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Engineering a self-navigated MnARK nanovaccine for inducing potent protective immunity against novel coronavirus

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Effective vaccines are vital to fight against the COVID-19 global pandemic. As a critical component of a subunit vaccine, the adjuvant is responsible for strengthening the antigen-induced immune responses. Here, we present a new nanovaccine that comprising the Receptor-Binding Domain (RBD) of spike protein and the manganese nanoadjuvant (MnARK), which induces humoral and cellular responses. Notably, even at a 5-fold lower antigen dose and with fewer injections, the MnARK vaccine immunized mice showed stronger neutralizing abilities against the infection of the pseudovirus (>270-fold) and live coronavirus (>8-fold) in vitro than that of Alum-adsorbed RBD vaccine (Alu-RBD). Furthermore, we found that the effective co-delivery of RBD antigen and MnARK to lymph nodes (LNs) elicited an increased cellular internalization and the activation of immune cells, including DCs, CD4\textsuperscript{+} and CD8\textsuperscript{+} T lymphocytes. Our findings highlight the importance of MnARK adjuvant in the design of novel coronavirus vaccines and provide a rationale strategy to design protective vaccines through promoting cellular internalization and the activation of immune-related pathways.

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Introduction

Multiple novel coronavirus vaccines have shown encouraging results and have been evaluated in preclinical or clinical trials \cite{1,2}. However, the insufficient production capacity would burden the herd immunity, and slow down the COVID-19 pandemic control steps \cite{3}. Besides optimizing the antigen, incorporating a suitable adjuvant into the subunit vaccine is one potential strategy to improve the weak RBD immunogenicity, decrease the number of vaccinations and antigen dosage \cite{4}, and induce potent neutralizing antibodies and cell-mediated immune responses to help arrest the COVID-19 pandemic \cite{3}, which is also important to avoid the risk of
vaccine-associated disease enhancement (VADE) caused by the deficient activation of CD8⁺ T cells [5,6]. As the only approved and safe adjuvant, Alum adjuvant facilitates antigen to produce a better immune response than free antigen. However, Alum-formulated vaccines are limited by the lack of cellular immune response. The manganese (Mn) nanoparticle adjuvants have shown great potential to activate the innate immune response and have been applied for cancer vaccines [7,8].

At present, the design and construction of vaccine systems that realize satisfactory antigen delivery (lymph node [LN]-targeting and efficient cytomembrane permeabilization) and activation of immune cells (dendritic cell (DC) and B cells) remain the major concerns for protein subunit-based vaccines [9,10]. The unique size and surface nature of functional nanomaterials facilitate to delivery of vaccine components (antigen and adjuvant) to key immune cells or lymphoid tissues [11–13] and improving the immune response to prevent infection [14–16]. The size-restrictive nature of LNs makes it arduous to deliver the vaccine specifically to immune cells [17]. Therefore, designing a simple approach to simultaneously realize the efficient delivery of antigen and adjuvant to LN, and activate the adaptive and innate immune responses is important for the novel coronavirus subunit vaccines.

The use of albumin can endow a vaccine with targeting abilities, for example, delivering an adjuvant, such as Evans blue or lipo-CpG, to LNs, thus promoting the induction of potent immune responses [10,18]. Notably, it’s also a good template to biomineralize small inorganic nanoparticles [19–21]. Inspired by the ability of albumin to potentiate vaccines, we set out to construct a nanovaccine via albumin to simultaneously deliver antigen and adjuvant to LNs. Exploiting the characteristics of albumin as a transporter and biotemplate, we proposed that an antigen-adjuvant-formulated nanovaccine (Fig. 1a), with a size of 10–100 nm, would accumulate in LNs after injection. To test this strategy, we constructed a nanovaccine against novel coronavirus, composed of the RBD antigen of the S1 protein and the manganese nanoadjuvant (MnARK), a negatively charged cubic manganese oxide nanoparticle known to potently activate the cGAS-STING pathway [22–24] and transport RBD antigens to LNs (Fig. 1b). Compared with the conventional Alum-adsorbed RBD vaccine (Alu-RBD), the nanovaccine (MnARK-RBD) significantly enhanced RBD-specific IgG (10-fold) and IgM (5-fold) responses in mice and improved the neutralization of novel coronavirus based on both pseudovirus (around 270-fold) and live virus (8-fold) evaluation systems. Our nanovaccine also induced a broader and stronger T cell response than the Alu-RBD vaccine, activating the cGAS-STING pathway and inducing a high-quality immunity.

