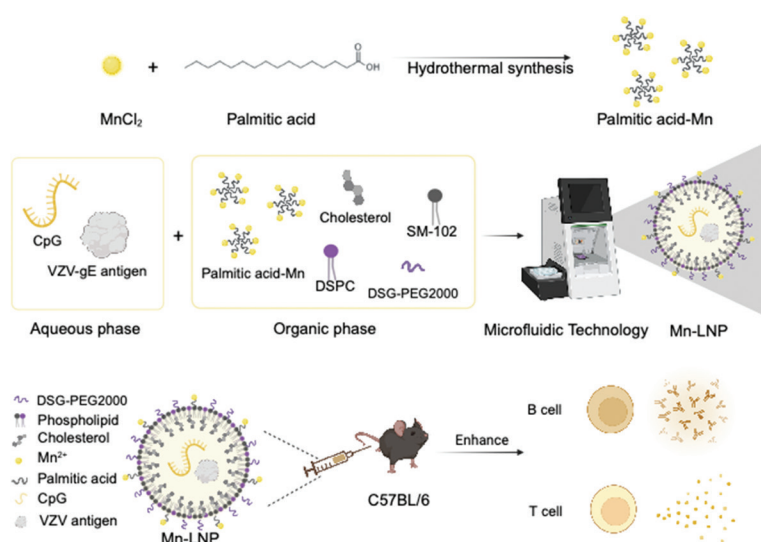




ORIGINAL ARTICLE

Manganese-Coordinated Lipid Nanoparticles for Co-Delivery of VZV Antigen and CpG Adjuvant Enhance Vaccine Efficacy

Graphical abstract



Highlights

- This study explored an Mn-LNP-delivered system to boost the efficacy of the varicella zoster virus (VZV) vaccine, on the basis of hydrothermal synthesis, microfluidic chip technology, and multiple immunological assays.
- We synthesized and characterized Mn-LNPs, which are spherical particles approximately 270 nm in diameter that encapsulated the VZV glycoprotein E (gE) antigen and CpG adjuvant in a 1:2 mass ratio. In a mouse model, the Mn-LNP-delivered VZV vaccine, compared with the aluminum adjuvant, significantly enhanced VZV gE antigen-specific CD4⁺ and CD8⁺ T cell responses (1.47-fold and 5.66-fold enhanced IFN- γ response for CD4⁺ T cells and CD8⁺ T cells; 2.25-fold and 2.64-fold enhanced TNF- α response for CD4⁺ T cells and CD8⁺ T cells; and 2.62-fold and 3.17-fold enhanced IL-2 response for CD4⁺ T cells and CD8⁺ T cells).
- Furthermore, the Mn-LNP-delivered VZV vaccine markedly enhanced the antigen-specific IgG2b subclass response in vivo. These results indicated that the Mn-LNP-delivered system has substantial potential in improving VZV vaccine efficacy.

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Brief statement

This study investigated a manganese-coordinated lipid nanoparticle (Mn-LNP) system for co-delivering varicella-zoster virus (VZV) glycoprotein E (gE) antigen and CpG adjuvant to enhance vaccine efficacy. By integrating Mn^{2+} with lipid nanoparticles, the researchers developed a stable, spherical delivery platform (270 nm diameter) that significantly increased both humoral and cellular immunity. In mouse models, the Mn-LNP-delivered VZV vaccine elicited robust Th1 immune responses, including a 5.66-fold increase in CD8⁺ T cell IFN- γ production and elevated antigen-specific IgG2b titers (1.29-fold vs. aluminum adjuvant). Mechanistically, Mn^{2+} acted synergistically with CpG in activating innate immune pathways, while the LNP structure ensured efficient co-delivery (79.9% encapsulation efficiency). These findings demonstrate that Mn-LNPs are a versatile platform that overcomes the limitations of conventional VZV vaccines, thus offering a promising strategy to balance immunogenicity and delivery efficiency for herpes zoster prevention.

Manganese-Coordinated Lipid Nanoparticles for Co-Delivery of VZV Antigen and CpG Adjuvant Enhance Vaccine Efficacy

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Abstract

Objective: Because varicella zoster virus (VZV) infections are continually increasing, vaccine efficacy must be enhanced. To date, the inefficient in vivo utilization of antigens and adjuvants has limited vaccine-induced immune protection against VZV infection. Therefore, improving the antigen and adjuvant utilization efficiency is crucial to enhance VZV vaccine performance.

Methods: To achieve favorable VZV antigen and adjuvant utilization efficiency, we constructed a co-delivery system based on manganese-coordinated lipid nanoparticles (Mn-LNPs). Palmitic acid was coordinated with manganese through hydrothermal synthesis. Furthermore, 1.6 molar palmitic acid-Mn was added to four commercial lipid nanoparticle components (SM-102:DSPC:CHOL:HP:DSG-PEG2000 in a 50:10:37.5:2.5 molar ratio) to encapsulate VZV glycoprotein E (gE) antigen and CpG adjuvant by using a microfluidic chip. We characterized Mn-LNP size and morphology through transmission electron microscopy and quantified the manganese on palmitic acid-Mn through X-ray photoelectron spectroscopy. We also evaluated the performance of the Mn-LNP co-delivery system in enhancing VZV vaccine-induced immune responses in a mouse model.

