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RESEARCH ARTICLE

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Varicella zoster virus mRNA vaccine candidate induced superior cellular immunity and comparable humoral and Fc-mediated immunity compared to the licensed subunit vaccine in a mouse model

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ABSTRACT

The threat of herpes zoster (HZ) is increasing, particularly in the elderly and immunocompromised individuals. Although two platform vaccines are currently available for HZ prevention, the low effectiveness of the live attenuated varicella-zoster virus vaccine (Zostavax®), and the high reactogenicity and limited supply of the AS01 adjuvant gE subunit vaccine (Shingrix®) indicate that, the development of more effective and safe vaccines is required. Compared to conventional vaccines, mRNA vaccines offer the advantages of faster production and generally do not require adjuvants. However, no authorized mRNA vaccine is currently available for HZ. Therefore, we aimed to prepare a gE mRNA vaccine and evaluate the immunogenicity compared with the two commercial vaccines in mice. The gE mRNA vaccine elicited a robust humoral immune response, as measured by an enzyme-linked immunosorbent assay and the fluorescent antibody to membrane antigen test. The mRNA vaccine binding antibody level was comparable to that of Shingrix® and significantly higher than that of Zostavax®. In contrast, in cellular immune responses, which were evaluated by ELISpot assays and intracellular cytokine staining assay, the VZV gE mRNA vaccine induced significantly higher responses than Zostavax® and Shingrix®. In addition, the antibody-dependent cellular phagocytosis activity of the gE mRNA vaccine was comparable to that of the commercial vaccines. However, the highest antibody-dependent cellular cytotoxicity response was achieved by Shingrix®, followed by gE mRNA and then Zostavax®. Our results demonstrate that the mRNA HZ vaccine candidate elicited robust immunogenicity, especially in cellular immunity, and shows a promising potential for HZ prevention.

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VZV: mRNA vaccine; humoral immunity; cellular immunity; Fc-mediated immunity

Introduction

Varicella-zoster virus (VZV) is a highly contagious neurotropic human alpha herpesvirus that primarily causes varicella infection, commonly known as chickenpox.[1] Reactivation of the dormant virus following a primary VZV infection leads to herpes zoster (HZ), which is characterized by a painful, blistering rash.[2] The most common serious complication associated with HZ is post-herpetic neuralgia (PHN), which causes persistent pain at the site of the HZ rash for weeks or even years after the rash resolves.[3] The increased incidence of HZ is associated with decreased cellular immunity to VZV, especially in the elderly, individuals infected with human immunodeficiency virus (HIV), and patients taking immunosuppressants, such as organ transplant recipients or patients with an autoimmune disease.[1,4–6]

To date, two platforms of HZ vaccines have been globally used: the live attenuated Zostavax® and the subunit Shingrix®. However, over time in older adults, Zostavax® loses its effectiveness and provides less protection against shingles, and, therefore, it has been discontinued in the United States since 2020.[7] On the other hand, Shingrix®, a subunit vaccine containing the VZV glycoprotein E (gE) protein, exhibits high protective efficiency and is more effective than Zostavax®.[8–10] However, despite its high efficacy, Shingrix® administration is associated with increased reactogenicity, including injection site pain and severe systemic reactions.[11] In addition, Shingrix® uses the AS01 supplement system, where the main ingredient, QS21, is derived from a rare plant that grows in South America, making it difficult to mass-produce and expensive.[12] Therefore, the development of a safer and more effective new-generation vaccine to prevent HZ is urgently required.

During the COVID-19 pandemic, mRNA vaccines were found to have unique advantages in inducing cell-mediated

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immunity (CMI).[13,14] The strong innate immune activation ability of mRNA vaccines may induce key protective adaptive immune responses, including CMI, more effectively than conventional vaccines.[15,16] Moreover, it has been reported that high levels of CMI are more effective than high antibody levels in preventing HZ and PNH.[17] Therefore, an mRNA vaccine may be effective for HZ prevention. It is well known that VZV gE is the most abundant and highly immunogenic VZV antigen with conserved T cell epitopes.[18,19] In endodomain of gE, AYRV motif mediates trafficking of gE to the *trans*-Golgi network (TGN) and the SSTT motif mediates C-terminal phosphorylation, and targeted mutations at each site enhance gE trafficking to the TGN and viral replication, respectively.[20] Recently, it has been reported that the untranslated region (UTR) of VZV mRNA affects the intracellular mRNA stability and half-life and plays an important role in protein expression and antigen translation.[21] Therefore, we designed a VZV gE mRNA vaccine with a mutation at the AYRV and SSTT sites of the C-terminal domain containing both 3′ and 5′ UTR regions and then evaluated its immunogenicity in mice.