Results

Design and construction of the MnARK nanovaccine

RBD can be fully exposed during the binding process between novel coronavirus and host cells and is readily recognized by the host immune system to produce immune responses [25]. Multiple recent reports have also supported the critical role of RBD in

![Fig. 1. Design of antigen/MnARK adjuvant co-delivered nanovaccine to combat novel coronavirus. (a) Schematic illustration of the construction of MnARK and the MnARK nanovaccine. First, MnARK is constructed by the in situ formation of Mn nanocubes via an albumin-template biomineralization process. Next, the RBD antigen is loaded onto the MnARK to form the nanovaccine. (b) Schematic representation of the utilization of the nanovaccine for protection from novel coronavirus infection. The vaccine is intramuscularly administered into the leg of BALB/c mice. The nanovaccine efficiently co-delivers antigens and adjuvants to LNs, and accumulates within antigen-presenting cells (APC), stimulating DC activation and antigen presentation to elicit potent, antigen-specific CD4⁺ and CD8⁺ T-cell responses and neutralizing antibodies. The nanovaccine activates the cGAS-STING pathway to generate humoral and cellular immunity.](image-url)
inducing anti-novel coronavirus neutralizing antibodies in various animal models and humans [26–28]. In the current study, we selected the RBD (amino acid residues 319–541) of the novel coronavirus spike protein as the vaccine antigen. We expressed the RBD antigen in the human-derived 293 T cell system to realize the natural protein glycosylation (N-glycosylation and O-glycosylation) and correct protein folding, both of which are crucial for inducing high-quality immune responses such as neutralizing antibodies against novel coronavirus [1,29]. Western blot analysis showed an obvious enrichment of a 35 kDa protein, which is consistent with the size of glycosylated RBD. The native LN-targeting ability of albumin facilitates vaccine accumulation in proximity to antigen-presenting cells (APCs) inside LNs [10,18].

In previous studies, we found that albumin is a facile biotemplate that can direct the formation of inorganic nanoparticles under mild conditions [30,31]. The physiochemical properties influence the stability, safety and biological effects of nanomaterials [12,32–34], and these properties are also critical to a vaccine. As shown in Fig. 2c, albumin programmed the formation of the manganese-based ‘ark’ (a cubic Mn3O4 nanoparticle with a diameter of ~9.77 ± 3.26 nm). The negative charge of MnARK facilitates the LN delivery of RBD [35], and the complementary surface charge between the albumin (negative charge) and RBD antigen (positive charge), favors the stable anchoring of the RBD antigen to MnARK. We confirmed the binding of the RBD antigen to MnARK by biolayer interferometry (BLI). The binding affinity was calculated to be about $K_D = 14.1$ nM (dissociation constant), with an association rate constant ($K_{on}$) of $9.13 \times 10^3$ M$^{-1}$ s$^{-1}$ and a dissociation rate constant ($K_{off}$) of $1.313 \times 10^{-4}$ s$^{-1}$ (Fig. 2h).

These findings indicate that the RBD antigen binds to MnARK with a high affinity. Subsequently, we optimized the binding ratio of MnARK and RBD antigen to formulate the nanovaccine. As shown in Figs. 2i and S1, the antigen loading efficiency of RBD antigen increased with increasing MnARK to RBD antigen ratio. When the mass ratio of MnARK to antigen reached 4:1, ~44.7% of RBD antigen anchored to MnARK to form a stable nanovaccine. The hydrodynamic diameter of MnARK increased from ~26 nm to ~55 nm after the formation of the nanovaccine, and the corresponding zeta potential decreased to $-18$ mV. The suitable size and negative charge of the nanovaccine predicts an efficient LNs-targeting delivery. The as formulated nanovaccine was stable at 4 °C and 25 °C for more than 42 days, presenting no significant changes in hydrodiameter or appearance (Fig. S1d).

