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Short Communication

Induction of antibody and interferon-γ production in mice immunized with virus-like particles of swine hepatitis E virus

Young-Jo Song[1], Woo-Jung Park[1], Seul-Kee Lee[1], Joong-Bok Lee[1], Seung-Yong Park[1], Chang-Seon Song[1], Sang-Won Lee[1], Kun-Ho Seo[2], Young-Sun Kang[3], Jae-Young Song[4], In-Soo Choi[1,5,*]

Departments of [1]Infectious Diseases, and [2]Public Health, College of Veterinary Medicine, [3]Department of Biomedical Science and Technology, SMART Institute of Advanced Biomedical Science, and [5]Veterinary Science Research Institute, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea
[4]Animal and Plant Quarantine Agency, Anyang 430-855, Korea

Virus-like particles (VLPs) composed of the truncated capsid protein of swine hepatitis E virus (HEV) were developed and immune responses of mice immunized with the VLPs were evaluated. IgG titers specific for the capsid protein of swine HEV were significantly higher for all groups of mice immunized with the VLPs than those of the negative control mice. Splenocytes from mice immunized with the VLPs also produced significantly greater quantities of interferon (IFN)-γ than interleukin (IL)-4 and IL-10. These newly developed swine HEV VLPs have the capacity to induce antigen-specific antibody and IFN-γ production in immunized mice.

Keywords: interferon-γ, swine hepatitis E virus, virus-like particle

Hepatitis E caused by hepatitis E virus (HEV) is a serious public health concern in developing countries where HEV is mainly transmitted through contaminated water [1]. Recently, autochthonous hepatitis E cases have been increasingly reported in industrialized countries. Most of these patients contracted the disease by consuming undercooked pork products [5]. HEV is now recognized as an emerging zoontic agent [12]. This virus belongs to the genus *Hepevirus* in the family *Hepeviridae* and is a non-enveloped virus with a positive sense, single-stranded RNA genome 7.2 kb in size [6].

The HEV genome contains three open reading frames (ORFs) [13]. ORF 1 is located at the 5' end of genome and encodes non-structural proteins. ORF 2 encodes a capsid protein that plays an important role in viral immune evasion and virion formation. ORF 3 overlaps with ORFs 1 and 2, and encodes an immunogenic small protein. The first animal strain of the virus, swine HEV, was isolated and characterized from a pig in the United States in 1997 [8]. The prototype strain of swine HEV is genetically related to the US strain of human HEV. Cross-species HEV infection between swine and nonhuman primates has been observed [7].

Virus-like particles (VLPs) lack genomes and are basically composed of viral structural proteins, rendering them non-infectious and incapable of reversion. Therefore, these particles are reputed to be very safe vaccine candidates. More importantly, they induce cellular immune responses as well as humoral immunity [3]. The purposes of the present study were to develop VLPs composed of the capsid protein of swine HEV and evaluate their immunogenicity in mice.

All experiments were performed under the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Konkuk University, Korea (permit no. KU12114). A DNA fragment encoding the truncated capsid protein of swine HEV (amino acids 112-608) known to contain the most immunogenic site was amplified by PCR using plasmid pHEV5137/7181 as a template [15]. The plasmid contains the full-length genotype 3 swine HEV ORF2 and was previously described in the literature [11]. Sf9 insect cells (Invitrogen, USA) were infected with recombinant baculovirus expressing the capsid protein to produce HEV VLPs. The resulting HEV VLPs were purified as previously described [10].

Female BALB/C mice 5~6 weeks old were divided into four groups (n = 10 per group). Mice in groups 1, 2, and 3 were intramuscularly injected with 100 μL (total volume)

of a solution containing 1, 5, or 10 μg of the HEV VLPs, respectively, homogenized with 10% aluminum hydroxide (Reheis, USA). Mice in group 4 received PBS as a negative control. The animals were immunized only one time.

Serum samples were collected by retro-orbital plexus puncture before immunization and 3 weeks after immunization. The samples were stored at −20°C prior to antibody titer analysis. Antibody titers were determined using an indirect enzyme-linked immunosorbent assay (ELISA) with purified HEV VLPs as an antigen. The mice were sacrificed 3 weeks after immunization to collect splenocytes and measure cytokine production. To analyze the cellular immune responses, lymphocytes isolated from the spleens of immunized and negative control mice were stimulated with purified HEV VLPs at a final concentration of 10 μg/mL. After 24 h, the cell culture supernatants were collected to measure the concentration of interleukin (IL)-4, IL-10, and interferon (IFN)-γ using commercially available cytokine-specific quantitative ELISA kits (R&D Systems, USA) according to the manufacturer's instructions. Antibody titers and cytokine production were measured in duplicate. Significant differences between the immunized and control groups were identified by Student's *t* test using Sigmaplot (ver. 12.0 Systat Software, USA). *P* values < 0.05 were considered statistically significant.

