCs in the blood. At 6 months, a significantly higher number of RBD-specific MBCs was maintained in the ferritin-NP-RBD group than in the RBD-SpyTag control group (Fig. 1f, g). Consistent with the enhanced MBC formation and maintenance, when mice were challenged with RBD vaccine antigen at day 210, the ferritin-NP-RBD group elicited a dramatically increased antibody recall response at days 217 and 231 that was more than 2000 times stronger than that in the control group (Fig. 1c). More importantly, the antisera at days 217 and 231 from the ferritin-NP-RBD group demonstrated significant neutralizing activity against live virus, with an average MN_{50} titer of $10^{4.0}$/ml for day 217 antisera and $10^{4.3}$/ml for day 231 antisera, whereas no neutralization was detected in the RBD-SpyTag immunization group (Fig. 1h, i). Thus, the ferritin-NP-RBD vaccine induced not only a persistent RBD-specific antibody response but also long-term protective memory.

Ferritin NPs have recently been used in SARS-CoV-2 vaccine design,[6] in which a similar approach was used as we previously reported and applied here.[3,4] Upon two immunizations, titers of ~10^5 RBD-specific anti-IgG were detected. In our study, average titers of 2.2×10^5 in antisera were induced upon two immunizations, and titers of ~10^6 in antisera were induced upon three immunizations. Given this impressive antibody response, we further monitored antibody persistence and the memory response over 7 months, which is, to the best of our knowledge, the longest

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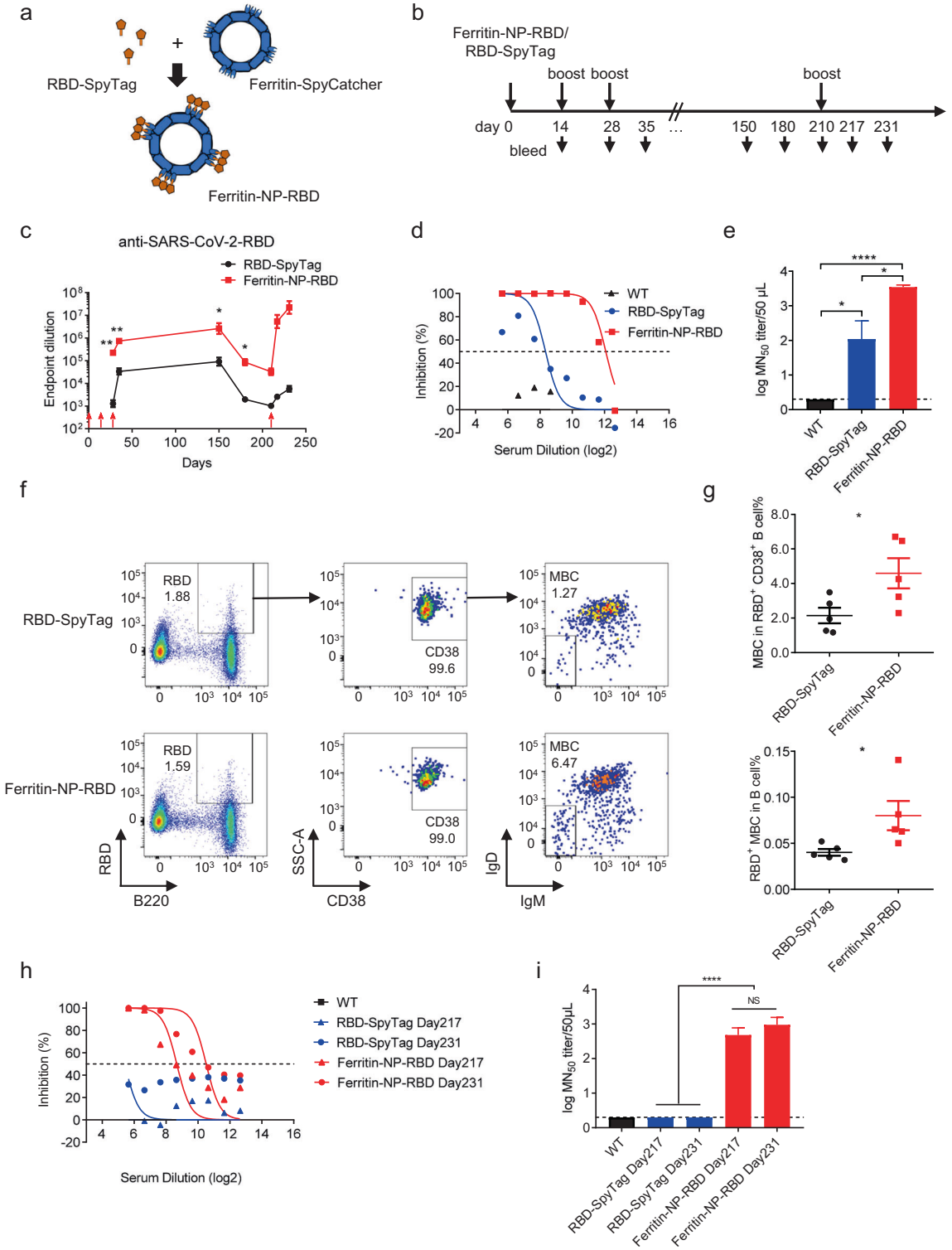

Fig. 1 The ferritin-NP-RBD vaccine induces a persistent antibody response and long-term memory. **a** Schematic illustration of ferritin-NP-RBD vaccine construction. **b** Naive WT C57BL/6 mice ($n = 5$) were subcutaneously immunized, boosted and bled at the indicated time points. **c** Anti-RBD responses were monitored and analyzed by ELISA. The red arrows indicate the immunization time points. **d**, **e** Live SARS-CoV-2 neutralization assay for sera collected from ferritin-NP-RBD- or RBD-SpyTag-immunized mice on day 35 or WT unimmunized mice. The inhibition (**d**) and MN50 titer (**e**) were calculated. **f**, **g** At 6 months after the first immunization, memory B cells in the peripheral blood were present (**f**) and statistically analyzed (**g**). Numbers adjacent to the outlined areas indicate the percentage of each gate. Data are shown as the mean ± SEM. **h**, **i** Live SARS-CoV-2 neutralization assay for sera collected from ferritin-NP-RBD- or RBD-SpyTag-immunized mice on day 217 and day 231 or WT unimmunized mice. The inhibition (**h**) and MN50 titer (**i**) were calculated

reported period for COVID-19 vaccine evaluation. The extended antibody persistence and well-boosted recall antibody response demonstrated in the current study support future success of ferritin-based COVID-19 vaccines.

Currently, multiple platforms are being used for SARS-CoV-2 vaccine development. Although vaccines come in different forms and are administered at different doses, our ferritin-based NP vaccine induced antibody titers (endpoint titer of 10^6) and live SARS-CoV-2-neutralizing activity roughly equal to those induced by the inactivated vaccine PiCoVacc,[7] mRNA-based vaccines[8,9] and an RBD-sc-dimer protein subunit vaccine.[10] In addition, more importantly, the current ferritin-NP-RBD vaccine induced a persistent antibody response and impressive long-term memory.

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AUTHOR CONTRIBUTIONS

W.W. conducted vaccine preparation, immunization, antibody titer measurements, and memory B cell determination; B.H. conducted antiserum neutralization assays; Y.Z. prepared vaccines; W.W., B.H., Y.Z., W.T. and M.Z. designed the experiments, analyzed the data, and wrote the manuscript; W.T. and M.Z. supervised the project; and M.Z. conceived the project.

ADDITIONAL INFORMATION

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Competing interests: The authors declare no competing interests.

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