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Original Article

Altered adjuvant of foot-and-mouth disease vaccine improves immune response and protection from virus challenge ☆

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ABSTRACT

Vaccines for foot-and-mouth disease (FMD) generally use oil adjuvants. For better immunization and safety, an adjuvant should be selected only after careful consideration. In this study, we produced vaccines for O, A, and Asia1 serotypes by mixing oil adjuvants, Emulsigen-D (ED), ISA 201, and ISA 206 with and without an aluminum hydroxide (AL) gel and measured their immunogenicity and safety to obtain information regarding critical differences (survival or weight loss) of vaccine quality in mice; the goal of this test was to overcome the difficulties associated with experiments large or medium-sized animals. The groups immunized with the vaccines containing only the oil adjuvants (ED, ISA 201, and ISA 206) had similar or higher levels of neutralizing antibodies and structural protein antibodies for the FMD virus (FMDV) than the groups immunized with the vaccines including the oil adjuvants mixed with the gel. However, in a challenge test using a mouse model, the protection rate showed the highest results in ISA 201 and ISA 206 mixed with AL. The mice immunized with vaccines containing ED showed temporary weight loss in the early postvaccination stages. Cell-mediated immunity was formed relatively strongly in the group vaccinated with vaccines including ISA 201 and ISA 206. We proposed that combinations of these adjuvants represent candidates for future FMD vaccines.

1. Introduction

Foot-and-mouth disease (FMD) is a viral infectious disease that forms vesicles in the mouth and hooves of artiodactyls, such as pigs, cattle, sheep, and goats, resulting in weight loss, reduced milk production, and growth delays. The disease can be spread rapidly not only by the excrement of infected animals, but also by contaminated feed, vehicles, and humans. Thus, the economic damage is substantial once an outbreak occurs. Therefore, FMD is subject to international regulations for the global trade of both livestock and their products [1,2]. The administration of vaccines is a highly effective method for preventing FMD. The selection of an appropriate adjuvant is the most important factor in determining the efficacy of these vaccines. To ensure a prompt and appropriate response to outbreaks of FMD, we previously investigated the immune response and protective effects to develop vaccines, with the aim of identifying the vaccine with the best immunogenicity and protection against the virus O/Andong/SKR/2010, which was isolated during FMD outbreaks in Korea in 2010 and 2011 [3]. In another study [3], we performed an experiment using ISA 201, ISA 206, Carbigen, Emulsigen-D (ED), and an aluminum hydroxide ($Al(OH)_3$; AL), gel in order to select adjuvants for pigs and dairy goats. The ED with AL gel resulted in stronger immunity and protective effects compared to ED only. Thus, it remains to be determined whether the oil-based adjuvants ISA 201 and ISA 206 mixed with the gel result in better immune responses.

In studies of vaccine developments for FMD, it is desirable that the adjuvants are applied directly to susceptible target animals. However, such experiments are time-consuming and costly in pigs and cattle. It is difficult to obtain precise decisive data like mortality and body weight variation in dealing with large or medium-sized animals because of subjective analysis of protection or safety. Hence, to establish a prompt and accurate comparison of the newly developed adjuvant using a mouse model, we measured the immunity on each serotype using the vaccines containing O, A, or Asia1

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serotypes, which have been used for disease control in Asian countries and examined the safety and protection capability of each adjuvant. The results were compared with those of pigs, the target animals.

2. Materials and methods

2.1. Virus purification and inactivation

The FMD viruses O/Andong/SKR/2010 and Asia1/MOG/05 were used for antigen preparation in a BHK21 cell line. For virus infection, the culture medium was replaced with serum-free Dulbecco's modified Eagle's medium (Cellgro, USA), and the cell was inoculated with the virus. After 1 h of incubation at 37 °C in an atmosphere of 5% CO_2, the extracellular virus was removed. Twenty-four hours after infection, the viruses were inactivated by 0.003 N of BEI for 24 h and concentrated with polyethylene glycol 6000 (81260; Sigma Aldrich, WI, USA). The virus was layered on 15–45% sucrose density gradients and centrifuged [4]. After ultracentrifugation, the bottom of the centrifuge tube was punctured, and 1 ml fractions were collected. The presence of FMD viral particles and the FMD viral protein in a sample of each fraction were tested with a lateral flow device (BioSign™ FMDV Ag, PBM, USA). The concentrated and inactivated FMDV, A22 Iraq antigen for type A supplied by Merial Co. Ltd (UK) was used to manufacture the vaccines for the immunity and safety tests.

2.2. Preparation of the vaccines

The concentrated O/Andong/SKR/2010, A22 Iraq and Asia1/MOG/05 antigens were diluted with a Tris-NaCl buffer (with a pH of 7.6) and then added to each adjuvant: Emulisgen-D® (ED; MVP Technologies, USA), Montanide™ ISA 201 VG (ISA 201; Seppic, France), Montanide ISA 206 VG (ISA 206; Seppic, France), and aluminum hydroxide gel (AL; Rehydragel® HPA; General Chemical, USA). The ratio of the adjuvant to the total volume was 20:80 for ED and 50:50 for both ISA 201 and ISA 206 (volume [v]/v). In the oil/gel adjuvant mixture, 10% of AL was added. The mixture was stirred at 300 rpm for 10 min at 30 °C in a water incubator to form a water-in-oil-in-water blend. The stability of the vaccines was tested using the dropping method [5]. To maintain the same amount of antigen per dose of vaccine, the same amount of antigen was prediluted to the same concentration before mixing it with the adjuvant.

