Immunological Characteristics of MAV/06 strain of Varicella-Zoster Virus Vaccine in an Animal model

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Research Article

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Abstract

**Background:** Varicella-zoster virus (VZV) is a pathogen that causes chickenpox and shingles in humans. Different types of the varicella vaccines derived from the Oka and MAV/06 strains are commercially available worldwide. Although the MAV/06 vaccine was introduced in 1990s, little was known about immunological characteristics.

**Results:** Here, we evaluated B and T cell immune response in animals inoculated with the Oka and MAV/06 vaccines as well as a new formulation of the MAV/06 vaccine. A variety of test methods were applied to evaluate T and B cell immune response. Plaque reduction neutralization test (PRNT) and fluorescent antibody to membrane antigen (FAMA) assay were conducted to measure the MAV/06 vaccine-induced antibody activity against various VZVs. Glycoprotein enzyme-linked immunosorbent assay (gpELISA) was used to compare the degree of the antibody responses induced by the two available commercial VZV vaccines and the MAV/06 vaccine. Interferon-gamma enzyme-linked immunosorbent spot (IFN-γ ELISpot) assays and cytokine bead array (CBA) assays were conducted to investigate T cell immune responses. Antibodies induced by MAV/06 vaccination showed immunogenicity against a variety of varicella-zoster virus and cross-reactivity among the virus clades.

**Conclusions:** It is indicating the similarity of the antibody responses induced by commercial varicella vaccines and the MAV/06 vaccine. Moreover, VZV-specific T cell immune response from MAV/06 vaccination was increased via Th1 cell response. MAV/06 varicella vaccine induced both humoral and cellular immune response via Th1 cell mediated response.

**Background**

Varicella-zoster virus (VZV) is one of the most common pathogens that affects humans [1, 2]. The virus causes chickenpox with its initial infection and herpes zoster (shingles, or simply zoster) after later reactivation in the body, induced by waning VZV-specific T cell response [1, 2]. Live-attenuated VZV vaccines have been developed and have been used for decades to prevent chickenpox [3–6]. A zoster vaccine, a highly concentrated form of VZV vaccine [7–9], has recently become available [10].

T cells are involved in pathogenesis of chickenpox as well as zoster [11]. In immune compromising conditions such as aging, the reduction of VZV-specific immune memory CD4 + T cells has been observed. The impaired immunity to VZV can lead to the reactivation of the initial infectious virus, which can be followed by a zoster outbreak [12–14]. In addition, T cell immunity is also crucial in primary VZV infection [15]. Children suffering from immune deficiencies with cellular immunity are readily infected by varicella, not likely with humoral immunity such as agammaglobulinemia [16–19].

There are 5 major clades and two provisional clades (VI and VII) of VZV that have been identified [20, 21]. Several studies have demonstrated a distinctive geographic distribution of the 5 major VZV genotypes [22, 23]: Clades 1 and 3 are common in Europe and North America; clade 2 has been found in Asia; clade 5 is common in India and Africa; and clade 4 is present in Europe and other areas. The Oka strain, the
vaccine strain used in live-attenuated VZV vaccine and zoster vaccine, was isolated in Japan and belong to clade 2 along with most other virus isolates from Japanese and Korean [24–26].

Another VZV vaccine strain, designated as MAV/06, was developed by attenuation of a wild-type isolate obtained from a Korean patient suffered with chickenpox in Seoul [3]. MAV/06 vaccine (Suduvax® as its trade name) has been commercialized in Korea since 1994 and globally since 1998. MAV/06 strain is genetically similar to Oka strain and is also clustered as clade 2 [25]. Although the MAV/06 strain has been used to produce VZV vaccines for more than 20 years, few studies have compared the characteristics of the immunological responses among different VZV strains.

A new MAV/06-based vaccine, BARYCELA®, has been developed and was approved in early 2020 by the Ministry of Food and Drug Safety in Korea. We evaluated the cross-reactivity of antibodies induced by the MAV/06 virus with VZV isolates of various genotypes. In addition, we compared both the humoral and cellular immunogenicity generated by MAV/06 vaccine to those of other VZV vaccines, including those derived from the Oka and MAV/06 viral strains.