Given the role of Mn in the activation of innate immune system, we evaluated the humoral profile of three Mn-based formulations ($\text{MnCl}_2$, Mn@HA (hyaluronic acid), MnARK). As shown in Fig. S2, all three Mn-based formulations showed significantly increased RBD-specific IgG levels compared with free RBD. Notably, the highest production of IgG was found in the MnARK nanovaccine group,
indicating that MnARK could trigger robust immunity response in vivo and might be superior to the MnCl₂ and Mn@HA.

**Dosage optimization of MnARK formulations**

Encouraged by the above results, we optimized the ratio of MnARK to RBD for inducing RBD-specific humoral and cellular responses in mice. As shown in Fig. S3, antigen-specific immune responses were MnARK dose-dependent in mice receiving either three injections (10 μg RBD per mouse per injection at differing ratios of RBD to MnARK) or two injections (50 μg RBD per mouse per injection at different ratios of RBD to Mn in MnARK) of nanovaccine. For the mice receiving three injections, 25 μg MnARK-RBD induced a significantly greater RBD-specific immunoglobulin (Ig) G (10–50 folds), IgM (5-fold) and T cell (>2-fold interferon (IFN)-γ production) responses than those of the 1 and 5 μg MnARK-RBD groups (Fig. S3a-c). Moreover, for the mice receiving two injections, 25 μg MnARK-RBD also induced significantly stronger vaccine-specific IgG (5-fold), IgM (5-fold) and T cell (6-fold IFN-γ production) responses than 1 and 5 μg MnARK (Fig. S3d-f). These results indicate that 25 μg MnARK-RBD is an optimized using dosage for enhancing RBD-specific humoral and cellular response in mice.

**MnARK nanovaccine requires a lower antigen dosage and fewer injections**

We next evaluated whether the MnARK-based nanovaccine can induce potent immunity at a relatively decreased dosage of antigen, which is an important indicator in the performance evaluation of a vaccine [36]. A lower dosage of antigen ensures favorable biosafety and decreases the cost of immunization. The MnARK nanovaccine (10 μg RBD and 25 μg MnARK) can induce a 5-fold stronger IgG and IgM response than that induced by either RBD alone (50 μg) or Alu-RBD (50 μg RBD and 175 μg aluminum; Fig. 3a–c). These results indicate that the MnARK-based nanovaccine requires at least a 5-fold lower dosage of antigen.

Effectiveness following fewer numbers of injections will decrease both invasiveness and the cost of a vaccine [37]. Thus, we assessed MnARK nanovaccine-induced immunity using fewer treatments. We compared the magnitude of the RBD-specific IgG/IgM response between mice receiving three injections of Alu-RBD (50 μg RBD and 175 μg aluminum) or RBD alone (50 μg) and mice receiving two injections of the MnARK nanovaccine (50 μg RBD and 25 μg MnARK). Two injections of the MnARK nanovaccine induced a 10-fold stronger IgG and 5-fold greater IgM response, compared with three injections of Alu-RBD or RBD alone.