Results: The Mn-LNPs comprised positively charged spherical particles of approximately 270 nm in diameter, which encapsulated VZV gE antigen and CpG adjuvant in a 1:2 mass ratio. The Mn-LNP co-delivery system, compared with aluminum adjuvant, significantly enhanced VZV gE antigen-specific CD4⁺ and CD8⁺ T cell responses (1.47-fold and 5.66-fold enhanced IFN- γ response for CD4⁺ T cells and CD8⁺ T cells; 2.25-fold and 2.64-fold enhanced TNF- α response for CD4⁺ T cells and CD8⁺ T cells; and 2.62-fold and 3.17-fold enhanced IL-2 response for CD4⁺ T cells and CD8⁺ T cells). Moreover, the Mn-LNPs, compared with commercial aluminum adjuvant, significantly enhanced the antigen-specific IgG2b subclass response.

Conclusion: The Mn-LNP co-delivery system efficiently delivered both VZV antigen and CpG adjuvant, and markedly improved the VZV vaccine-induced T cell response. Thus, this Mn-LNP co-delivery system has promising potential in promoting VZV vaccine efficacy.

Key words: VZV vaccine, Co-delivery, Manganese, CpG, Lipid nanoparticles

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INTRODUCTION

VZV, a highly contagious member of the α -herpesvirus family, can lead to serious varicella and zoster infections in clinical settings [1]. Initial VZV infection causes chickenpox in children, who frequently present with rash, fever, and general discomfort [2,3]. After the initial infection, the virus can remain dormant in the nervous system. When the host immune system weakens, VZV can reactivate and lead to serious zoster infection [4,5]. To prevent this disease, two vaccine types against varicella and zoster have been approved for human use [6–8]. Merck's Zostavax vaccine is used primarily for zoster prevention in older people [9]. However, the aluminum hydroxide adjuvant is not used in the Zostavax vaccine due to it tends to promote a humoral response instead of the cellular response critical for controlling zoster [8]. Shingrix, an AS01B-adjuvanted recombinant VZV gE protein vaccine manufactured by GlaxoSmithKline, has shown potential efficacy in preventing shingles and its complications [10]. However, the need for bedside mixing and the restricted availability of the QS-21 component have hindered its widespread application [11,12]. Therefore, new strategies are necessary to enhance the VZV vaccine-induced immune response and protective efficacy.

To improve VZV vaccine efficacy, effective *in vivo* co-delivery of antigen and adjuvant is critical. Lipid nanoparticles (LNPs) have shown great potential in enhancing the immunogenicity and efficacy of mRNA vaccines, because of their mRNA packaging ability, efficient *in vivo* delivery efficacy, and adjustable drug release properties [13–16]. However, almost all existing LNPs have been used for packaging and delivering nucleic acids rather than proteins. Here, we sought to develop an LNP-based system to co-deliver VZV protein antigen and nucleic acid adjuvant (CpG), thereby broadening the application scenarios of LNPs [17,18].

Encapsulation of the VZV gE antigen by Mn-LNPs prevented its enzymatic hydrolysis and premature degradation, and precisely delivered it to antigen-presenting cells (APCs) such as dendritic cells (DCs). LNPs increase the contact between antigens and APCs, and enhance antigen internalization efficiency. The CpG adjuvant specifically activates Toll-like receptor 9 (TLR9), thereby leading to IRF7 activation. The CpG adjuvant promoted the VZV gE antigen-specific T-cell immune response by inducing a strong Th1 cell-mediated immune response and the production of cytotoxic T cells. Moreover, we added palmitic acid-coordinated manganese (palmitic acid-Mn) as a carrier component to construct Mn-LNPs inducing a potent immune response. Manganese ions (Mn^{2+}) activate pattern-recognition receptors in DCs, such as Toll-like receptors (TLR4/9) and the cGAS-STING pathway, thereby increasing DC maturation markers (CD80/CD86 and MHC-II) and promoting the presentation of MHC complexes to T cells [19–23]. Our Mn-LNP co-delivery system for the VZV protein vaccine, compared with

conventional aluminum adjuvants, markedly enhanced the cellular (5-fold) and IgG2b (1.3-fold) response.

MATERIALS AND METHODS

Adjuvants and reagents

Palmitic acid and $MnCl_2$ were purchased from Shanghai Adamas Reagent Co., Ltd.; 1,2-distearoyl-SN-glycero-3-phosphocholine (DSPC) and heptadecan-9-yl-8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate (SM-102, HUO) were purchased from Xiamen Sanobang Biotech Co., Ltd.; and 1, 2-distearoyl-RAC-glycerol-3-methoxypolyethylene glycol (DSG-PEG2000) and cholest-5-en-3 β -ol (CHO-HP) were purchased from Aivito (Shanghai) Pharmaceutical Technology Co., Ltd. The CpG ODN adjuvant was purchased from Huapu Biotechnology (Hebei) Co., Ltd. Aluminum hydroxide and VZV gE protein were synthesized by the Institute of Medical Biology, Chinese Academy of Medical Sciences. Horseradish peroxidase labeled sheep anti-mouse IgG was purchased from Anolun (Beijing) Biotechnology Co., Ltd. A BCA protein concentration detection kit was purchased from Shanghai Biyuntian Biotechnology Co., Ltd.

Synthesis of palmitic acid-Mn

First, we used a 6.36:1 molar ratio of $MnCl_2 \cdot 12H_2O$:palmitic acid. We precisely weighed 184 mg $MnCl_2 \cdot 12H_2O$ and dissolved it in 3.68 ml N, N-dimethylformamide (DMF) solvent. This procedure enabled the preparation of solution A, in which the concentration of Mn^{2+} was 50 mg/ml. Subsequently, we accurately weighed 58.8 mg palmitic acid and dissolved it in 1.96 ml DMF solvent, thereby obtaining solution B, with a palmitic acid concentration of 30 mg/ml. After fully mixing solution A and solution B, we added 1 ml triethylamine solution as a catalyst to enhance the intensity of the complexation reaction. Concurrently, 500 mg of polyvinylpyrrolidone-K30 (PVP-K30) was added as a dispersant within the reaction system. We then supplemented the reaction mixture with 20 ml absolute ethanol and 30 ml DMF as the solvent system. The resultant mixture was shaken well with a vortex mixer to ensure uniform distribution and then subjected to ultrasonication for 1 hour. The liquid reaction mixture was then transferred to a hydrothermal reaction autoclave at 150°C for 24 hours. After the mixture had cooled to room temperature naturally, the reaction product was isolated. The product was subjected to a series of washing and redispersion steps with absolute ethanol as the washing solvent. Specifically, it was washed three times under centrifugation at 11,000 rpm, then resuspended in absolute ethanol for subsequent experimental use.

