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Cross-protection induced by *Toxoplasma gondii* virus-like particle vaccine upon intraperitoneal route challenge

Dong-Hun Lee[a], Ah-Ra Kim[a], Su-Hwa Lee[a], Fu-Shi Quan[b,*]

[a] Department of Biomedical Science, Graduate School, Kyung Hee University, Seoul, Republic of Korea
[b] Department of Medical Zoology, Kyung Hee University School of Medicine, Seoul, Republic of Korea

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ABSTRACT

The inner membrane complex sub-compartment has a critical role in *Toxoplasma gondii* endodyogeny. In this study, we investigated the protection upon intraperitoneal route (IP) challenge induced by the virus-like particles (VLPs) vaccine containing *Toxoplasma gondii* IMC ISP (RH strain) (Type I). Intranasal immunization with the VLPs in mice elicited enhanced systemic and mucosal *Toxoplasma gondii*-specific IgG, IgG1, IgG2a and IgA antibody responses, and CD4+ and CD8+ responses. Immunized mice significantly reduced *T. gondii* cyst burden and size in brain, resulting in cross-protection upon *T. gondii* (ME49) (Type II) challenge infection. These results indicate that the IP route challenge infection induced by *T. gondii* IMC ISP VLPs might be a very good target for vaccination representing novel approach to reduce infection.
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1. Introduction

T. gondii infections occur throughout the world, and infection rates differ significantly by country (Pappas et al., 2009). Humans and other warm-blooded animals are its hosts (Dubey, 2004). Approximately one-third of all humans have been exposed to this parasite. Although usually asymptomatic in immunocompetent adults, it can cause severe disease manifestations and even death in immunocompromised subjects. Effective drug are not widely available, and there is no licensed vaccine. Currently, the strategy of toxoplasmosis control is the chemotherapy targeting the acute phase of infection. However, the drugs have toxic effects and do not eliminate the cysts, therefore the patient can have reactivations (Rodriguez and Szajnman, 2012; Bhopale, 2003; Hassan et al., 2014). Regardless of the vaccine construct, the vaccines have not been able to induce protective immunity when the organism is challenged with *T. gondii*, either directly or via a vector (Jongert et al., 2009).

Toxoplasma gondii possess an unusual double membrane structure located directly below the plasma membrane named the inner membrane complex (IMC) coupled to a supporting cytoskeletal network (Harding and Meissner, 2014). IMC sub-compartment proteins (ISPs) have recently been shown to play a role in asexual *T. gondii* daughter cell formation. Three proteins, IMC sub-compartment protein (ISP) 1, ISP2, and ISP3, were initially identified and found to localize to distinct sub-compartments of the IMC in *T. gondii* (Beck et al., 2010). These three proteins localize in the different regions of complex. Disruption of ISP2 caused a significant loss in parasite fitness and a severe defect in endodyogeny, the form of internal cell budding in which two daughter cells are formed within the intact mother parasite (Beck et al., 2010). Since these three ISP1, 2 and 3 are largely conserved in amino acid sequences, we hypothesized that humoral or cellular immunity-inducing VLPs containing IMC ISP3 could target all three IMC ISP.

Virus-like particle (VLP) vaccines are genetically engineered complexes of multiple copies of protein antigens in a particulate virus-like structure. Viral proteins presented as VLPs or recombinant vaccines are highly immunogenic and induce protection (Zhan et al., 2007; Murawski et al., 2010; Takimoto et al., 2004; Yu et al., 2008). Therefore, it is hypothesized that VLPs containing *Toxoplasma gondii* IMC will induce strong *Toxoplasma gondii*-specific immune responses and immunity. VLPs consisting of the respiratory syncytial virus (RSV) fusion F protein and attachment G glycoprotein together with influenza M1 protein have been successfully developed, showing spherical particle shapes of VLPs. In this study, we developed VLPs consisting of the influenza M1 protein as a core protein together with IMC of *T. gondii*. Immune responses and protection against *T. gondii* (ME49) challenge were determined in mice immunized with these VLPs.

* Corresponding author.
E-mail address: fsquan@khu.ac.kr (F.-S. Quan).

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2. Materials and methods

2.1. Parasites, cells and antibodies

Toxoplasma gondii RH and ME49 strains were maintained by serial intraperitoneal passage (RH) or oral passage (ME49) in Balb/C mice. *Spodoptera frugiperda* Sf9 cells were maintained in suspension in serum-free SF900 II medium (GIBCO-BRL) at 27 °C in spinner flasks at 70–80 rpm. Horseradish peroxidase (HRP)-conjugated goat anti-mouse immunoglobulin A (IgA) and G (IgG), IgG1, and IgG2a were purchased from Southern Biotech (Birmingham, AL, USA).

2.2. Toxoplasma gondii *antigen*

T. gondii RH tachyzoites were harvested from the peritoneal cavity of the mice 4 days after infection. Cellular debris was removed and the parasites were sonicated, and *T. gondii* antigen was prepared as described (Fang et al., 2010).