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**Fig. 3.** IgG and IgM neutralizing antibody response induced by MnARK nanovaccine. 
(a) The reduction of antigen dosage. The mice were immunized with three injections of the nanovaccine (10 μg RBD), Alu-RBD (50 μg RBD) or RBD alone (50 μg RBD). (b and c) Three injections of MnARK (25 μg) enabled a low dosage of RBD (10 μg) to induce stronger IgG (b) and IgM (c) responses than a high dosage of RBD (50 μg) with an aluminum adjuvant or RBD (50 μg) alone. (d–f) The decrease in injection number. The mice were immunized by two injections of the nanovaccine (50 μg RBD) or three injections of Alu-RBD (50 μg RBD) or RBD alone (50 μg RBD). Using the same dosage of RBD (50 μg), two injections of the MnARK nanovaccine induced stronger IgG (e) and IgM (f) effects than three injections of Alu-RBD or RBD alone. 
(g) The effects of the nanovaccine on inducing neutralizing antibodies. (h and i) In both evaluation systems of pseudotyped virus (h) and live virus (i), the nanovaccine (25 μg MnARK / 50 μg RBD) induced a significantly higher titer of neutralizing antibodies than RBD (50 μg) alone or Alu-RBD (175 μg aluminum / 50 μg RBD). The data are shown as the mean ± SD. Statistical significance was tested with a two-tailed, unpaired Student’s t-test. *significant difference (P < 0.05).
in mice (Fig. 3d–f). These results indicate that the MnARK nanovaccine can produce a stronger humoral response after receiving fewer injections.

**MnARK nanovaccine improves the neutralizing antibody response**

To determine whether the MnARK nanovaccine can enhance the neutralization against novel coronavirus, we evaluated the neutralizing antibody response induced by the MnARK nanovaccine in mice in the subsequent experiments (Fig. 3g). We divided the BALB/c mice into four groups: MnARK-RBD (10 μg RBD/25 μg MnARK), Alu-RBD (50 μg RBD/175 μg aluminum), RBD alone (50 μg RBD) and saline (control). Each group contained six mice, each of which received three injections at an interval of 3 weeks. Mouse serum samples from all groups were detected by neutralization assays using both pseudotyped and live virus systems. In the pseudovirus system, 60-fold diluted serum samples from the MnARK-RBD group exhibited a greater than 90% inhibitory concentration (IC), i.e., the IC50 value was > 60-fold serum dilution (Fig. 3h). The serum dilution capable of inhibiting 50% (IC50) was 540, which far higher than that of the Alu-RBD group (~20; Fig. 3h). The use of RBD alone fails to realize 50% neutralization even in the case of a 20-fold dilution. In the live virus evaluation system, the IC50 value was > 8 in the MnARK-RBD group. In contrast, both Alu-RBD and RBD alone were unable to realize 50% neutralization even at a 2-fold dilution (Fig. 3i). The above results indicate that the MnARK nanovaccine can induce an enhanced neutralizing antibody response against novel coronavirus.

**Co-internalization of MnARK and antigen drives activation and antigen-presentation programs in DCs**

To verify whether the MnARK nanovaccine induces potent antibody and T cell responses, we evaluated the ability of the MnARK nanovaccine to target LNs, enter APCs and activate DCs. These represent the critical steps in determining the magnitude of antibody and T cell responses [38]. To assess lymphatic delivery in vivo, Cy5-tagged RBD antigen or MnARK nanovaccine was intramuscularly injected into the right leg of BALB/c mice. As shown in Fig. 4b, MnARK induced a greater depot of RBD antigen at the injection site, compared with that of RBD alone, and the antigen persisted for over 3 days at the site. The axillary and inguinal LN were harvested for imaging 12, 24, 48 and 72 h post-injection (Fig. 4c). At 12 h, compared with the Cy5-labeled RBD antigen group, the MnARK nanovaccine elicited stronger fluorescence signals in the LN and weaker fluorescence signals in the liver (Fig. S4). Quantitative analysis revealed that the MnARK nanovaccine had accumulated in LNs approximately two times more efficiently than the free RBD antigen 12 h after injection (Figs. 4d and 55), indicating that the MnARK nanovaccine promotes in vivo delivery of antigen to LN for subsequent uptake by DCs in LN. The effective retention of nanovaccine in LNs at 12 h induced an approximately 2-fold greater internalization of RBD in DCs than the free RBD, eliciting a time-dependent antigen internalization and exocytosis process in DCs (Fig. 4e). In the group that received the MnARK nanovaccine, the expression of MHC-I and CD86 on the surface of DCs significantly increased in comparison with DCs from mice that received RBD alone, indicating an effect of the MnARK nanovaccine in activating DCs in vivo (Fig. 4f and g). Then, we evaluated the cytotoxicity profile of MnARK and Mn2+ to DCs by the CCK-8 assay. Compared with Mn2+, those MnARK nanoparticles showed no significant cytotoxicity to DCs in vitro even at the concentration of 0.5 mM (Fig. 56). Afterward, the efficiency of DC maturation was assessed using CD80 and CD86 as the markers. Notably, compared to the DC maturation levels achieved by free Mn2+, MnARK could induce a higher level of DC maturation (Fig. 56). DCs determine the presentation of T cell epitopes [36,39]. Thus, the activation of DCs can dramatically facilitate a vaccine’s ability to induce potent T cell responses [40]. Furthermore, we examined the expression of three critical indicators (major histocompatibility complex (MHC) class II molecules, CD69 and CD86) on the surface of B cells to evaluate the maturation of B cells [41,42]. B cells from the mouse group receiving the MnARK nanovaccine exhibited a significantly greater expression of MHC-II, CD69 and CD86 than those in the RBD group, indicating that MnARK nanovaccine can promote the maturation of B cells in vivo (Fig. S7). To understand the process of nanovaccine uptake and immune regulations route, the FITC-tagged nanovaccine were imaged through both confocal and synchrotron radiation hard X-ray nano-resolution computed tomography (nano-CT), to observe the RBD and MnARK distribution in DCs. As shown in Fig. 4h and Supplementary Movie 1, 2, 3, RBD and MnARK exhibited a time-dependent antigen internalization process in DCs. This validated the possibilities of intracellular antigen delivery by MnARK. The enhanced LN delivery and DC uptake suggest the possibility of antigen-specific immune responses.