To verify the immunogenicity, we evaluated the acquired immunity, including antibody titers and T cell immunities, and the Fc-mediated effector functions, such as antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC). In the pathogenesis of HZ, viruses spread through the nerve fiber to the skin epithelium, and the viruses mainly exist within the cells with low viremia.[22,23] Therefore, neutralizing antibodies are not effective for the clearance of reactivated VZV; instead, neutrophils, macrophages and natural killer cells mainly infiltrate the affected skin lesions during the early phase of HZ, and then T cells infiltrate later.[24] In the process of virus-infected cell clearance, antibodies mediate this clearance via the Fc-mediated effector function with innate immune cells that possess Fc receptors.[25] It has also been reported that individuals with defects in NK cells, the effector cells of ADCC, suffer severe, recurrent, and often fatal VZV infections.[26] Therefore, in this study, we comprehensively investigated the immune responses induced by VZV gE mRNA vaccine candidate and compared it with the commercially available live attenuated Zostavax® and gE subunit Shingrix® as control vaccines.

Materials and methods

Design and synthesis of the VZV gE mRNA vaccine

The DNA template for the VZV gE mRNA vaccine comprised a DNA fragment encoding the gE protein of VZV YC03 strain (GenBank Accession No. KJ808816)[27] was synthesized by GenScript bio (Piscataway, NJ, USA). The gE (ORF68) codon was mutated on the AYRV motif to AARV and the SSTT motif to AEAADA in the C-terminal domain and then optimized for synthesis (Figure 1(a)). The DNA template was cloned into a plasmid vector with backbone sequence elements of a T7 promoter, 5′- and 3′ UTR, and a 100 nucleotide poly (A) tail interrupted by a linker (A50LA50, 20 nucleotides) (Figure 1(b)). The DNA was purified and quantified using a spectrophotometer (NanoDrop™, Thermo-Fisher Scientific, Waltham, MA, USA), and then transcribed in vitro with an EZ™ T7 high Yield In Vitro Transcription kit (Enzynomics, Daejeon, South Korea), a Cap 1 capping analog (SMARTCAP®, ST PHARM, Seoul, South Korea), and N1 methyl pseudouridine-5′-triphosphate (m1ΨTP; TriLink, CA, USA) to replace uridine-5′-triphosphate (UTP). After transcription, the RNA was purified via lithium chloride precipitation and dsRNA was eliminated using cellulose-based purification. RNA characteristics was assessed by the concentration, purity, and gel electrophoresis (Table S1, Figure S1).

LNPs were prepared according to a previously reported protocol.[28] In brief, all lipid components (SM-102; 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); cholesterol; and 1,2 dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000)) were dissolved in ethanol at a molar ratio of 50:10:38.5:1.5, respectively, and mRNA-gE-LNPs were dissolved at a charge ratio of N/P = 6 in a 50 mM sodium citrate buffer (pH 4) solution. LNPs were formulated using enCELL-Master V2 (ENPARTICLE, Busan, Korea) by mixing the aqueous and organic solutions at a ratio of 3:1. The LNP solution was concentrated by ultrafiltration using the Amicon Ultra Centrifugal Filter (UFC9030, Merck Millipore, MA, USA), according to the manufacturer's instructions. The size, polydispersity index, and encapsulation efficiency of the mRNA vaccine were all confirmed to be suitable (Table S2, Figure S2).

Western blot (WB)

A549 cells were transfected with VZV gE mRNA using Solfect™ (Biosolyx, Daegu, Republic of Korea) according to the manufacturer's instruction. Twenty-four hours after transfection, the cell lysates were loaded onto polyacrylamide gels and transferred to nitrocellulose membranes (Amersham Biosciences, USA). Subsequently, the membrane was incubated with the specific anti-VZV gE monoclonal antibody (mAb, Clone M1, Millipore, #MAB8612, USA), followed by incubation with a goat anti-mouse IgG/HRP (Invitrogen, #62-–6520, USA).

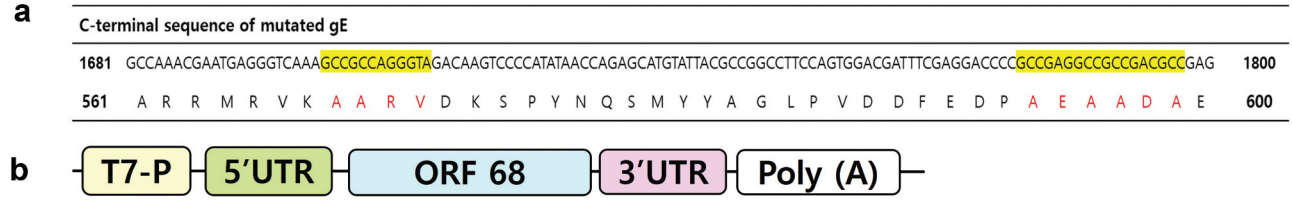

Figure 1. Design of varicella-zoster virus (VZV) gE mRNA vaccine. (a) DNA and amino acid sequences of gE C-terminal mutation. (b) Schematic diagram of the components of mRNA nucleic acid sequence synthesis.