Preparation of Mn-LNPs

Mn-LNPs were prepared with a microfluidic device (INano L, Micro&Nano Biologics Co. Ltd., China). The 1.5 mg palmitic acid-Mn synthesized in the previous step

was mixed with 2.78 mg SM-102, 0.71 mg DSPC, 1.215 mg CHO-HP, and 0.3 mg DSG-PEG2000 (molar ratio of 50:10:37.5:2.5) in 0.5 ml ethanol as the organic phase. Subsequently, 0.3 mg VZV gE antigen and 0.6 mg CpG adjuvant (mass ratio of 1:2) were dissolved in 1.5 ml 1× PBS buffer as the aqueous phase. Mn-LNPs were obtained by mixture of the organic phase and the aqueous phase [1:3 (v/v)] in a microfluidic device at a flow rate of 9 ml/min. Finally, Mn-LNPs were added to a 10 kD tube for ultrafiltration 100 times with 1× PBS buffer. After ultrafiltration, we measured the concentration of free protein in 100 µl Mn-LNPs with a BCA protein assay kit (Biyuntian Biotechnology Co., Ltd). First, we mixed the Mn-LNPs sample with a protein quantification dye (BCA kit) and measured the concentration of unencapsulated VZV gE protein (UgE). Second, we added a surfactant (Triton X-100) to disrupt the LNPs, released the encapsulated VZV gE protein, and calculated the total amount of VZV gE protein (TgE) in the sample according to fluorescence. The encapsulation efficiency of Mn-LNPs was calculated with the following formula: encapsulation efficiency % = [(TgE - UgE)/TgE × 100].

Finally, we observed the particle morphology under a transmission electron microscope after filtration through a 0.22-micron membrane. We used a Zetasizer Nano ZS90 instrument to measure the average particle size and zeta potential of the Mn-LNPs.

Animal experiments

C57BL/6 mice were provided by, and housed under standard laboratory conditions at, the Laboratory Animal Center, Institute of Medical Biology, Chinese Academy of Medical Science, under laboratory animal license SYXK (Yunnan) K2018-0007.

Immunization and sampling

C57BL/6 mice (6–8 weeks old) were divided into three groups (n = 5 per group) receiving the Mn-LNP-delivered VZV vaccine, aluminum adjuvanted VZV vaccine, or vehicle control without processing. The mice were immunized by intramuscular injection on days 0, 14, and 28. Blood samples were collected from the tail veins 7 days after the second immunization and 14 days after the last immunization. Samples were kept at room temperature for 3 hours and centrifuged at 1200 rpm for 10 min. The sera were then collected and stored at -20°C for ELISA. On day 56 after the first immunization, we euthanized the mice and harvested their spleens for subsequent flow cytometry and ELISpot assays.

ELISA

First, we coated 96-well plate with VZV protein at a concentration of 2 µg/ml with 100 µl per well, and incubated it overnight at 4°C. Afterward, we removed the solution from the plate and washed the wells four or five times with PBST (0.01 mol/l PBS + 0.05% Tween), then tapped the plate to remove excess moisture. Next, we

blocked the wells with PBS containing 1% BSA and incubated the plate at 37°C for 2 hours. We then removed the solution, washed the wells four or five times with PBST, and tapped the plate to dry the wells. Subsequently, we added HRP-labeled goat anti-mouse IgG/IgG1/IgG2a/IgG2b/IgG3 and incubated the plate at 37°C for 1 hour. We then removed the solution, washed the wells four or five times with PBST, and tapped the plate to dry the wells. Finally, we added 100 µl TMB substrate mixed with hydrogen peroxide (H₂O₂) to each well and incubated the plate at 37°C for 10 min. To stop the reaction, we added 100 µl 1 mol/l H₂SO₄ to each well. We then measured the absorbance at 450 nm and 630 nm with a microplate reader.

ELISpot analysis

We performed an IFN-γ-based ELISpot assay with a Mouse IFN-γ ELISpot PLUS kit (ELISpot kit, Mabtech) to determine antigen-specific T cell responses according to the kit instructions. After being washed four times with sterile PBS (200 µl per well), the plates were blocked with DMEM containing 10% FBS (200 µl per well) for at least 30 min at room temperature. Freshly extracted splenocytes (5 × 10⁵ cells per well) from Mn-LNP-vaccinated mice at day 56 after immunization and stimulant (2 µg/ml) were simultaneously added to the plates. Unstimulated cells served as a negative control. The plates were incubated at 37°C under 5% CO₂ for 36 hours. Subsequently, the cells were removed, and the plates were washed five times with PBS (200 µl per well). The biotinylated IFN-γ detection antibody, streptavidin-alkaline phosphatase conjugate, and substrate were subsequently added to the plates in a stepwise manner, according to the manufacturer's instructions. When spots had clearly emerged, development was stopped by rinsing of the plates with deionized water. The numbers of spots were determined with an automatic ELISpot reader.