**Specific T-cell response and intracellular cytokine secretion**

We proceeded to evaluate the enhancement of the RBD-specific T cell response induced by the MnARK nanovaccine. The vaccine-specific IFN-γ production was 2-fold and 6-fold stronger in mice receiving three and two MnARK nanovaccine injections, respectively, in comparison to those receiving Alu-RBD or RBD alone (Fig. 5a, b). We also used multicolor flow cytometry to more comprehensively analyze MnARK nanovaccine-induced cellular immune responses in the four mouse groups. Six markers (IFN-γ, TNF-α, IL-2, CD134, CD137, CD69) expressed by CD4+ and CD8+ T cells were selected as indicators for evaluating the performance of MnARK nanovaccine-induced cellular immune response [39,43]. All of these proteins are critical for the clearance of novel coronavirus or novel coronavirus infected cells. In the CD4+ T cell subgroup, the expression of all six proteins was significantly enhanced (P < 0.05) in the MnARK nanovaccine group, in comparison to both the Alu-RBD and RBD alone groups (Fig. 5e, f). Similarly, in the CD8+ T cell subgroup, the expression of IFN-γ, IL-2, CD134 and CD137 were significantly greater in the MnARK nanovaccine group than in either the Alu-RBD or RBD alone groups (P < 0.05). There were no significant differences in the expression of TNF-α and CD69 between the MnARK nanovaccine group and the Alu-RBD or RBD alone groups (P > 0.05; Fig. 5g, h). These results demonstrate that the MnARK nanovaccine can induce a stronger and broader cellular immune response than the antigen alone or the conventional alum-containing formulation. Considering memory CD8 T cells are a critical component of protective immunity, and inducing effective memory T-cell responses is a major goal of vaccines against chronic infections, we examined the phenotype of the memory CD8 T cells in mice at day 56 post-infection (Fig. S8). Flow cytometry results revealed that high dosage of MnARK induced differentiation of effector memory (TEM, CD44hiCD62Llow), whereas low dosage of MnARK increased proliferative capacity of central memory (TCM, CD44+CD62Lhi). This conversion from TEM to TCM correlates with enhanced protective immunity, IL-2 production, and homeostatic turnover resulting in long-term antigen-independent maintenance. Furthermore, after different treatments, we did not observe major alterations in body weight and blood biochemical parameters (Fig. S9 and Tables S1–S3). Hematoxylin and eosin (H&E) staining images confirmed no obvious damage or inflammation infiltration in the major organs, suggesting favorable biosafety of the nanovaccine (Fig. 510).
Mechanism of MnARK-induced novel coronavirus immune responses