**Methods**

**Viruses and cells**

MRC-5 cells were purchased from ECACC (European Collection of Authenticated Cell Cultures) and maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen Life Technologies) supplemented with 10% fetal bovine serum (FBS; Gibco) and sodium pyruvate. VZV YC strains and Jena strains were kindly provided from Dr. Hosun Park (Youngnam University, Korea) and Dr. Andreas Sauerbrei (Jena University, Germany), respectively. The 8 VZV YC isolates were clade 2 genotypes [27] and the 6 Jena VZV isolates were clustered into the major VZV clades 1, 3 and 5 [21] (Table 1). VZV viruses were propagated in MRC-5 cells, and titer for infectious viruses were determined with plaque assay on MRC-5 cells.
Table 1
Virus isolates used in this study.

| Strain name | Strain origin | Clade | Sampling date | Patient Information (Sex, Age) | Accession No. | Reference |
|-------------|---------------|-------|---------------|--------------------------------|---------------|-----------|
| YC01        | Zoster        | 2     | 19 June 2012  | M, 40                          | KJ767491.1    | Kim et al. 2017 |
| YC02        | Zoster        | 2     | 18 July 2012  | M, 3                           | KJ767492.1    | Kim et al. 2017 |
| YC03        | Zoster        | 2     | 07 Aug. 2012  | F, 8                           | KJ808816.1    | Kim et al. 2017 |
| YC04        | Zoster        | 2     | 02 May 2013   | F, 76                          | -             | Kim et al. 2017 |
| YC05        | Zoster        | 2     | 01 July 2013  | F, 52                          | -             | Kim et al. 2017 |
| YC06        | Zoster        | 2     | 09 July 2013  | M, 73                          | -             | Kim et al. 2017 |
| YC07        | Zoster        | 2     | 22 July 2013  | M, 80                          | -             | Kim et al. 2017 |
| YC08        | Zoster        | 2     | 24 July 2013  | M, 66                          | -             | Kim et al. 2017 |
| Jena 4 (432/2008) | Zoster | 1 | 07 Feb.2008 | M, 57 | JN704695.1 | Sauerbrei et al. 2012 |
| Jena 6 (1883/2007) | Varicella | 1 | 07 Nov.2007 | F, 4 | JN704694.1 | Sauerbrei et al. 2012 |
| Jena 12 (2308/2003) | Varicella | 3 | 22 Nov.2003 | F, 5 | JN704699.1 | Sauerbrei et al. 2012 |
| Jena 16 (52/2007) | Zoster | 3 | 08 Jan.2007 | M, 18 | JN704701.1 | Sauerbrei et al. 2012 |
| Jena 26 (446/2007) | Varicella | 5 | 15 Mar.2007 | M, 1 | JN704707.1 | Sauerbrei et al. 2012 |
| MAV/06      | Varicella     | 2     | 1989          | M, 3                           | JF306641.2    | Lee et al. 2011 |

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Animal experiment

Male Hartley guinea pigs weighing 200 to 250 g were purchased from Japan SLC (Japan). Thirty guinea pigs were injected subcutaneously in the scruff of the neck with the MAV/06 vaccine containing
approximately 45,000 plaque forming units (PFU) 2 times with a 3-week interval between injections. Blood was collected by cardiac puncture three weeks after second immunization. Sera were pooled and stored at -70°C until tested for cross-reactivity.

Five to six-week-old female C57BL/6 mice were purchased from Orient Bio (Korea). 4 heads of C57BL/6 female mice were used for the mouse experiment. MAV/06 vaccines were prepared with low (~600 PFU/0.1 mL), medium (~2,000 PFU/0.1 mL), and high (~4,000 PFU/0.1 mL) viral titers. Commercialized vaccines were used as positive control: Suduvax® (Vx1) at a minimum of 1,400 PFU/0.5 mL and Zostavax® (Vx2) at a minimum of 19,400 PFU/0.5 mL. Animals were immunized intramuscularly with 0.1 mL of vaccine formulations in thigh muscle of the hind limb 2 times with a 3-week interval between injections after random allocation. Animals were sacrificed two weeks after the second immunization. The sera and spleen were collected from the sacrificed animals.

The sample size was determined by previous experiments, and in previous experiments it was determined that this sample would be appropriate. All animals were anesthetized with isoflurane using closed chambers. Animals were monitored every day and 20% of weight loss was considered for humane endpoints. According to the internal guideline, 20% of weight loss was considered for the criteria. During this study, there were no cases of animals that died.