Immunofluorescence assay (IFA)

VZV gE mRNA transfected A549 cells and mock-transfected A549 cell (transfection reagent only without gE mRNA, negative control) were fixed with 2% paraformaldehyde (Biosesang, Republic of Korea) for 10 min at RT. Subsequently, the cells were blocked with 10% goat serum for 1 h at RT, followed by incubation overnight with anti-VZV-gE mAb at 4 °C. The following day, the cells were incubated with goat anti-mouse IgG-Alexa Fluor 488 (Invitrogen, Carlsbad, CA, USA) for 1 h at RT. Finally, the cells were counterstained with DAPI and analyzed using a confocal fluorescent microscope (K1-fluo; Republic of Korea).

Immunization

All experimental procedures performed using animals in this study were reviewed and approved by the IACUC of Ewha Womans University College of Medicine (EWHA MEDIACUC 22–009-t). The animals were housed in an animal biosafety level 2 facility with ad libitum access to food, water, and environmental enrichment. Because HZ is caused by the reactivation of VZV, patient with HZ have prior immunity to VZV.[1,29] To mimic a similar immunological setting, 6-week-old C57BL/6 mice were primed with a single dose of live attenuated varicella vaccine BARYCELA® (GC Biopharma, Yongin-si, South Korea) by subcutaneous administration 4 weeks before zoster vaccination (Figure 2). Then, followed by two intramuscular doses of VZV gE mRNA vaccine candidates (3 weeks apart) and Shingrix® (4 weeks apart) or a single subcutaneous dose of Zostavax®. Two weeks after the final immunization, all mice were euthanized via CO_2 inhalation, and the sera and splenocytes were collected for further immunological analysis (Figure 2).

Enzyme-linked immunosorbent assay (ELISA)

To detect anti-VZV IgG, serum samples were tested using an AccuDiag™ Varicella IgG ELISA Kit (Diagnostic Automation/Cortez Diagnostics, Inc., CA, USA). ELISA was performed according to the manufacturers' instructions, and a secondary antibody, HRP-conjugated goat anti-mouse IgG (Invitrogen), was used. Results were expressed as the Immune Status Ratio (ISR) values, which were calculated according to the manufacturers' instructions. The ISR values were interpreted as follows: samples with an ISR value ≤ 0.9 were considered negative, an ISR value 0.91–1.09 were deemed equivocal, and an ISR value ≥ 1.1 were considered positive.

Fluorescent antibody to membrane antigen (FAMA) test

FAMA antigen preparation and the overall FAMA experimental procedures were performed as previously described.[30] Briefly, MRC-5 cells (ECACC, London, UK) were infected with the VZV strain YC03 at a multiplicity of infection of 0.005, and was used as FAMA antigen. Cell-free VZV-infected mouse serum and DPBS were used as positive and negative controls, respectively. Two-fold serially diluted serum and control samples were incubated with 2×10^5 FAMA antigen cells for the primary antibody reaction. Goat anti-mouse IgG-Alexa Fluor 488 antibody (Invitrogen) was used as the secondary antibody. A specific clear fluorescence with complete ring structure around the cell surface graded ≥ "1 + " was regarded as FAMA-positive using an Axioscope fluorescence microscope (Carl Zeiss, Jena, Germany).[30]

Enzyme-linked immune absorbent spot (ELISpot) assay

The frequencies of mouse interferon-γ (IFN-γ) and interleukin-2 (IL-2) secreting T cells were assessed using commercial ELISpot kits (Mabtech Ab, Stockholm, Sweden) according to the manufacturer's instructions. In brief, immunized mouse splenocytes were seeded in duplicate at 2.5×10^5 cells/well on ELISpot plates. The splenocytes were stimulated with irradiation-inactivated VZV-infected MRC-5 cell lysate antigen and normal MRC-5 cell lysate antigen which were prepared following the method for VZV antigen production by Microbix Biosystems (Cat No. EL-03-02 and ELC-29-02, Toronto, Ontario, Canada)[31] for 24 h at 37°C.[31,32] Concanavalin A and culture media were used as positive and negative control, respectively. Spots were developed with the BCIP/NBT substrate and counted using a CTL-ImmunoSpot S6 Versa Analyzer (CTL-ImmunoSpot, Cleveland, OH, USA).[33] Afterward, outliers were removed using the interquartile range (IQR) rule and statistically analyzed.