To explore the molecular mechanism of the MnARK nanovaccine’s improvement of RBD-specific immune responses in vivo, we analyzed the transcriptomes of splenocytes isolated from mice treated with the MnARK nanovaccine (25 μg MnARK/10 μg RBD; Fig. 6a). Using principal component analysis (PCA) to assess overall sample-to-sample distance, the transcriptomes of the mice treated...
Fig. 5. Enhancement of the T-cell response by the MnARK nanovaccine. (a) After three injections, the nanovaccine (10 μg RBD) induced a stronger T cell response (IFN-γ production) in mice, in comparison with Alu-RBD (50 μg RBD) or RBD alone (50 μg RBD). (b) Using the same dosage of RBD (50 μg), two injections of the nanovaccine induced a stronger T cell response (IFN-γ production) than three injections of Alu-RBD or RBD alone. (c and d) Flow cytometry analysis of CD4+ T cells, which express interleukin (IL)-2, CD137, CD134, tumor necrosis factor (TNF)-α, IFN-γ and CD69, from mice receiving nanovaccine, Alu-RBD or RBD alone. (e and f) Flow cytometry analysis of CD8+ T cells, which express IL-2, CD137, CD134, TNF-α, IFN-γ and CD69, from mice receiving nanovaccine, Alu-RBD or RBD alone. The data are shown as the mean ± SD. Statistical significance was tested with a two-tailed, unpaired Student’s t-test. *P < 0.05.
Fig. 6. Molecular mechanism of the stimulation of the antigen-induced immune response by the MnARK adjuvant. (a) Illustration of the transcriptome analysis of splenocytes isolated from mice treated with nanovaccine or RBD alone. (b) Principal component analysis (PCA) between the nanovaccine group and the RBD group. (c) Heat map based on differentially expressed genes (DEGs) which are identified by two criteria: (1) fold change in expression is ×2; (2) FDR adjusted p-value is <0.05. (d) Visualized enrichment networks by Enrichment Map based on the gene ontology enrichment analysis (GOEA) using BINGO. (e) Visualized protein-protein interaction network by Cytoscape software based on weighted gene co-expression network analysis (WGCNA). (f) RT-PCR analysis of the STING-related and MAPK-associated genes. (g) Western blot analysis of the activation of the cGAS-STING pathway in DC2.4 cell after treatment with RBD or nanovaccine for 24 h. (h) Schematic illustration of the activation of cGAS-STING pathway. The data are shown as the mean ± SD. Statistical significance was tested with a two-tailed, unpaired Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001.
with the MnARK nanovaccine were distinguishable from those of mice treated with RBD only (Fig. 6b). In total, we obtained 462 differentially expressed genes (DEGs; fold change > 2, FDR adjusted P-value < 0.05). A heat map of the DEGs visually confirmed the significantly different expression profiles between the MnARK nanovaccine (127 up-regulated genes) and RBD-treated mice (Fig. 6c). To link DEGs to biological processes, we performed gene ontology (GO) enrichment analysis (GOEA) using BiNGO and visualized the enrichment networks using EnrichmentMap (Fig. 6d). This GO-term network was divided into 10 functional clusters/modules, all of which were related to immune processes, such as MAPK-mediated immunoregulation, immune receptor expression, intracellular signal transmission, acute inflammatory response ionizing radiation and antigen receptor-mediated cytotoxicity, among others. To investigate the network connections among DEGs, we applied weighted gene coexpression network analysis (WGCNA) and visualized the network using Cytoscape software. The expression of six cytokine-encoding genes (TNF-α, IL-2, IFN-γ, CD69, CD134 and CD137), which are critical for the inhibition and clearance of novel coronavirus, as well as antibody expression genes, were analyzed in the connection network. We extracted the coexpression network and identified the connections among these genes (Fig. 6e). Using the gene set enrichment analysis (GSEA), we found the majority of genes from nanovaccine-injected mice are related to the positive regulation of the expression of type I interferons (Fig. S11a and b). Considering the close correlation between type I interferons and cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway [44], we tried to verify whether the MnARK nanovaccine can regulate immune responses via the cGAS-STING pathway [8,23].