All experiments including the procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee in GC Pharma (approval No. 2018002). In addition, we confirm that all methods were carried out in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Rodent Experiments provided by the IACUC (Institutional Animal Care and Use Committee), and all methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

**Plaque reduction neutralization test**

Plaque reduction neutralization tests (PRNT\(_{50}\)) were performed as previously described [28]. Briefly, 2-fold dilutions of heat-inactivated guinea pig sera, from 4-fold through 128-fold, were each mixed with an equal volume of diluted VZV isolates at 100 PFU/0.1 mL. The mixtures were incubated for 1 hour at 37°C. Two hundred microliters of the mixture were added to \(6 \times 10^5\) MRC-5 cells seeded in wells of 6-well culture plates. The plates were incubated for 60 minutes at 37°C with agitation. DMEM with 2% FBS was overlaid and incubated for 5 days. After removing the overlays, a 0.5% crystal violet solution in 25% methanol was added to the cells. The number of stained plaques were counted. PRNT\(_{50}\) titers were determined as the reciprocal of the serum dilution that demonstrated a 50% reduction in plaque counts.

**Fluorescent antibody to membrane antigen (FAMA) test**

FAMA assay was performed with slight modification from previously described methods [29]. Briefly, MRC-5 cells were grown to confluency in 175T flasks and infected with VZV isolates at 0.003 m.o.i. When cytopathic effects were observed in ~50–60% of the cells, the cells were washed three times with PBS. Infected cells were detached from the flasks and incubated with serially-diluted sera at room temperature
for 30 min. After washing with PBS, cell preparations were incubated with Alexa Fluor®488 goat anti-
guinea pig IgG (Invitrogen) and mounted on slides. After overlaying with mounting media containing
DAPI, the slide was covered with a cover-glass and observed under a fluorescence microscope (Nikon).
Titers were defined as the reciprocal of the highest dilution causing bright fluorescent ring around the
surface of cells. Titers of ≥ 1:4 were considered positive. Pooled sera from MAV/06 immunized guinea
pigs were pre-treated with MRC-5 cells before incubation with virus-infected cells in order to remove
immune responses to cellular debris within vaccine ingredients. Non-specific reactions to mock-infected
cells were confirmed using both vaccine groups.

Glycoprotein enzyme-linked immunosorbent assay
(gpELISA)

Microplate (Corning) were coated with 1 µg/mL of VZV glycoprotein (QED BIO) in PBS at 4°C overnight.
Plates were washed with wash buffer (0.05% Tween in PBS) and the wells blocked with ELISA assay
buffer (1% BSA, 0.1% Tween in PBS) for 2 hr. A thousand-fold dilution of sera from immunized mice were
added to the wells and incubated for 2 hrs. Plates were washed and incubated with HRP-conjugated
secondary antibody mouse IgG (Southern Biotech) for 1 hr. After the final wash, TMB substrate (KPL) was
added to each well and incubated for 15 min. The reactions were stopped with TMB stop solution (KPL)
and the 450 nm absorbance determined using a spectrophotometer (Molecular Devices, USA) and data
were analyzed using SoftMaxPro (Molecular Devices, USA).

Interferon-gamma enzyme-linked immunosorbent spot
(IFN-γ ELISpot) assay

Spleen cells were isolated from the immunized mice and suspended at a final concentration of 5 x 10^6
per mL in RPMI 1640 medium (Gibco) supplemented with 10% (vol/vol) heat-inactivated FBS (Gibco),
antibiotic-antimycotic solution (anti-anti; Gibco), 2 mM Glutamax (Gibco), 1 mM sodium pyruvate (Gibco),
and 55 µM 2-mercaptoethanol (Gibco). Multiscreen-HA filter plates were coated with anti-mouse IFN-γ
capture antibody (R&D systems) at 4°C overnight and blocked with RPMI 1640 medium with 10% FBS.
Cells were added with intact virions (VZV lysate; Mycrobix), recombinant glycoprotein E (gE), glycoprotein
I (gI) (gE and gI from Peptron), IE63 (Genscript), and overlapped peptide (IE63 OLP from JPT), and
incubated overnight at 37°C. The plates were washed with PBS (3x) and biotinylated anti-mouse IFN-γ
detection antibody (R&D systems) was added. After washing, streptavidin was conjugated and 3-amino-
9-ethylcarbazole (AEC; BD) were added to the plates. The reaction was stopped by rinsing with tap water.
Spot-forming units (SFU) were read using ELISPOT reader (Autoimmune Diagnnositika). Adjusted SFU
were obtained by subtraction of mock-stimulated counts (mock lysate for VZV lysates, medium for
recombinant protein, and DMSO for OLP).