Cells stimulated with a 400× Cell Activation Cocktail (without brefeldin A) (BioLegend Cat #423301) served as a positive control, whereas the negative control used the same volume of DMSO.

Flow cytometry analysis of T cells

We collected the spleens and finely ground them in 5 ml 1× PBS in a Petri dish, then filtered the samples through a 70 µm nylon mesh filter plate (Corning). The resulting cell suspension was centrifuged (1800 rpm, 5 min, 4°C). After removal of the supernatant, we added 2 ml ACK lysis buffer to lyse red blood cells for 5 min, after gentle shaking to ensure thorough mixing. We added 5 ml 1× PBS to terminate the lysis process. Subsequently, we centrifuged the mixture, removed the supernatant, and resuspended the pellet to obtain a clear single-cell suspension. We then transferred 1 × 10⁶ cells to a 96-well plate and performed centrifugation again. Subsequently, we added complete RPMI 1640 medium (Gibco) and stimulants to the plates, and incubated them at 37°C

for 6 hours to allow for cellular responses to occur. Cell viability was assessed with a Zombie NIR™ Fixable Viability Kit. Cells were washed and blocked with anti-CD16/32 antibodies, and stained with the following surface antibodies: CD8a-Alexa Fluor 700 (clone 53-6.7; catalog no. 100730; BioLegend), CD4-FITC (clone RM4-5; catalog no. 100510; BioLegend), CD3-Brilliant Violet 510 (clone 17A2; catalog no. 100234; BioLegend), and CD69-Brilliant Violet 421 (clone FN50; catalog no. 310910; BioLegend). After 30 min incubation at 4°C, the cells were washed twice with cell staining buffer, fixed, and permeabilized with a BD Cytofix/Cytoperm Fixation/Permeabilization Kit (catalog no. 554714). The cells were stained for 30 min at 4°C with the following intracellular antibodies: IL-2-Brilliant Violet 605 (clone JES6-5H4; catalog no. 503829; BioLegend), TNF- α -PerCP/cyanine5.5 (clone MP6-XT22; catalog no. 506322; BioLegend), and IFN- γ -APC (clone XMG1.2; catalog no. 505810; BioLegend) in Permeabilization/Wash (Perm/Wash) buffer.

For flow cytometry, the positive control consisted of cells stimulated with 400× Cell Activation Cocktail (without brefeldin A) (BioLegend Cat #423301) and 1000× brefeldin A (BioLegend Cat #420601). The negative control used the same volume of DMSO and brefeldin A without the activation cocktail. The gating strategy used in the flow cytometry analyses was as follows. Lymphocytes were identified with FSC-A and SSC-A, and this was followed by doublet exclusion with SSC-A versus SSC-H. Live T cells were selected by gating on Zombie-negative and CD3-positive populations. These cells were further divided into CD4⁺ and CD8⁺ T cells according to respective markers. The percentages of IFN- γ , TNF- α , and IL-2 expressing cells were analyzed within each subset. This step-by-step approach ensured precise identification and analysis of T cell populations and their cytokine profiles. Finally, samples were sorted with a BD FACS Fortessa instrument, and the data were analyzed in FlowJo v10.8.1 software.

Biocompatibility assessment

In addition to assessing the efficacy of the Mn-LNP-delivered VZV vaccine, we evaluated its biocompatibility *in vivo*. Mice were divided into two groups: a control group and an Mn-LNP-delivered VZV vaccine treated group. After 7 days of continued observation, the animals were euthanized for sample collection. We evaluated biocompatibility by monitoring changes in body temperature and body weight; analyzing routine blood biochemical parameters; and examining histopathological effects on major tissues and organs, including the heart, liver, spleen, lungs, and kidneys.

Statistical analysis

In GraphPad Prism (GraphPad, San Diego, CA, USA), we performed univariate analysis of variance (ANOVA)

for comparisons between groups. $P < 0.05$ was considered statistically significant.

Ethics statement

All procedures and experiments performed were approved by the animal ethics committee of the Institute of Medical Biology, CAMS, under animal ethics review number DWSP202008 021.

RESULTS

Synthesis and characterization of palmitic acid-Mn

We used hydrothermal synthesis to formulate palmitic acid-Mn by mixing MnCl₂ and palmitic acid in a 4:1 ratio as the substrates (Fig 1A). The manganese content in the palmitic-Mn was 15.09%, as quantified with X-ray photoelectron spectroscopy (XPS), thus confirming the successful coupling of palmitic acid with manganese (Fig 1C). Palmitic acid-Mn has one hydrophobic terminus and one hydrophilic terminus; consequently, the molecules assembled through intermolecular hydrophobic-hydrophilic interactions in aqueous solution. We observed uniform spherical particles through transmission electron microscopy (TEM) (Fig 1D). Dynamic light scattering indicated that the hydrodynamic diameter of palmitic acid-Mn was approximately 309 nm, the polydispersity index was 0.268, and the zeta potential was approximately +4.23 mV.

Formulation of the Mn-LNP-delivered VZV vaccine

We used commercial microfluidic chips to formulate the Mn-LNP-delivered VZV vaccine. We mixed palmitic acid-Mn with SM-102, DSPC, CHO-HP, and DSG-PEG2000 in ethanol as an organic phase with a molar ratio of 50:10:37.5:2.5. As aqueous phases, VZV gE antigen and CpG adjuvant with a mass ratio of 1:2 were dissolved in 1× PBS buffer. Subsequently, we formulated the Mn-LNP-delivered VZV vaccine by mixing the two phases in a microfluidic device (Fig 1A). After ultrafiltration, spherical Mn-LNP-encapsulated VZV vaccine particles were observed via TEM (Fig 1E). The hydrodynamic diameter of the vaccine particles was approximately 270 nm, and the polydispersity index was 0.248. The zeta potential of vaccine particles was −1.15 mV. Additionally, we used 2% Triton X-100 to demulsify the vaccine particles, thus exposing the VZV gE antigen encapsulated in Mn-LNPs. Through comparison with the concentration of free protein before and after demulsification, determined with a BCA protein assay kit, we calculated that the efficiency of Mn-LNPs in encapsulating VZV gE antigen was 79.9%.