Intracellular cytokine staining (ICS) assay

Splenocyte (1×10^6 cells) were stimulated with Concanavalin (Sigma, St Louis, MO, USA) and VZV gE peptide (JPT peptide technologies, Berlin, Germany) for 2 hours in a 37℃ incubator. Afterward, the cells were stimulated with brefeldin A (eBioscience™, San Diego, CA, USA) and incubated with

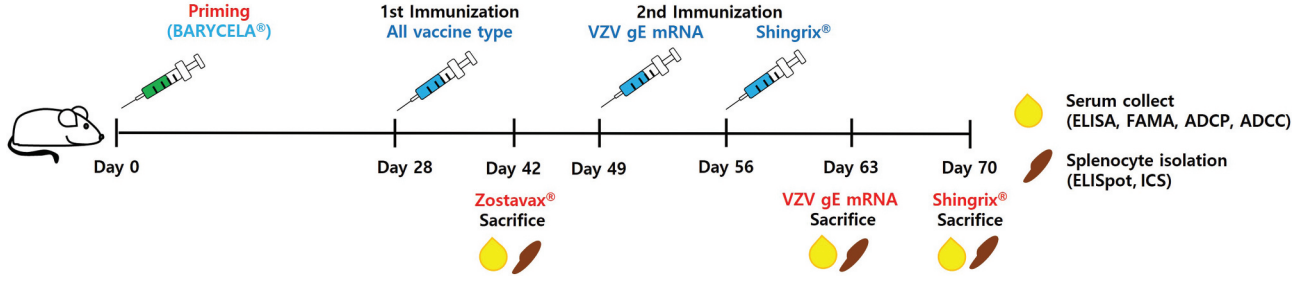

Figure 2. Immunization schedule of VZV gE mRNA vaccines in C57BL/6 mice. Female mice were primed on day-0 with varicella vaccine and then vaccinated on days-28 with Zostavax®, Shingrix®, and VZV gE mRNA vaccine. Second dose of mRNA vaccine and Shingrix® were administrated at day-49 and − 56, respectively. Sera and spleens were collected on the day of sacrifice.

T cell markers anti-CD3 APC-Cy7, anti-CD4 BV510, anti-CD8 PE-Cy7 and Live/Dead dye (Invitrogen, Carlsbad, CA, USA). Next, the cells were reacted in Fix/Perm buffer for 20 min, incubated with cytokine antibodies; anti-IL-2 PE, anti-IFN-γ APC, and anti-TNF BV421 and then analyzed by flow cytometer (CytoFLEX, Beckman Coulter, Fullerton, CA, USA). All the antibodies were purchased from BD Biosciences (San Jose, CA, USA).

Antibody dependent cellular phagocytosis assay (ADCP)

RAW 264.7 cells (ATCC, TIB-71) were used as effector cells in the ADCP analysis. VZV-infected MRC-5 cells as the target cells were labeled with 3 μM of 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFSE; Thermo Fisher Scientific, Eugene, USA) in DPBS at 37°C for 10 min. The effector cells were harvested, and then co-cultured with the target cells in the presence of serum (1:2000) and incubated for 3 h at 37°C. Before flow cytometry analysis, CD11b antibody (BD Pharmingen™, San Diego, CA, USA) conjugated with allophycocyanin (APC) was used to label the effector cells. ADCP activity (%) was quantified as the percentage of RAW 264.7 cells that phagocytosed VZV-infected MRC-5 target cells ($CFSE^+$ $CD11b^+$ double positive cells). Samples were analyzed by flow cytometer (CytoFLEX, Beckman Coulter, Fullerton, CA, USA).

Antibody dependent cellular cytotoxicity assay (ADCC)

ADCC activity was assessed using mFcγRIV ADCC Reporter Bioassay Kit (Promega, Madison, WI, USA). Jurkat-FcγRIII-NFAT-Luc reporter cells were used as effector cells and VZV-infected MRC-5 cells were used as targets. Briefly 20,000 target cells were seeded into 96 well plates along with 25 μL of diluted mouse serum. Then 10,000 mouse effector cells (E:T ratio = 1:2) were thawed and immediately added to each well. After incubation for 3 h, the Bio-Glo reagent was added to the plate along with an equal volume of the pre-existing medium for luminescence measurement using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis

The data were analyzed by GraphPad Prism 10.0.2 (GraphPad Software Inc., San Diego, CA, USA). Multiple comparisons were performed using a one-way ANOVA and the means of each group were compared with those of all other groups. Asterisks represent the p-value classification: $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$.

Results

VZV gE mRNA in vitro expression

We confirmed in vitro translation of the VZV gE mRNA by delivery into A549 cells. The purified VZV protein was then analyzed by western blot, confirming the expression of the 95 kDa gE protein (Figure 3(a)). In addition, to verify the in vitro expression, we conducted IFA on the A549 cells post-mRNA transfection, and significant gE expression levels were detected by the presence of green fluorescence (Figure 3(b), Figure S3).