Cytokine bead array (CBA)

Splenocytes were stimulated with intact virions at 37°C for three days. Supernatants were harvested and
subjected to cytometric bead array (CBA, BD Biosciences) analysis to detect levels of Th1/Th2/Th17
cytokines, tumor necrosis factor alpha (TNF-α), IFN-γ, interleukin 2 (IL-2), IL-4, IL-6 IL-10, IL-17A.

**Statistical analysis**

All results are expressed as the means ± standard errors of the means and compared by two-way ANOVA. Statistical analysis was performed using GraphPad Prism™ software (GraphPad, San Diego, CA, USA), and statistical significance was defined as a p value (*p < 0.05, **p < 0.01, ***p < 0.001).

**Results**

**MAV/06 vaccine elicits cross-reactive antibodies to multiple wild-type VZV isolates**

Guinea pigs were immunized on Day 0 and 21 subcutaneously in the scruff of the neck with new MAV/06 vaccine. On Day 42 post-immunization, blood was collected and sera were evaluated for capacity to neutralize the infectivity of virion and infected cells with multiple wild-type VZV isolates. Eight YC isolates, isolated in Korea, were projected into clade 2 genotype [27] and six Jena isolates were clustered into major VZV genotype 1, 3 and 5 respectively [21] (Table 1). The neutralization titer against MAV/06 virus was 78 ± 1. The titers against clade 2 viruses, which MAV/06 belongs to, was 159 ± 61 and those for other clades including clade 1, 3, 5 was 129 ± 52 (Fig. 1A, Table 2). FAMA test, which is to detect antibodies against virus infected cells, is the gold standard method to measure protective antibodies against VZV [30, 31] since VZV is transmitted by cell-to-cell spread. As shown in Fig. 1B, FAMA titer was 128 for MAV/06 itself and the titer showed 192 ± 68 for clade 2 viruses and 179 ± 70 for other clades, respectively (Fig. 1B, Table 3). The neutralization activities against virion and infected cells did not show significant difference between clade 2 and other virus clades. These data demonstrate that MAV/06 vaccine immunization induces broad cross-clade antibody responses.
Table 2

Result of PRNT\textsubscript{50} titer against multiple VZV isolates.

| Strain name | PRNT\textsubscript{50} Mean | SD |
|-------------|-----------------------------|----|
| YC01        | 156 159 179 165 13          |
| YC02        | 304 244 217 255 45          |
| YC03        | 99   91   82   91   8      |
| YC04        | 162  209 202 191 25        |
| YC05        | 197  158 203 186 24        |
| YC06        | 178  147 189 172 22        |
| YC07        | 149  136 177 154 21        |
| YC08        | 57   55   63   58   4      |
| Jena 4      | 160  148 149 153 6         |
| Jena 6      | 128  146 128 134 11        |
| Jena 12     | 64   58   57   60   4      |
| Jena 16     | 196  192 203 197 6         |
| Jena 26     | 101  92  105  99  7        |
| MAV/06      | 78   77  80   78   1       |

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Table 3
Result of FAMA titer against multiple VZV isolates.

| Strain name | Clade | Titer |
|-------------|-------|-------|
| YC01        | 2     | 256   |
| YC02        | 2     | 256   |
| YC03        | 2     | 128   |
| YC04        | 2     | 256   |
| YC05        | 2     | 128   |
| YC06        | 2     | 128   |
| YC07        | 2     | 256   |
| YC08        | 2     | 128   |
| Jena 4      | 1     | 128   |
| Jena 6      | 1     | 128   |
| Jena 12     | 3     | 256   |
| Jena 16     | 3     | 128   |
| Jena 26     | 5     | 256   |
| MAV/06      | 2     | 128   |

MAV/06 vaccine induced humoral immune responses comparable to commercialized VZV vaccine

C57BL/6 mice were immunized subcutaneously with MAV/06 vaccine or commercialized vaccines two times with three weeks’ interval. Blood was gathered 2 weeks after immunization and sera were tested to detect VZV specific antibodies.

Despite the value and broad use of FAMA as a “gold-standard” assay, the VZV gpELISA is an acceptable alternative to detect VZV gp-specific antibodies as a result of the strict parameters of the FAMA test [31]. Since the titer of varicella and zoster vaccine is claimed as “minimum” dose, it is impossible to directly compare the new MAV/06 vaccine with commercialized Oka strain and MAV/06 vaccine.