2.3. *Constructions of rBV expressing* Toxoplasma gondii *IMC and influenza M1*

Total RNA of *T. gondii* (RH) tachyzoites was extracted (RNeasy Mini kit; Qiagen, Valencia, CA, USA). Complementary DNA (cDNA) was synthesized and *Toxoplasma gondii* IMC gene was amplified by polymerase chain reaction (PCR) from cDNA with primers 5-AAAGAATTCACCATGGGAGCTGTCAGCTCG-3 and 5-TTACTCGAGCTATGCCTTCAGCTTCAA-3 (EcoRI and XhoI underlined). A cDNA fragment containing the gene was cloned into pFastBac vector (Invitrogen, Carlsbad, CA, USA) as described previously (Quan et al., 2010). The accession number of the IMC protein in NCBI is HQ012578. Influenza M1 gene was obtained as described (Quan et al., 2010).

2.4. Generation of recombinant baculovirus (rBV)

Transfection of DNA containing *T. gondii* IMC or influenza M1 was done using Cellfectin II (Invitrogen) with SF9 cells as recommended by the manufacturer, followed by transformation of pFastBac containing *T. gondii* IMC or M1 with white/blue screening. The rBVs were derived using a Bac-to-Bac expression system (Invitrogen) according to the manufacturer's instructions.

2.5. Production of VLPs

Sf9 insect cells were co-infected with recombinant rBVs expressing *T. gondii* IMC or M1. VLPs released into the cell culture supernatants were harvested and purified through a 15%-30%-60% discontinuous sucrose gradient at 28000 rpm for 1 h at 4 °C. VLP bands between 30% and 60% were collected and then diluted with PBS and pelleted at 28000 rpm for 1 h at 4 °C. VLPs were resuspended in PBS overnight at 4 °C.

2.6. Characterization of VLPs

VLPs containing *T. gondii* IMC and influenza M1 were characterized by Western blots and electron microscopy. Antibody to determine *T. gondii* IMC in VLPs was prepared from *T. gondii* ME 49 infected mice. Anti-M1 antibody was used to determine M1 protein content. Negative staining of VLPs was performed followed by transmission electron microscopy, which was done at the Korea Advanced Institute of Science and Technology.

2.7. Immunization and challenge

Balb/c mice, 6–8 week old, (NARA Biotech, Seoul, Korea) were intranasally immunized twice with 100 μg total VLP protein at 4-week intervals (n = 6 per group). Blood samples were collected by retro-orbital plexus puncture before immunization and at 1, 2 and 4 weeks after priming and boosting. For challenge studies, naïve or immunized mice were infected with *T. gondii* ME49 intraperitoneally with 20 cysts in 100 μl PBS at 1 month after boosting. Body weight changes and survival were observed daily, and cysts in the brain were counted. Two independent experiments were performed. All animal experiments and husbandry involved in the studies presented in this manuscript were conducted under the guidelines of the Kyung Hee University IACUC.

2.8. Antibody responses in sera, feces and intestines

Blood and feces samples were collected at weeks 1 and 4 before and after challenge infection. Intestine samples were collected at week 4 postchallenge. IgG, IgG1, IgG2a and IgA antibody responses were determined by ELISA. Plates were coated with 100 μl of *T. gondii* RH (4 μg/ml) per well in coating buffer at 4 °C overnight. Feces samples were incubated in PBS at 37 °C for 1 h and the supernatants were collected after centrifugation at 2000 rpm and stored −20 °C until use. Intestines were collected at 4 weeks after postchallenge infection. The small intestine site for each mouse was 10 cm beneath the stomach. The collected intestine was incubated in PBS at 37 °C for 1 h. The intestinal mucus was collected and centrifuged at 2000 rpm for 10 min as described previously (Chu et al., 2014). The supernatant was stored at −20 °C until use.

2.9. Antibody-secreting cell response

Spleen was used to detect antibody-producing cells (ASC). *T. gondii* (RH) (2 μg/ml in 100 μl) was used to coat 96-well culture plates (SPL), and freshly isolated cells from the spleen (1×10^6 cells/well) were added to each well and incubated for 3–4 days at 37 °C with 5% CO_2. Parasite-specific IgG, IgG1, IgG2a and IgA antibodies secreted into the culture medium and bound to the coated antigens were determined as previously described (Chu et al., 2014).

2.10. Cellular responses

Mouse spleen was collected 1 month after challenge, and single-cell suspensions were prepared from each spleen. Cells were cultured and stimulated with 100 μl of 0 or 2 μg/ml *T. gondii* RH. Supernatants of spleen cell cultures were used to determine cytokines interferon-gamma (IFN-γ), interleukin (IL)-6 and IL-10 using OptEIA sets (BD Bioscience, San Jose, CA, USA). To determine CD4+ and CD8+ T cells in the spleen, cells were stained with CD4 and CD8 markers (BD Biosciences) and analyzed using a FACScan flow cytometer (BD, Mountain View, CA, USA). Results were analyzed using WinMDI 2.9 software (De Novo Software, Los Angeles, CA, USA).

2.11. Statistics

All parameters were recorded for individuals within all groups. Statistical comparisons of data were carried out using the *t*-test of the SigmaPlot (Systat Software, La Jolla, CA, USA). A P value < 0.05 was considered to be significant.

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