As shown in Fig. 6f and g, RT-PCR and western blot analysis results demonstrate that the MnARK nanovaccine can indeed activate the cGAS-STING pathway in DC2.4 cells to enhance RBD-specific humoral and cellular immune responses. Such a conclusion is also consistent with the data of our transcriptome analysis and previous reports [45]. Multiple cGAS-STING pathway key genes (interferon-induced transmembrane protein 10 (IFitm10), IFN, TNF-α) show connections with the MAPK-mediated immunoregulation functional cluster which has been identified in our transcriptome analysis. Taken together, these data indicate that the MnARK nanovaccine enhances immune responses via the cGAS-STING pathway which may be closely connected to MAPK-mediated immunoregulation (Fig. 6f).

Discussion

In summary, we have developed a MnARK-based nanovaccine for preventing novel coronavirus infection. Our nanovaccine is a programmable platform, whose synthesis is facile, with the ability to enhance the co-delivery efficiency of RBD antigen and a nanoadjuvant to LNs. We stably and efficiently co-loaded antigen with the MnARK adjuvant on a nanovaccine, without any complicated chemical conjugation or genetic recombination processes, to enhance antigen-specific antibody and T cell responses. The natural targeting property of albumin and the size of the nanovaccine make the formulation amenable to efficient draining and retention in LNs, leading to enhanced uptake and the activation of DCs, which are responsible for enhancing antigen-specific immune responses. The use of the MnARK nanovaccine in mice elicited a potent humoral and cellular immune response, even when reducing the antigen dosage and number of injections. Importantly, a potent neutralizing antibody response induced by MnARK nanovaccine strongly supports its promising potential for realizing a satisfactory protective immunity against novel coronavirus. Moreover, the low cost of nanovaccine components further allows its clinical investigation in our next studies.

The MnARK nanovaccine presents some unparalleled advantages over other types of vaccines. For example, in comparison with DNA, mRNA and virus vector-based vaccines, the MnARK nanovaccine uses the RBD protein antigen to induce antigen-specific immune responses in a reduced time frame, bypassing the transcription/translation process of antigens inside host cells. In contrast to inactivated virus-based vaccines, the MnARK nanovaccine comprises a clearly defined set of components, thus avoiding any unpredictable side effects and risks caused by some unknown vaccine components. The combination of an adjuvant with the RBD protein in a MnARK nanovaccine may be an ideal strategy for the development of vaccines to combat novel coronavirus or other coronaviruses.

CRediT authorship contribution statement

All authors contributed to the article and approved the submitted version. All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Chunying Chen, Ye Liu and Yaling Wang conceived and designed the experiments. Ye Liu, Yuping Xie and Guoyang Liao designed and produced immunogens. Yuping Xie, Jia Luo, Liangnian Wei, Jia Zhou, Chunyan He, Yufang Zhang, Ye Zhang and Sisi Chen performed animal experiments. Jia Luo, Liangnian Wei, Jia Zhou, Yun Chen and Lijuan Shen carried out antibodies and T cell response experiments and analysis. Ye Liu, Yunfei Ma, and Ruixin Wang analyzed the transcriptomes data. Mengyu Guo, Xuhao Hu, Nasha Qiuyi and Ying Liu performed western and PCR experiments. Yaling Wang, Xi Chen, Ziwei Chen, Xinyi Lu, Yanyan Cui and Lichun Mao performed preparation, TEM, XRD, DLS and BLI analysis of nanovaccine. Yaling Wang and Kai Zhang designed and performed nano-CT data collection and analysis. Chunying Chen, Ye Liu, Yaling Wang and Mengyu Guo wrote the manuscript. All authors discussed the results and commented on the manuscript.
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