VZV gE mRNA vaccines induce VZV-specific humoral immune responses

Anti-VZV IgG was measured by ELISA and the mean ISR values of each vaccine group were 3.85 ± 0.71 for Zostavax®, 6.84 ± 0.97 for Shingrix®, and 5.55 ± 0.79 for the gE mRNA vaccine. Although the Shingrix® group had the highest ISR value, the value of the mRNA vaccine group did not significantly differ from the Shingrix® group ($p = .0853$), but the ISR level of the gE mRNA vaccine group was significantly higher than the Zostavax® group ($p = .0133$) (Figure 4(a), Figure S4(a)). VZV-specific protective antibodies were measured using the FAMA test, and the GMTs (geometric mean titers) of each vaccine group were 203.2 ± 1.43, 2,580.3 ± 1.43, and 812.8 ± 1.43, for Zostavax®, Shingrix®, and the gE mRNA vaccine, respectively (Figure 4(b), Figure S4(b)). In summary, the VZV gE mRNA vaccine elicited a stronger humoral immune response than Zostavax® and a similar or slightly lower humoral immune response compared to Shingrix®.

Cell-mediated immune response by VZV mRNA vaccines

T-cell responses were measured by ELISpot assay and ICS. ELISpot assay were performed for IFN-γ and IL-2 and were

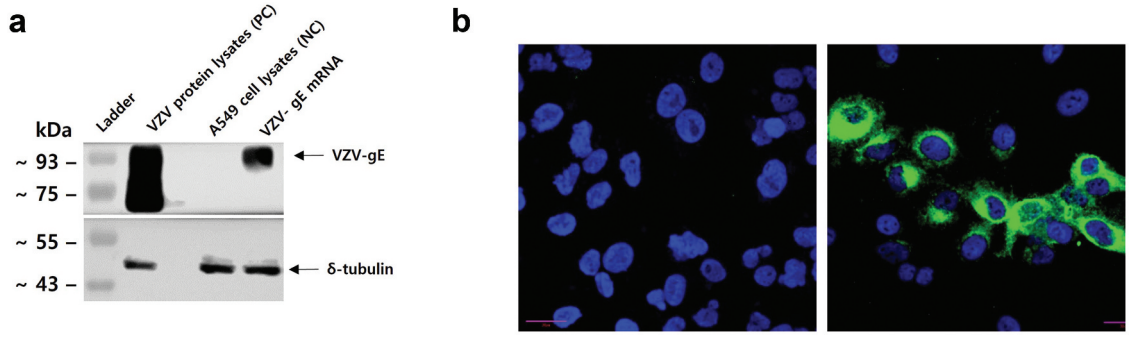

Figure 3. In vitro expression gE protein of VZV mRNA vaccine. (a) Western blot analysis confirming gE protein expression in mRNA-transfected A549 cells. Lane 1: VZV-infected MRC-5 cell lysate (positive control, PC); Lane 2: A549 cell lysate (negative control, NC); Lane 3: VZV gE mRNA-transfected A549 cell lysate (b) IFA verifying the in vitro expression of the VZV gE mRNA vaccine construct in the A549 cells. Left: mock A549 cell, Right: VZV-gE mRNA transfected A549 (nucleus: blue, gE: green). gE, glycoprotein E; VZV, varicella zoster virus.

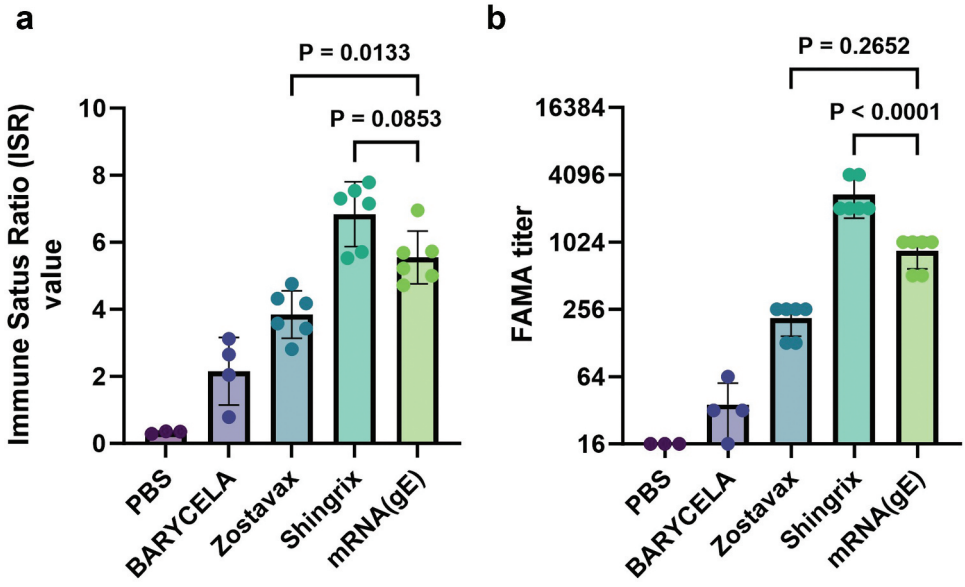

Figure 4. Humoral responses of VZV gE mRNA vaccines in C57BL/6 mice. (a) Anti-VZV binding antibodies were detected by ELISA. Data were analyzed using a one-way ANOVA test and are presented as the mean ± SD. (b) Protective antibodies were analyzed by the FAMA test. Data were analyzed using a one-way ANOVA test and are presented as the geometric mean titer ± geometric SD factor. Error bars indicate the standard deviations (SD).