The Mn-LNP-delivered VZV vaccine enhances the VZV gE antigen-induced T cell response in mice

The vaccine-elicited T-cell response is critical in mitigating the clinical manifestations of VZV [24]. We assessed the cellular immune response in a C57BL/6 mouse model in four groups ($n = 5$ per group): Mn-LNP-delivered

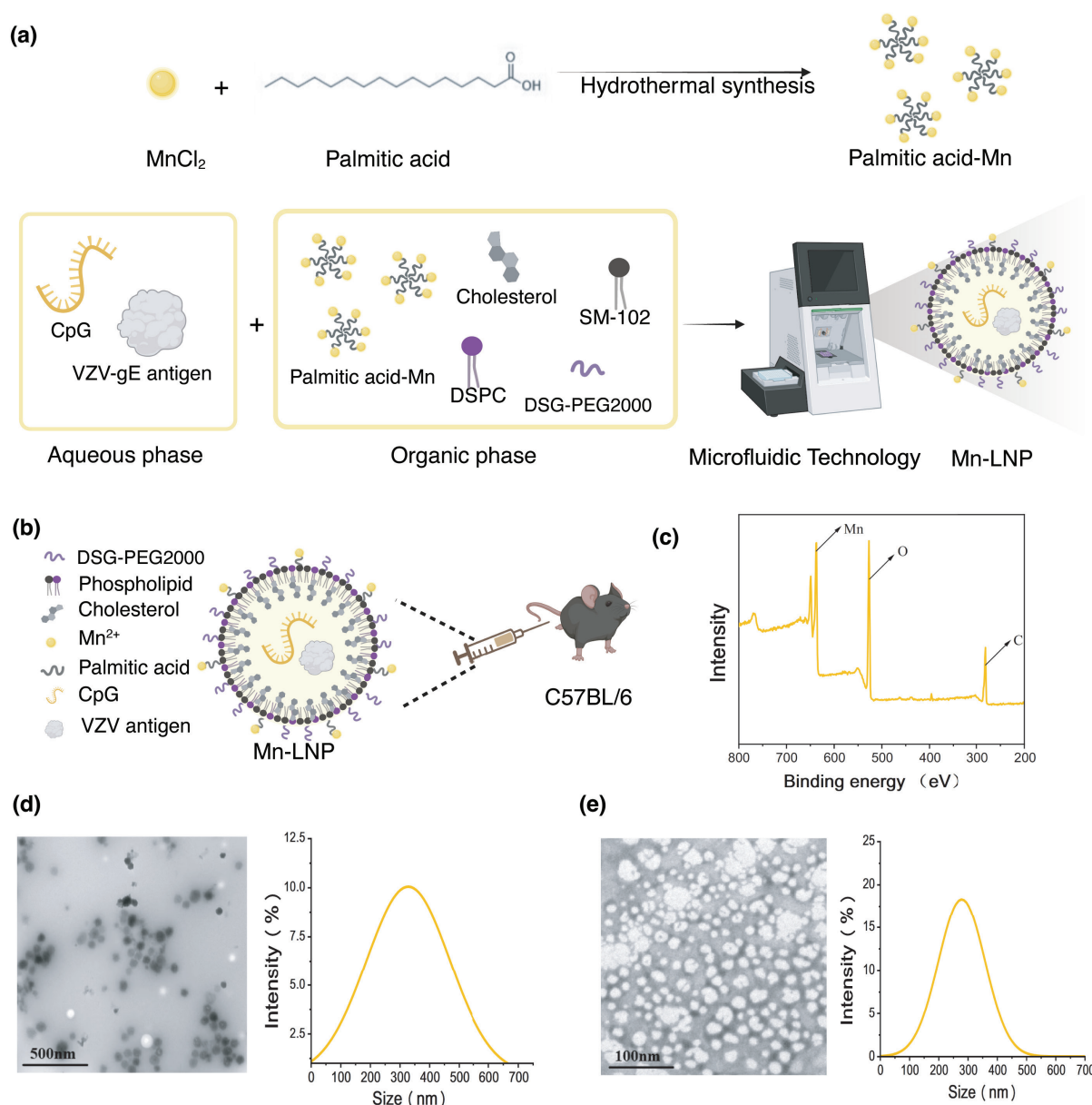


FIGURE 1 | Synthesis and characterization of Mn-LNP-based nanoparticles. (A) Schematic illustration of the fabrication process of Mn-LNP nanoparticles. (B) Composition and immunity of Mn-LNP nanoparticles in C57BL/6 mice. (C) XPS image of palmitic acid-Mn. (D) TEM image and particle size of palmitic acid-Mn (scale: 500 nm). (E) TEM image and particle size of Mn-LNP-delivered VZV vaccine nanoparticles (scale: 100 nm).

VZV vaccine, aluminum adjuvant assisted VZV vaccine, empty Mn-LNPs, and vehicle control. Fresh splenocytes collected on day 56 were subjected to ELISpot analysis. As indicated by the IFN- γ spots, the Mn-LNP co-delivery system outperformed the aluminum adjuvant and yielded 1.35-fold the number of spots (Fig 2A). Furthermore, flow cytometry indicated that the Mn-LNP-delivered VZV vaccine, compared with commercial aluminum adjuvant, enhanced the IFN- γ response for CD4⁺ T cells and CD8⁺ T cells by 1.47-fold and 5.66-fold, respectively; the TNF- α response for CD4⁺ T cells and CD8⁺ T cells by 2.25-fold and 2.64-fold, respectively; and the IL-2 response for CD4⁺ T cells and CD8⁺ T cells by 2.62-fold and 3.17-fold, respectively (Fig 2B). These results suggested that the

Mn-LNP-delivered VZV vaccine induced a strong Th1 cell response.