VLPs were generated by Sf9 cells infected with recombinant baculovirus. The particles were purified by sucrose layer gradient ultracentrifugation and detected by Western blot analysis (53-kDa bands) using a capsid-specific antibody (panel A in Fig. 1) or directly visualized with a transmission electron microscope (panel B in Fig. 1). No capsid-specific antibodies were detected prior to immunization in any of the mice treated with the VLPs. Antibodies against the capsid protein of swine HEV appeared in all the VLP-immunized mice. Animals that received either the lowest (1 μg) or highest (10 μg) dose of the VLPs produced similar antibody titers (panel A in Fig. 2). These results indicated that the low dose of the VLPs was sufficient for inducing the production of high antibody titers.

The expression patterns of IFN-γ, IL-4, and IL-10 by splenocytes isolated from the immunized mice were monitored. The Th1-type cytokine IFN-γ was more prominently expressed than the Th2-type cytokines IL-4 and IL-10 in mice immunized with the VLPs. On the other hand, expression of all three cytokines was negligible in the control mice. The maximum amounts of IL-4 and IL-10 produced by the mice immunized with the VLPs were about 17 and 182 pg/mL, respectively (panels B and C in Fig. 2). The maximum concentration of IFN-γ generated in mice immunized with the VLPs was about 1,295 pg/mL (panel D in Fig. 2). These data indicated that the VLPs can produce a potent IFN-γ and Th1-type immune response.

VLPs are composed of self-assembled, genome-free viral structural proteins. VLP-based vaccines are highly immunogenic and induce anti-viral immune responses due to the dense organization of viral proteins in spherical forms [4]. It is impossible to generate revertants from VLPs; this is an important safety feature of VLPs. Therefore, these particles are considered one of the most promising new vaccine candidates. Strong humoral immune responses to structural proteins incorporated into the VLPs are commonly detected with many VLPs
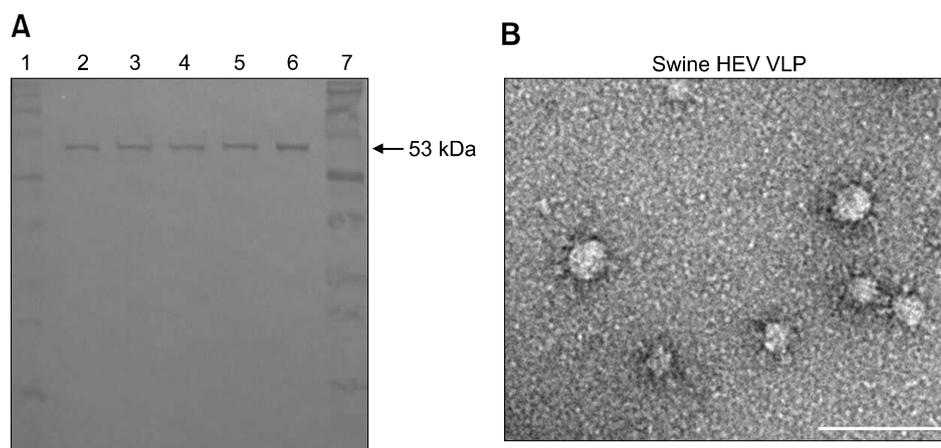
Fig. 1. Identification of swine hepatitis E virus (HEV) virus-like particles (VLPs) by Western blotting and transmission electron microscopy. (A) VLPs derived from the swine HEV capsid protein were purified on a sucrose gradient and five fractions were collected. Presence of the VLPs was identified by Western blotting with a polyclonal rabbit anti-swine HEV capsid protein antibody. Capsid-specific bands (53 kDa) were produced by samples from all fractions. Lanes 1 and 7 contain a standard protein marker, and lanes 2∼5 contain VLPs derived from the capsid protein. (B) Morphology of the HEV VLPs particles viewed with transmission electron microscopy. Scale bar = 100 nm.
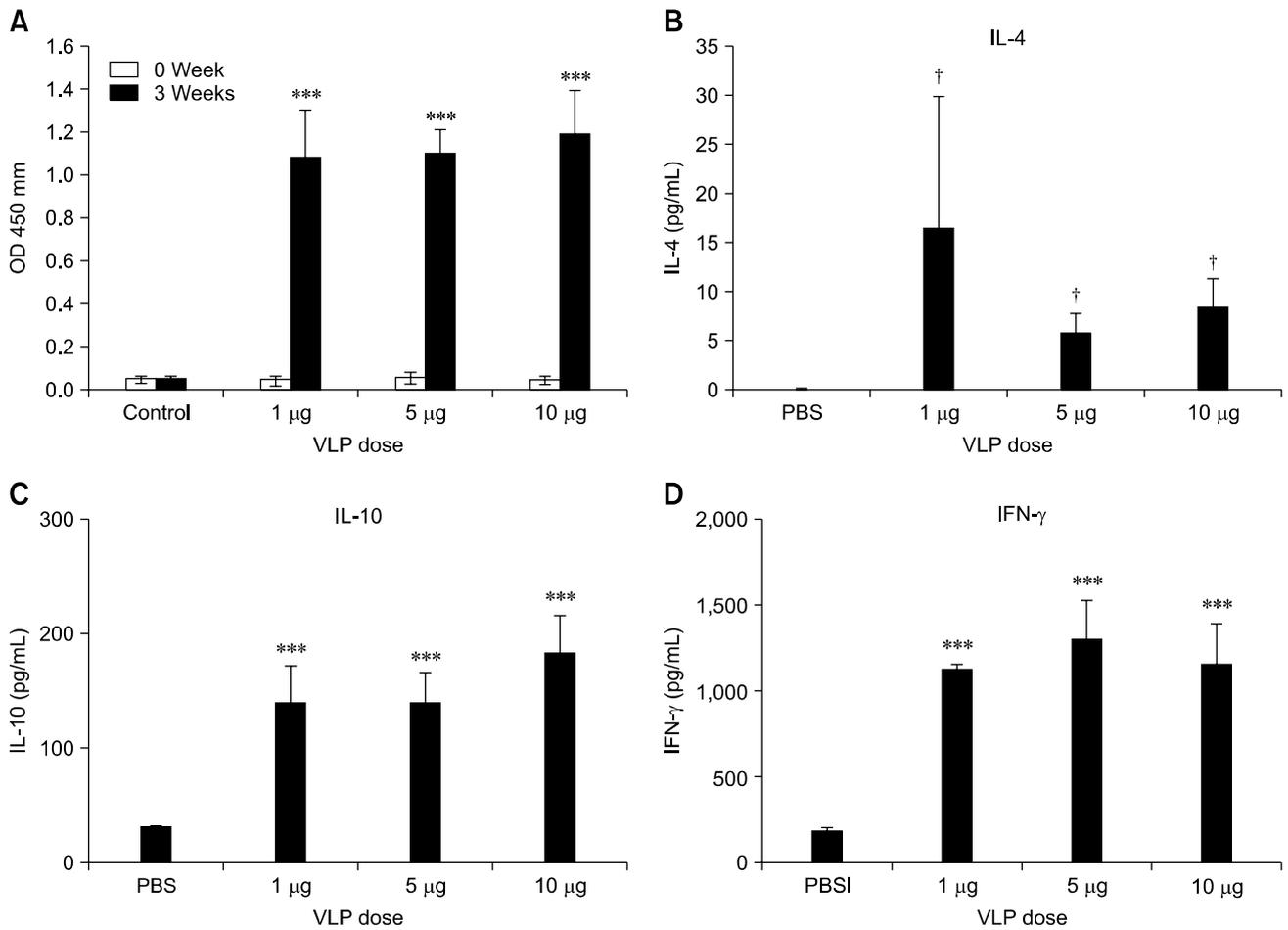
Fig. 2. Antibody titers and cytokine concentration for mice immunized with HEV VLPs. Blood samples were obtained from the mice before immunization and 3 weeks after immunization (0 and 3 weeks as shown in the insert). (A) Antibody titers for the serum samples collected at 3 weeks from mice immunized with 1, 5, or 10 μg of the VLPs were significantly higher (***$p < 0.001$) than those found for the negative control mice injected with PBS. Splenocytes taken from mice immunized with the VLPs and negative control mice at 3 weeks after immunization were stimulated with the VLPs for 24 h. Cell culture supernatants were then collected and the concentrations of IL-4, IL-10, and IFN-γ were determined. (B) The amount of IL-4 produced by mice immunized with the VLPs was significantly higher ($^{\dagger}p < 0.05$) than that from the negative control group. (C) The quantity of IL-10 produced by mice immunized with the VLPs was significantly higher (***$p < 0.001$) than that generated by the negative control group. (D) The concentration of IFN-γ produced by mice immunized with the VLPs was significantly higher (***$p < 0.001$) than that of the negative control group.