statistically analyzed except for the outlier. The average IFN-γ spot count was 28.2 ± 12.1, 113.0 ± 40.6, and 368.5 ± 106.4 in Zostavax®, Shingrix®, and the gE mRNA vaccine, respectively (Figure 5(a), Figure S5(a)). In addition, the average IL-2 spot count was 12.7 ± 13.3 in the Zostavax®, 61.3 ± 23.2 in the Shingrix®, and 171.7 ± 45.1 in the gE mRNA vaccine (Figure 5(b), Figure S5(b)). Therefore, the ELISpot results showed significantly higher responses for both IFN-γ and IL-2 in the gE mRNA group compared to the other vaccine groups. Polyfunctional T cell responses were analyzed by ICS. The gE-specific CD4 + T cells producing IFN-γ, IL-2, and TNF were detected in mRNA and Shingrix® groups but not in Zostavax® (Figure 5(c), Figure S5(c)). The gE mRNA vaccine showed higher reactivity than Shingrix®, even though the statistical significance was showed only in IL-2 producing CD4 + T cells. However, there were no significant responses in

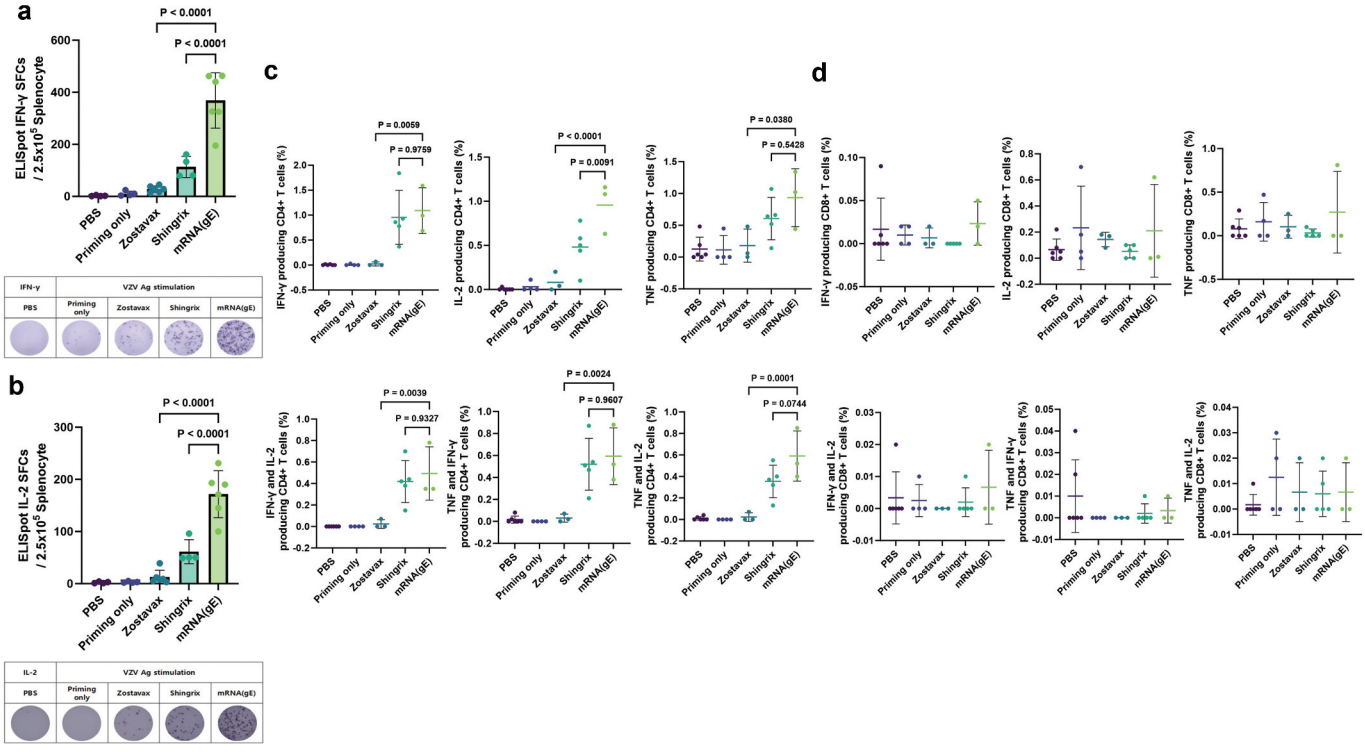

Figure 5. VZV gE mRNA vaccines induce cellular immune responses in C57BL/6 mice. (a) Spot forming counts (SFCs) of IFN-γ-producing splenocytes and (b) SFCs of IL-2 producing splenocytes after VZV antigen stimulation. (c, d) ICS was performed to measure the percentage of single or double cytokine secreting CD4 + , CD8 + splenocytes. The data were analyzed using a one-way ANOVA test and are presented as the mean ± standard deviations (SD). Error bars indicate SD.

CD8 + T cells in all vaccine groups (Figure 5(d), Figure S5 (d)).[34] This indicates that the gE mRNA vaccine group produced higher CMI than the control vaccine groups.

VZV gE mRNA vaccine elicited Fc-mediated functional antibody response via ADCP and ADCC

We evaluated the vaccine-induced Fc-mediated IgG responses using ADCP and ADCC assays. The average ADCP activity was 24.65 ± 3.08 for Zostavax®, 27.50 ± 1.66 for Shingrix®, and 25.77 ± 1.02 for the gE mRNA vaccine, revealing similar responses in the three groups (Figure 6(a), Figure S6(a)). However, these values were significantly higher than those in the PBS group (10.60 ± 1.70) or the priming-only group (14.28 ± 3.02). Therefore, all HZ vaccines induced Fc-mediated phagocytosis. The ADCC results are represented as, the mean relative luminescence unit (RLU) levels, which were 3,074 ± 382, 4,635 ± 1221, and 3,351 ± 595 in Zostavax®, Shingrix® and gE mRNA groups, respectively (Figure 6(b), Figure S6(b)). The RLU levels of the PBS and priming-only groups were 1,503 ± 276 and 2,887 ± 295, respectively. The ADCC activities of Shingrix® was significantly higher than the other groups and all HZ vaccines induced significantly higher ADCC than that of the PBS. Therefore, it was confirmed that the gE mRNA vaccine-induced antibodies preserve Fc effector functions capable of eliminating VZV-infected cells.