The Mn-LNP-delivered VZV vaccine enhances the VZV gE antigen-specific IgG2b antibody response in vivo

To determine whether the co-delivery of both VZV gE antigen and CpG ODN by Mn-LNP might elicit potent humoral responses in vivo, we immunized mice with the Mn-LNP-delivered VZV vaccine, aluminum adjuvant assisted VZV vaccine, empty Mn-LNPs, or vehicle control on days 0, 14, and 28 (Fig 3A). Serum collected on day 42 was subjected to ELISA analysis to detect VZV antigen-specific IgG, IgG1, IgG2a, IgG2b, and IgG3 titers (Fig

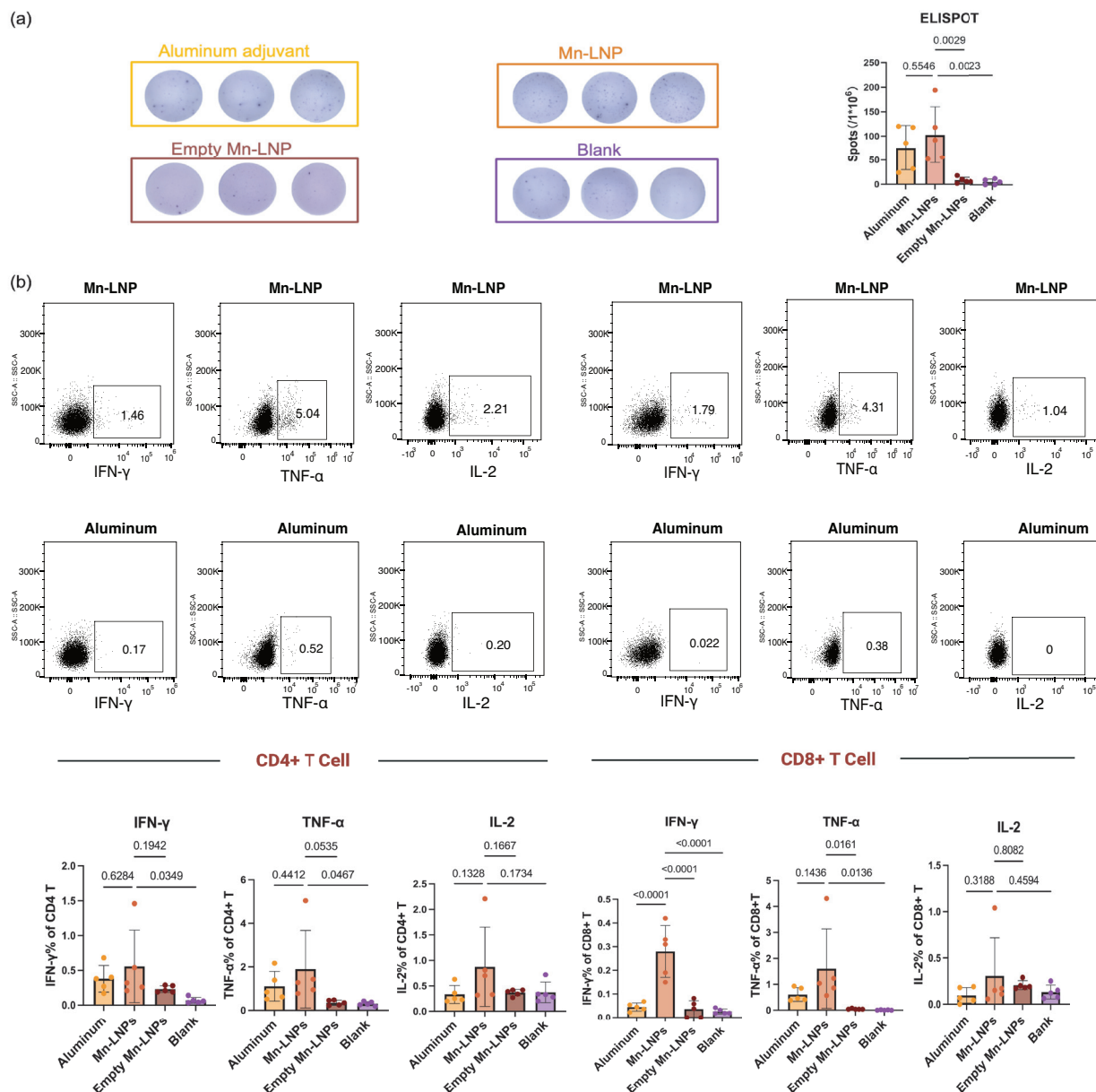


FIGURE 2 | Cellular immunity induced by the Mn-LNP-delivered VZV vaccine. (A) ELISpot analysis of IFN- γ -producing T cells in splenocytes from mice on day 56. (B) Flow cytometry analysis of T cells (IFN- γ , TNF- α , and IL-2) in splenocytes from mice on day 56. Univariate analysis of variance (ANOVA) was used for comparison between groups, and $P < 0.05$ was considered statistically significant.