Regardless, the gpELISA titer of VZV gp-specific antibodies induced by the low dose of new MAV/06 Vaccine (equals to minimum dose of new vaccine) was similar to commercialized Varicella vaccine (Vx1), and the high dose of MAV/06 Vaccine was similar to that of commercialized Zoster vaccine (Vx2)
both in priming status (Fig. 2A) and boosting status (Fig. 2B). Antibodies induced by the new MAV/06 vaccine demonstrated a dose-dependent response in priming (Fig. 2A) while the difference was not significant (ns) in boosting status (Fig. 2B).

**MAV/06 vaccine potently increased VZV-specific T cell response**

Vaccines containing live organisms are likely to induce cellular immune response as well as humoral immune response [32]. Moreover, T cell mediated immunity in VZV infection had an impact on prognosis for chickenpox and shingles [33]. For these reasons, VZV-specific T cell response was evaluated in new MAV/06 vaccine immunized mice and compared with commercial live vaccine immunized mice.

Splenocytes from immunized mice were stimulated with VZV virions and IFN-γ secreting T cells were detected in a MAV/06 dose-dependent manner (Fig. 3A). Protein and overlapping peptides for gE, gI and IE63, which is identified as T cell epitopes, were prepared and incubated with splenocytes from vaccine inoculated mice. As shown in Fig. 3B and 3C, those stimulated cells which is secreting effector cytokine IFN-γ, to a greater or lesser extent compared with commercialized vaccines, and responded better to gE epitope compared with other epitope. This indicated that MAV/06 vaccine is a potent inducer of VZV-specific T cell response.

**MAV/06 vaccine induced Th1 skewed immune response**

Three subsets of CD4+ T helper cells (Th1, Th2 and Th17) have been identified on the basis of cytokine profiles [34, 35]. Th1 cytokines are IL-2, IFN-γ, and TNF-α, and those cytokines promote proliferation, differentiation and activation of macrophage, NK cells, Th1 cells and cytotoxic T cells. Th2 cytokines are IL-4 and IL-6 which stimulates B cell proliferation and maturation into plasma cells. Th17 cytokine is IL17A, IL21 and IL-22, and Th17 contributes progression of autoimmune and inflammatory diseases. Splenocyte from immunized mice were incubated with each VZV virion, cytokine profiles in supernatant were analyzed with CBA. IFN-γ, and TNF-α cytokine secreted significantly and the secretion was to a greater or lesser extent comparable in MAV/06 vaccine and commercialized vaccines (Fig. 4E-4F) while IL-4, IL-6, IL-17A, IL-2 cytokine was not detected by MAV/06 or commercialized vaccine stimulation (Fig. 4A-4D). The cells also secreted IL-10 as auto-regulator of Th1 cell activation (Fig. 4G). Taken together, these results indicate that new MAV/06 vaccine induced a Th1 type polarized response comparable to commercialized vaccines.

**Discussion**

In this study, we confirmed that the newly developed MAV/06 vaccine triggers humoral immunity by production of antibodies exhibiting cross-reactivity to various VZV virus clade antigens from clade 1, 2, 3, 5. Furthermore, new MAV/06 vaccine induces cell-mediated immunity through Th1 cell response.
Currently, there exists 7 different clades of VZV worldwide and most of the varicella vaccine viruses come from 5 types of clades [20, 21]. Commercially available VZV vaccines are manufactured from Oka strain and MAV/06 strain from clade 2. Although it is common that confirming reactivities of antibodies induced by vaccination and antigens which is same as the vaccine when conducting PRNT\textsubscript{50} or FAMA tests, few studies have shown cross-reactivity of vaccine-induced antibodies among different virus clades. Cross-reactivity of antibodies induced by vaccination against various clades of VZV is important to estimate the prophylactic effectiveness against wild-type VZV in the field.

From the PRNT\textsubscript{50} and FAMA studies, we demonstrated that the newly developed MAV/06 vaccine triggers humoral immunity by the production of antibodies exhibiting cross-reactivity to viruses from VZV clades 1, 2, 3, and 5. A study conducted with Oka strain illustrated that Oka vaccine induced antibody response to a wild-type VZV that prevailed in Germany [36]. The extent of antibody reactivities to clade 1, 2, 3, and 5 viruses was similar to that of the MAV/06 virus. These results are similar to the results from the study conducted in Germany (Fig. 1) [36]. This study was conducted with the MAV/06 strain that used both in Suduvax and a newly developed Varicella vaccine (Trade name: BARYCELA inj.) with upgrade formulation by GC Pharma. It is the first investigation demonstrating that MAV/06 vaccination induced antibody responses against various VZV clades worldwide as well as clade 2 viruses that prevail in Asian countries, including the Republic of Korea and Japan.