including ones derived from the influenza virus [2]. Several kinds of animal-based VLPs have been developed as experimental vaccines [3]. However, no study describing the creation of VLPs composed of the swine HEV capsid protein has been published. VLPs derived from swine HEV generated in the present study induced IgG production in immunized mice.

It is well known that neutralizing or neutralizing-of-binding (NOB) antibodies recognize the protruding (P) domain of the HEV capsid protein [9]. Even though we did not observed the presence of neutralizing antibodies, VLPs generated in our study contained the P domain that included amino acids 456-606. Therefore, we expect the antibodies produced by immunization with the swine HEV VLPs would include neutralizing or NOB antibodies.

Exposure to the swine HEV VLPs also induced a high level of IFN-γ production in the vaccinated mice. When spleen cells from the animals were stimulated with the VLPs *in vitro*, they produced a much higher concentration of IFN-γ compared to IL-4 or IL-10. Similar immune responses were found to be induced by several kinds of VLP-based vaccines including ones produced from influenza and human papilloma viruses [2,4]. In part, strong Th1 type-specific immune responses could be attained by interaction of the VLPs with dendritic cells, leading to stimulation of CD8$^+$ T cells [14]. In conclusion, we developed novel VLPs derived from the swine HEV capsid protein. The particles were highly immunogenic in mice, and strongly induced humoral as well as cellular immune responses. Our findings demonstrated that VLPs are a

suitable vaccine candidate for controlling swine HEV infection.

Conflict of Interest

There is no conflict of interest.

References

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