3B). We used the three samples from the vehicle control group as negative controls. Antibody titers in the empty Mn-LNP group that were lower than those in the control (negative control) group were assigned the values of the negative control. Compared with the aluminum adjuvant group, there was no significant difference in the titers of varicella-zoster virus antigen-specific IgG and IgG3 in Mn-LNPs group. The titers in Mn-LNPs group of VZV antigen-specific IgG1 and IgG2a were significantly lower than those in the aluminum adjuvant group ($P < 0.05$). Notably, the Mn-LNP-delivered VZV vaccine induced 1.29-fold VZV antigenspecific IgG2b titers compared to aluminum adjuvant group, with a statistically significant difference ($P < 0.05$). These results demonstrated

the efficacy of Mn-LNPs in promoting antigen-specific humoral responses.

Favorable biocompatibility of the Mn-LNP-delivered VZV vaccine

For 12 hours after injection, the mice immunized with the Mn-LNP-delivered VZV vaccine were observed every 4 hours. By observing the behavioral and physiological responses of the mice, we preliminarily assessed any signs of poisoning. The mice in the Mn-LNP group did not exhibit behaviors such as anorexia, convulsions, twitching, or rapid breathing. After the day of immunization (day 0), we measured and recorded the body temperatures and weights of the mice in each group daily. As shown

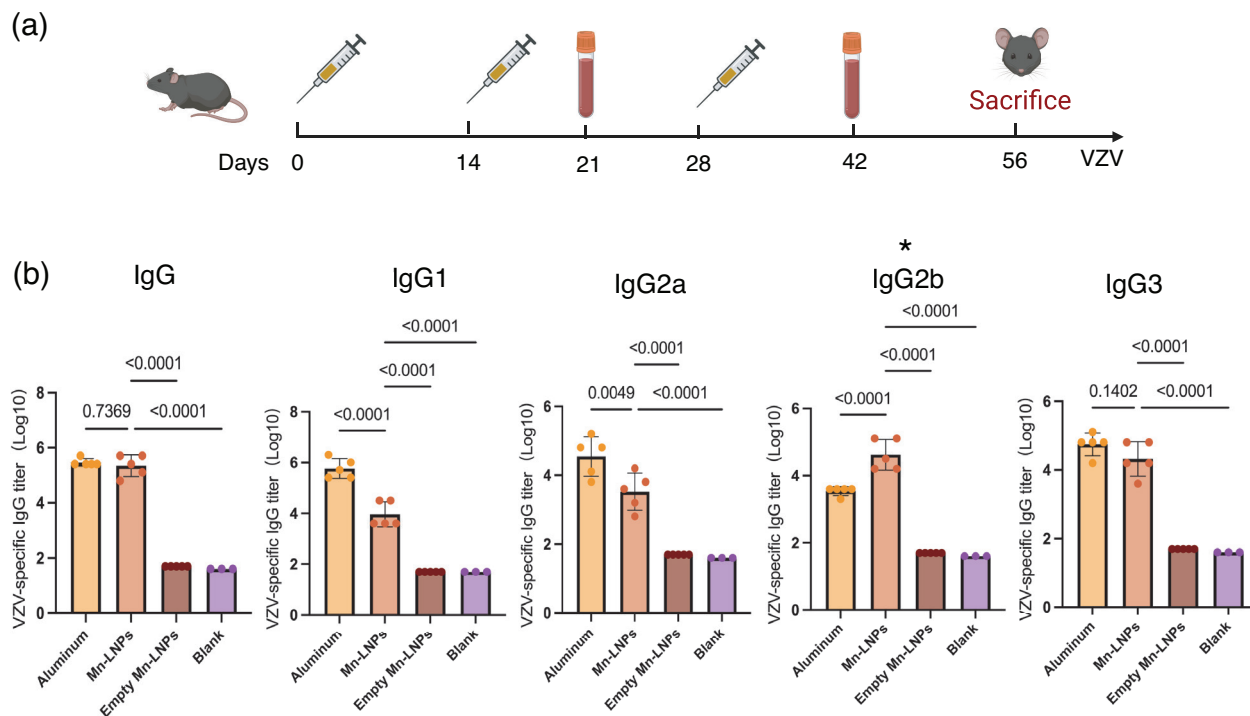


FIGURE 3 | Humoral immunity effects of the Mn-LNP-delivered VZV vaccine. (A) Schematic diagram of the C57BL/6 mouse immunization schedule. (B) Antibody titer (IgG, IgG1, IgG2a, IgG2b, and IgG3) of mouse serum, measured with ELISA 56 days after immunization. Univariate analysis of variance (ANOVA) was used for comparison between groups, and $P < 0.05$ was considered statistically significant.