Discussion

HZ is caused by the reactivation of a latent virus rather than an external viral infection, therefore, regardless of specific preventive measures or social systems, many people who were infected with chickenpox in childhood suffer from reactivation symptoms and various complications.[1] Reports have indicated that aging reduces VZV-specific CMI to a threshold that cannot inhibit viral reactivation; therefore, the severity and complications of HZ increase with age.[1,35] Recently, the elderly population in South Korea has been rapidly increasing, and as a result, the demand for the shingles vaccine is also rising.

gThere have been efforts to develop more effective and safe HZ vaccines than commercially available vaccines.[33,36–38] After the COVID-19 pandemic, the immunogenicity of several mRNA HZ vaccines was evaluated in mice, guinea pigs, and non-human primates.[21,34,36,39–42] Considering the nature of mRNA and LNPs, the mRNA vaccine is a strong type I interferon-polarizing innate immunity inducer and effective adjuvant; therefore, it induces greater T cell immunity compared to conventional vaccines.[43] Many mRNA HZ vaccines exhibited comparable antibody and T cell responses compared with Shingrix®.[21,34,36,40] In particular, the optimization of the gE sequence through signal peptide replacement, C-terminal modification, and the insertion of an mRNA-stabilizing motif, improved the immunogenicity and induced greater T cell immune responses compared with Shingrix®.[39,41]

In this study, a VZV mRNA candidate vaccine was produced after C-terminal mutations, optimization of the VZV YC03 strain gE sequence, and UTRs inserted into the gE, and then encapsulated, with LNP. The size, polydispersity index, and encapsulation efficiency of the mRNA vaccine were all confirmed to be suitable (Table S2, Figure S2). Similar to other mRNA HZ vaccine studies, the humoral immune responses evaluated by ELISA and FAMA test were comparable to Shingrix® and higher than Zostavax®, and the cellular immune responses assessed by ELISpot assays and ICS were higher than both Shingrix® and Zostavax®.