Live-attenuated vaccines exhibit immunological strength that enables antibody induction and other immune reaction such as T cell immune response [32]. Previous studies of VZV vaccines implied the importance of T cell immunity after vaccination [1, 2, 9, 11–14, 33]. The occurrence of herpes zoster and the observed concomitant reduction of cell-mediated immunity (CMI) in individuals with high titers of VZV antibody indicate that the reduction in CMI is the primary cause of herpes zoster. CMI evaluation were performed using the VZV vaccine developed from the Oka strain and reported CMI induction by the VZV vaccine [33, 37]. We investigated MAV/06 vaccine-induced CMI responses and demonstrated the vaccine evoked VZV-specific B cell and T cell immune responses that were comparable to that of the commercial vaccines. In addition, the new MAV/06 vaccine-induced T cell response was found to be mediated by Th1 rather than Th2 or Th17 cell responses, implying the MAV/06 vaccine functions via intracellular virus clearance as have been shown for other previous studies [38]. These results indicate that the live-attenuated MAV/06 vaccine induces both humoral and cellular immunity in live organism.

Our study provided the explanation to the previously reported vaccine efficacy via immunological characterization of MAV/06 strain and will contribute to future studies for vaccine efficacy or effectiveness after new MAV/06 vaccine.

**Conclusions**

This study indicated that MAV/06 strain vaccination triggers B cell immune response to various VZV clades and T cell immune response via Th1 cells in animal model.
Declarations

Acknowledgments

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Author Contributions

Conceptualization: Shin DH.

Methodology: Shin DH, Kim EM, Nam HJ.

Formal analysis: Shin DH, Kim EM, Nam HJ.

Validation: Nam HJ, Nan HY, Shin YC.

Resources: Shin DH, Kim EM, Nam HJ.

Visualisation: Shin YC.

Supervision: Lee JW.

Writing – original draft: Shin YC, Shin DH.

Writing - review & editing: Shin YC, Kim EM, Nan HY, Lee JW.

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GC Pharma was the funding source. MOGAM Institute & GC Pharma were involved in this study and analysis.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval

All experiments including the procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee in GC Pharma (approval No. 2018002). In addition, we confirm that all methods were carried out in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals and the Guidelines and Policies for Rodent Experiments provided by the
IACUC (Institutional Animal Care and Use Committee), and all methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

Competing interests

Shin DH, Shin YC, Kim EM, Nam HJ, Lee JW employees of the GC group of companies. Involvement of GC employees did not compromise the scientific integrity of this work.

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Figures

Figure 1

**Evaluation of cross reactivity of antibodies induced by MAV/06 vaccine.** Guinea pig (n = 30) were immunized subcutaneously two times at three weeks interval with MAV/06 vaccine. Sera were collected by cardiac puncture 3 weeks after second immunization. (A) PRNT<sub>50</sub> titer against multiple VZV isolates (included genetic clade 1 to 5) were measured by plaque reduction neutralization assay in triplicate. (B) FAMA, which is the gold standard method to detect protective antibody against VZV, were performed with MRC-5 cell infected with multiple VZV isolates.

Figure 2

**VZV specific antibody responses after MAV/06 vaccine immunization.** Mice were immunized intramuscularly two times at three weeks interval. MAV/06 vaccines were prepared with different concentration, and commercialized vaccines for chickenpox and shingles were used as positive control. Mock lysate and PBS were used as negative control. (A) Sera were collected 3 weeks after first
immunization and (B) 2 weeks after second immunization, and VZV specific antibody were measured with gpELISA.

**Figure 3.**

**VZV specific T cell responses after MAV/06 vaccine immunization.** Splenocyte were collected from immunized mice with MAV/06 or commercialized vaccines. (A; VZV lysate) Cells were pulsed with VZV virion or (B) recombinant proteins or (C; gE, gI and IE63) overlapping peptides as its component. Interferon-γ secreting T cells were measured by IFN-γ ELISPOT. Spot counts were adjusted by baseline spot with mock-pulsed (mock lysate for VZV lysate, medium for recombinant protein, and DMSO for OLP).

**Figure 4.**

**Multiple cytokine analysis of VZV-specific immune cells.** Spleen cells were prepared from immunized mice with MAV/06 or commercialized vaccines. Cells were incubated with VZV lysate for 3 days, and supernatants were analyzed with Th1/Th2/Th17 cytokine bead array. Cytokine profiles were adjusted by subtracting result of mock lysate stimulated cell.