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Influence of adjuvant formulation on the induced protection of mice immunized with total soluble antigen of *Trichinella spiralis*

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Abstract

Vaccination of pigs against the helminth nematode *Trichinella* could be a good alternative to prevent the risk of human infection. In order to develop an efficient and safe vaccine, the choice of the adjuvant is an important issue. In this study, two adjuvants were selected to prepare vaccines based on total soluble *Trichinella spiralis* muscle larvae (ML) antigen: Montanide® ISA70 water in oil emulsion and Montanide® IMS nanoparticles. Aluminium hydroxide was used as a reference adjuvant. The immune response was checked by ELISA of parasite antigen specific IgG1 and IgE. Finally, protection induced in vaccinated mice was measured after a *T. spiralis* challenge by counting ML burdens. The results clearly showed an impact of adjuvants on the specific IgG1 and IgE antibody responses against *T. spiralis*. Differences were observed between the rates of protection induced according to the type of formulation, although the three adjuvants tested were able to enhance the humoral immune response. This work demonstrated the need to use an adjuvant to obtain a specific IgG1 and IgE responses directed against the total soluble extract of *T. spiralis*.

Keywords: *Trichinella*; Vaccination; Adjuvant; Immune response

1. Introduction

A major problem in the development of vaccines against helminth parasites is due to the complexity of those organisms compared to bacteria or viruses (Meeusen and Piedrafita, 2003). Developing recombinant antigens is a more promising strategy for the design of efficient vaccines. However, as purified, recombinant or synthetic antigens are less immunogenic than inactivated vaccines or crude antigens, the use of potent and safe adjuvants is necessary to improve the immune response and to protect against the parasite. Whereas aluminium hydroxide (AlOH) and emulsions are the most currently used adjuvants in veterinary vaccines, some more recently developed adjuvants (nanoparticles) exhibit interesting proper-

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ties for induction of both short and long term immunity. Water in oil emulsions (W/O) are potent adjuvants which induce a strong and long term immune response (Bahnemann and Mesquita, 1987).

Some vaccination trials against *Trichinella spiralis* have been completed successfully in mice using crude antigen (Sun et al., 1994; Raefa et al., 1996) or peptide (Robinson et al., 1995; McGuire et al., 2002) in the presence of Freund complete adjuvant (FCA). Other types of adjuvants have been tested in mice without improving the immune response induced using FCA (Robinson et al., 1994). Of those tests conducted in large animals, only one test has been performed in swine using newborn larvae antigens with FCA which gave good protection of animals and production of antibodies (Marti et al., 1987). In a previous study, we screened new generations of adjuvants like nanoparticles, microbeads and Montanide® emulsions of oil and water associated in different ways: water in oil (W/O), water in oil in water (W/O/W) or oil in water (O/W) (Aucouturier et al., 2001). Using those formulations, the results of a study with crude *Trichinella* antigens demonstrated that W/O emulsions were potent adjuvants which induced a strong humoral response against *Trichinella*; however, the protection against the parasite generated by those adjuvant formulations was not assessed in this work.

The objective of the present study was thus to assess the protection induced by different formulations of adjuvants (W/O Montanide® emulsions, nanoparticles) during vaccination trials in mice immunized with total soluble antigen from *Trichinella* muscle larvae (ML) of *T. spiralis*. The efficacy was checked by analysis of the systemic protective humoral response evaluated determined by the titer of specific antibody levels of IgG1 and IgE. Protection induced by the vaccination was evaluated by analysis of *T. spiralis* ML burdens after a challenge infection.

2. Material and methods

2.1. Animals

Female OF1 mice weighting 18–20 g were purchased from IFFA-CREDO (Arbresle, France) and housed under specific pathogen free conditions with over pressured and filtered atmosphere and sterilized food and water. These precautions were taken to avoid contact with any other intestinal parasite that could have influenced the immune response. During the trials, mice were fed without restriction.

2.2. Formulations

The water in oil formulation tested was achieved with Montanide® ISA70 adjuvant. Montanide IMS 1312 ® VG formulation based on nanoparticles was also tested. These formulations were issued by SEPPIC (Paris, France). The aluminium hydroxide (AlOH) (Alhydrogel 85″, Superfos Biosector) was used as a reference in terms of adjuvant effect. Two negative controls were used in this study: an antigen in saline solution (antigen control) and an absolute control (unvaccinated mice).

2.3. Vaccination

Groups of five mice were vaccinated with a crude soluble antigen from *T. spiralis* ML (ISS 104). Antigen concentration was previously optimized to 5 μg/mouse in order to observe the best adjuvant effect defined as the antigenic concentration giving the highest specific antibody titer when combined with an adjuvant and giving no immune response when administered without adjuvant. Mice were immunized by subcutaneous injection at days 0 and 28 with 100 μl of vaccine or control solution per animal.

2.4. Challenge protocol

At day 56, each mouse was infected with 300 ML of *T. spiralis* (ISS 104). The larvae were collected by artificial digestion from mice previously infected as describe below.

2.5. Titration of cytokines and antibody in mice sera

Blood samples were harvested at 0, 14, 28, 42, 56, 58, 60, 64 and 90 days post-immunization (dpi). *Trichinella* specific IgG1 and IgE antibody levels in pools of sera were assessed by indirect ELISA.