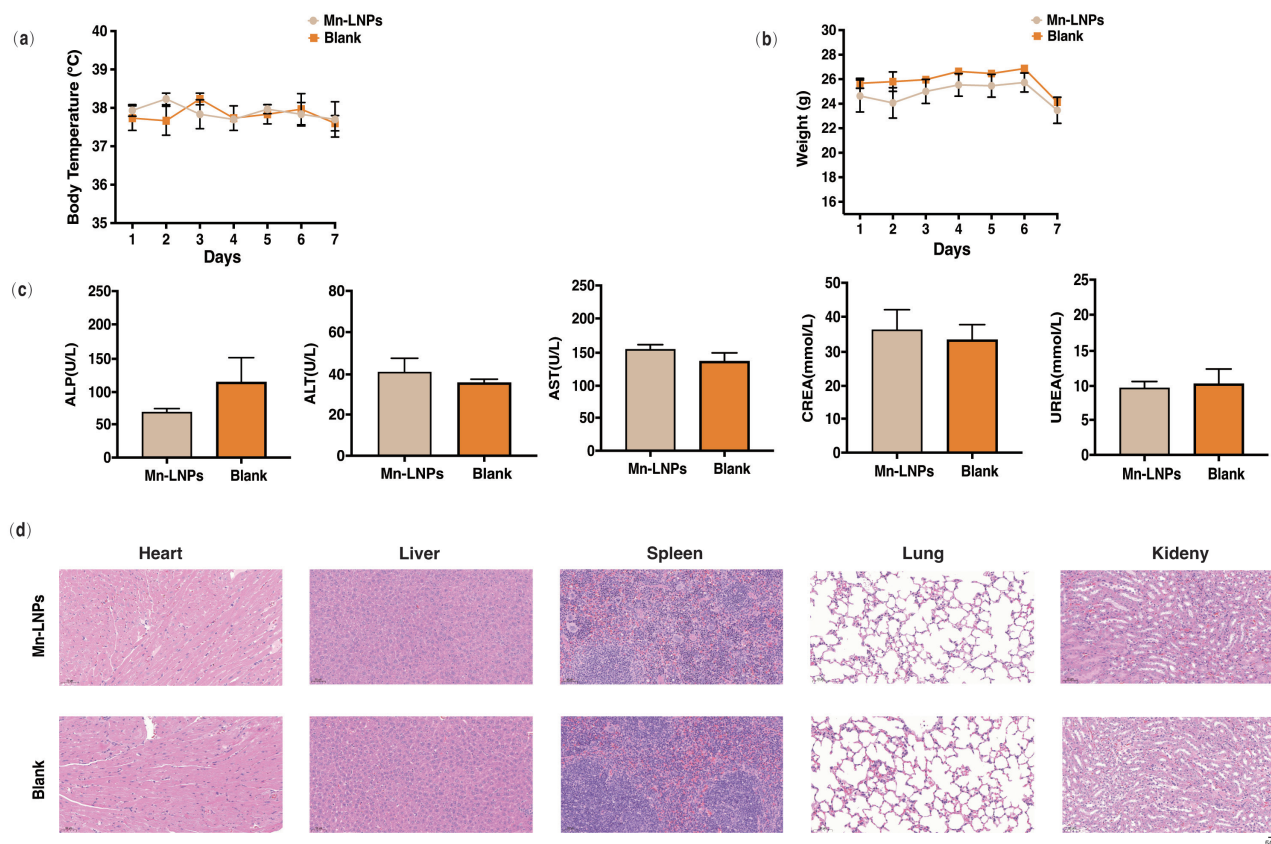


FIGURE 4 | Biocompatibility assessment of the Mn-LNP-delivered VZV vaccine. (A) Body temperature changes post immunization (7-day monitoring). (B) Weight changes post immunization (7-day monitoring). (C) Index of routine serum biochemical tests. (D) Histopathological evaluation of major organs (heart, liver, spleen, lungs, and kidneys) with hematoxylin and eosin (H&E) staining.

in Fig 4A and 4B, we observed no significant differences in the body temperatures and weights of the mice in the Mn-LNP group and vehicle control group. Thus, the Mn-LNP-delivered VZV vaccine did not lead to differences in body temperature and body weight.

With an automated biochemical analyzer, we analyzed serum samples from mice on day 7 after immunization with the Mn-LNP-delivered VZV vaccine. As shown in Fig 4C, no statistically significant differences in the serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, or creatinine were observed between the Mn-LNP group and the vehicle control group. Therefore, the Mn-LNP-delivered VZV vaccine neither induced hepatic inflammation nor disrupted renal metabolism in mice.

We performed hematoxylin and eosin staining on heart, liver, spleen, lung, and kidney tissues from the Mn-LNP and control groups. Histopathological examination revealed no inflammatory cell infiltration or tissue necrosis in the Mn-LNP-treated mice (Fig 4D). Thus, the Mn-LNP-delivered VZV vaccine caused no significant organ damage and exhibited excellent biocompatibility.

DISCUSSION

In this study, we constructed an Mn-LNP-delivered VZV vaccine for effective co-delivery of VZV gE antigen and CpG adjuvant. The Mn-LNP-delivered VZV vaccine significantly enhanced both humoral and cellular immunity in a C57BL/6 mouse model. Compared with the aluminum adjuvanted VZV vaccine, the Mn-LNP-delivered VZV vaccine significantly increased the titers of VZV antigen-specific IgG2b, thus indicating that Mn-LNPs strongly promoted humoral immunity. Furthermore, ELISpot and flow cytometry analyses revealed that the production of IFN- γ by CD8⁺ T cells significantly increased after immunization with the Mn-LNP-delivered VZV vaccine. Therefore, the Mn-LNPs not only effectively induced humoral immunity but also activated a robust Th1-type cellular immune response, which is crucial for generating memory effects and providing long-term immune protection [25,26].

The inefficient use of antigens and adjuvants in vivo is a substantial issue in inducing immune protection against VZV infection with vaccines [2,8,27]. Numerous formulation approaches based on LNPs have been developed to achieve highly efficient delivery of mRNA vaccines [13,28,29]. On the basis of previous research on manganese adjuvants, manganese synergistically enhances adjuvant effects when combined with LNPs [23,30]. The Mn-LNP-delivered VZV vaccine designed by integrating these two aspects not only achieved a high delivery efficiency but also synergistically enhanced the immune response with the manganese adjuvant and CpG adjuvant.

The Mn-LNP-delivered VZV vaccine constructed herein has substantial potential for immune enhancement. One limitation of this study is that we evaluated only the VZV gE

antigen with the Mn-LNP-delivered delivery system. Future research may explore the application of Mn-LNP-delivered system to different antigens, to advance the development of new vaccines and potentially provide new strategies for the prevention and treatment of other viral infections.

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CONFLICTS OF INTEREST

All authors declare that they have no competing interests.

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