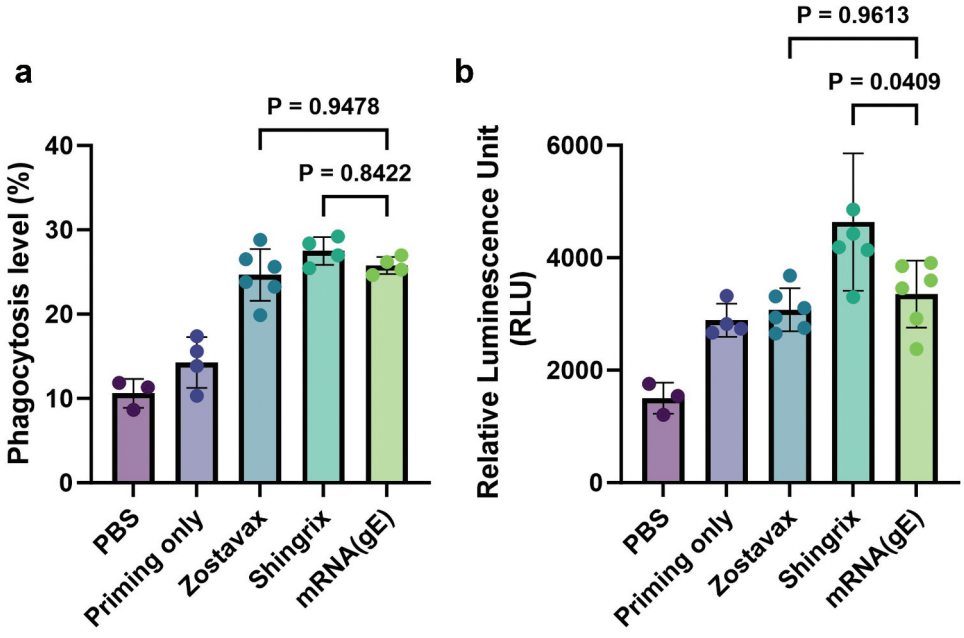

Figure 6. Fc-mediated immune response measured by ADCP and ADCC. (a) The ADCP activity of vaccinated sera was measured using RAW 264.7 cells as effector cells and CSFE-labeled VZV-infected MRC-5 cells as target cells. The ADCP activity was analyzed by the percentage of macrophage marker and CSFE double positive cells using flow cytometry. (b) ADCC activity of vaccinated sera was measured using mFcγRIV ADCC Reporter Bioassay Kit. Jurkat-mFcγRIV-NFAT-Luc reporter cells were used as effector cells and VZV-infected MRC-5 cells were used as targets. ADCC activity was measured by relative luminescence unit (RLU) levels. The data were analyzed using a one-way ANOVA test and are presented as the mean ± standard deviations (SD). Error bars indicate (SD).

Fc receptor-dependent antibody function provides a direct link between the innate and adaptive immune systems by combining the robust antiviral activity of the innate effector cells with the diversity and specificity of adaptive humoral responses.[25] ADCP mainly promotes the phagocytosis of viruses or virus-infected cells by macrophages, whereas ADCC promotes the cytolysis of virus-infected cells by NK cells.[25] Therefore, the Fc-mediated immune response may be advantageous for protection against VZV infection via the clearance of infected cells. Recently, Huang et al. performed an antibody-dependent complement deposition (ADCD) and antibody-dependent neutrophil phagocytosis (ADNP) using an mRNA vaccine candidate (ZOSAL) in mice, and the ADCD and ADNP activities of ZOSAL were similar to that of Shingrix®.[39] In our study, we measured ADCP activities using macrophage, and all three different platform HZ vaccines exhibited similar ADCP activity levels; however, they were significantly higher than the negative control (PBS group) or priming only group. Park et al. reported that antibodies induced by Shingrix® vaccines provided a higher ADCC than those induced by Zostavax® vaccines, and younger adults (50–60 years old) showed higher ADCC activities than old adults (≥70 years old).[44] Our ADCC data showed similar results, where Shingrix® exhibited the highest ADCC activity, followed by the mRNA vaccine and then Zostavax®. In conclusion, we investigated the immunogenicity of our mRNA-based VZV gE vaccine in mice and found that it induced superior cellular immune responses than commercial vaccines as well as comparable antibody and Fc-mediated immune responses. These findings provide promising applications for further pre-clinical and clinical studies.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Credit authorship contribution statement

Eun-Jeong Jang: methodology, formal analysis, visualization, and writing of the original draft. **Sivilay Xayaheuang**: methodology and formal analysis. **Ji-Young Hwang**: methodology and formal analysis. **Yunhwa Kim**: formal analysis. **Kyung-Min Lee**: formal analysis. **Seok-Tae Choi**: formal analysis. **Hye Won Kwak**: resources and formal analysis. **Jae-Hwan Nam**: conceptualization. **Keun Kim**: resources and formal analysis. **Boomi Yoon**: resources and methodology. **Jae Hyang Lim**: methodology and resources. **Ho Seong Seo**: methodology **Chang-Hoon Woo**: methodology and resources. **Hosun Park**: conceptualization, investigation, resources, funding acquisition, and writing. All the authors have read and agreed to the published version of the manuscript.

Data availability statement

Data supporting the findings of this study are available from the corresponding author, H.P., upon reasonable request.

Declaration of generative AI in scientific writing

No AI technology was used in the scientific writing

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