2.3. Immunization and FMDV challenge in the mice

In the first study, eight-week-old female BALB/c mice were divided into seven test groups ($n = 4$ in each group), and a nonvaccinated control (NVC) group ($n = 2$). The test groups were as follows: Carbigen, ED, ED + AL, ISA 201, ISA 201 + AL, ISA 206, and ISA 206 + AL. With the exception of the control group, all the mice were inoculated intramuscularly with an experimental vaccine containing 1 μg of inactivated antigen (O/Andong/SKR/2010), and they were inoculated once more with the same method seven weeks later. The serum of each mouse was collected 0, 2, 4, 6, 7, and 9 weeks after vaccination. Nine weeks after the first vaccination, all the mice were stimulated with 1 μg of purified virion antigen (O/Andong/SKR/2010) for cell-mediated immune responses, and cytokine assays were performed on blood samples collected 24 h after the stimulation.

In the second study, eight-week-old female C57BL/6 mice ($n = 3$) were used to compare the seven vaccinated groups (ED, ED + AL, ISA 201, ISA 201 + AL, ISA 206, ISA 206 + AL, and FMD trivalent vaccine [Aftopor® Trivalent, Merial, France]) and the nonvaccinated group. All the mice were vaccinated intramuscularly with an experimental vaccine containing 1 μg of inactivated antigen (A22 Iraq). On the 28th day after vaccination (dpv), all the mice were challenged with $10^{6.0}$ of $TCID_{50}$/0.1 ml of A22 Iraq by the intraperitoneal route. Sera were collected 0, 2, 4, and 6 weeks after vaccination and 3 days after the challenge, and antibodies to the virus and the FMDV structural proteins (SPs) were measured. For the safety test, the safety of the ED, ED + AL, ISA 201, and ISA 201 + AL vaccines was tested in eight-week-old female C57BL/6 mice. The FMDV trivalent vaccine was used as a control. In the safety test, each group ($n = 8$) received a subcutaneous vaccination containing 50 μl of each adjuvant. Each mouse was weighed daily for 10 days after vaccination.

In the third study, eight-week-old female C57BL/6 mice were divided into the seven groups (ED, ED + AL, ISA 201, ISA 20 + AL, ISA 206, ISA 206 + AL, and FMD trivalent vaccine [Aftopor® Trivalent, Merial, France]) ($n = 8$) and the nonvaccinated group ($n = 4$). First, to observe early immune reaction, the mice were vaccinated with 0.2 μg of inactivated antigen (Asia1/MOG/05) and challenged with 100 median lethal dose (LD_{50}) of Asia1/Sha/89 (Asia1 Shamir) after one week fater vaccination. To observe mid-term immune reaction, the challenge was performed in an identical manner four weeks after vaccination. The survival rate was checked for 10 days after the challenge. To investigate long-term immune reaction, the mice were challenged 12 weeks after vaccination and were observed for 10 days after the challenge.

2.4. Immunization and FMDV challenge in pigs

For the pig experiment, the pig serum employed in the previous study was used [3]. The 22 female pigs (eight-week-old) were divided into five test groups ($n = 4$ each group; Carbigen, ED, ISA 201, ISA 206, ED with AL [ED + AL]) and an unvaccinated control group ($n = 2$). All the pigs were inoculated with an experimental vaccine containing 10 μg of antigen per dose (2 ml). The serum of the pigs was collected into serum-separating tubes 0, 7, 14, 21, and 28 days after vaccination, and neutralizing antibodies were measured. At 28 days after vaccination, test pigs were challenged with $10^{5.0}TCID_{50}$/0.1 ml of FMDV O/Andong/SKR/2010 from the vesicles of infected pigs by intradermally injecting it into the foot pad. The sera collected one day after the challenge and 10 days after the challenge were used to measure the SP antibodies for the FMD virus and IL-12 and IL-4 cytokine secretion, respectively. In the pig test, the sera of only the ED, ISA 201, and ISA 206 groups were used after excluding the Carbigen and ED + AL.

2.5. Structural protein (SP) ELISA and nonstructural protein (NSP) ELISA

For the detection of SP antibodies, PrioCHECK FMDV type A and type O (Prionics AG, Schlieren-Zurich, Switzerland) were used. Nonstructural protein (NSP) antibodies were detected with a PrioCHECK FMDV NSP (Prionics AG, Schlieren-Zurich, Switzerland) ELISA kit in serum samples of mice. The absorbance of the ELISA plate was converted to the percent inhibition (PI) value. When the PI was 50% or above, the mice were regarded as antibody positive.

2.6. Virus neutralization test

Titers of neutralizing antibodies in the serum were measured with a virus neutralization test. Serum samples were collected from the animals after the vaccinations and the virus challenge. The serum samples were heat inactivated at 56 °C for 30 min. Following incubation of the test serum with FMDV 100 $TCID_{50}$/0.1 ml for 1 h, LF-BK cells were added to the plate and incubated