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Toxoplasma gondii: Immunological response of sheep to injections of recombinant SAG1, SAG2, GRA1 proteins coupled to the non-toxic microparticle muramyl dipeptide

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ABSTRACT

Background: Toxoplasma gondii is an important cause of reproductive loss in sheep and has a large economic impact, worldwide, because of infections in humans and animals. The immune response in sheep was evaluated, following administration with four different combinations of recombinant proteins derived from *T. gondii*.
Methods: 25 three-year-old non-pregnant Coopworth ewes were vaccinated twice by intramuscular injection with either the recombinant surface antigen 1 (SAG1), 2 (SAG2) or excretory/secretory antigen (GRA1) each individually conjugated to non-toxic muramyl dipeptide (MDP). Blood samples were collected from all animals prior to the first injection and once per week until five weeks after the second injection. Immunoglobulins (Ig) G1 and IgG2 level were measured using an ELISA test. IFN-γ were estimated using the commercially available bovine IFN-gamma test kit (Bovigam, Prionics AG).
Results: Administration of the recombinant GRA1 enhanced both IFN-γ production from peripheral blood cells when cultured *in vitro* with *Toxoplasma* antigen, and GRA1-specific IgG2 antibody level present in serum. Unlike GRA1 SAG1 did not stimulate IFN-γ production in the same test system.
Conclusion: These results indicate the potential of recombinant GRA1, as a vaccine candidate to protect sheep against *T. gondii* infection.

1. Introduction

Toxoplasma gondii is an obligate, intracellular, apicomplexan parasite that has a global distribution and infects nearly all mammals and avian species. Toxoplasmosis is of veterinary importance. *T. gondii* is a major cause of embryonic and foetal death, abortion, stillbirth and neonatal death in sheep and goats and is responsible for heavy economic losses to the sheep industry worldwide (Williams et al., 2005; West 2002; Buxton et al., 2007). A vaccine against toxoplasmosis in sheep is commercially available in New Zealand, the United Kingdom and France, and it is based on a live, attenuated tachyzoite – S48 strain (Toxovax®, Intervet Schering-Plough, New Zealand). However, this vaccine is expensive, may cause side effects and has a short shelf life. Furthermore, this vaccine can revert to a pathogenic strain and this makes it a poor vaccine candidate for humans (O'Connell et al., 1988; Wilkins et al., 1988; Wilkins and O'Connell 1992). To circumvent these negative features, current research is focused on developing safe vaccine candidates from DNA or protein derived from *T. gondii* which exhibit sufficient antigenicity.

Studies in experimental animals models have shown that the correlate for protection is a strong cellular immune response; *T. gondii*-specific IFN gamma (IFN-γ) and cytotoxic T-lymphocyte production. Moreover, IFN-γ was shown to be the central mediator of protection during both acute and chronic phases of toxoplasmosis (Suzuki et al., 1988; Gazzinelli et al., 1991; Tait and Hunter 2009).

Regarding the importance of effective vaccination in the animals and humans, many studies have been conducted in order

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to find more effective methods, for example using adjuvants with the vaccination procedure. Muramyl dipeptide (MDP) is one of the adjuvants which have been used widely as an active component in Freund's complete adjuvant. Prabhakar et al., in 1998, revealed that MDP may act as an immunodominant antigen which stimulates cellular and humoral responses presumably through production of proinflammatory cytokines (Hemmi et al., 2000; Prabhakar et al., 1998) and the induction of IFN-γ production (Cooper et al., 1993; Flynn et al., 1993). The importance of this molecule is underscored by the fact that MDP, common to gram-positive and gram-negative classes of bacterial peptidoglycans, was shown to be detected by nucleotide oligomerization domain 2 (NOD2), (Kanneganti et al., 2007).

The identification of immunostimulatory/modulatory properties of muramyl dipeptide (MDP) led to immunopharmacological studies aimed at clinical application of MDP as a chemically defined, fully active immunoadjuvant (Inohara et al., 2003; Kufer et al., 2006). Structural modifications of MDP and its derivatives have been extensively studied in an attempt to increase adjuvant activity and boost the immune response effectively for clinical use in the treatment of cancer and vaccination. Because of its toxicity and poor pharmacokinetic profile, *i.e.* the rapid clearance of MDP from the body (Traub et al., 2006) MDP itself is not suitable for clinical use. The non-toxic MDP microparticle used in this study has been designed to enhance specific immune responses to native proteins, recombinant proteins, synthetic peptides and other immunogenic materials, *i.e.* (Gelder 2006; Gelder and Webster 2009).

The most promising vaccine candidates of *T. gondii* include the surface antigens (SAG), secreted dense granules (GRA), rhoptry (ROP) and microneme (MIC) proteins. Several studies have demonstrated protective immune responses induced by native and recombinant proteins as well as DNA vaccine versions of SAG, GRA, ROP and MIC proteins (Letscher-Bru et al., 2003; Ismael et al., 2003; Martin et al., 2004).

The main surface antigen of *T. gondii*, SAG1, is expressed on the surface of free tachyzoites and participates in the initial contact and attachment of the parasite to the host cell (Mineo and Kasper 1994). In addition, numerous studies have shown that vaccination with purified natural SAG1 (Debard et al., 1996; Bhopale 2003) with recombinant SAG1 produced by *Escherichia coli* (Letscher-Bru et al., 2003; Petersen et al., 1998), elicits a specific immune response and protection in animal models against *T. gondii* infection (Jongert et al., 2009). Moreover, a recent study showed that administration of DNA vaccine based on SAG1 to rodents induced a humoral immune response, and a plasmid encoding SAG1 gene can provide partial protection against challenge with the virulent RH strain of *T. gondii* (Angus et al., 2000; Liu et al., 2010).

A second surface antigen, SAG 2 (p22), known to be involved in the invasion process as an attachment ligand (Grimwood and Smith 1996), exhibits good antigenicity and immunogenicity (Li et al., 2000). Recombinant SAG2 significantly protected BALB/c mice from lethal challenge (Mishima et al., 2001; Yang et al., 2004, Lau and Fong 2008).

Dense granule antigen 1 (GRA1) appears to be a promising vaccine candidate as a member of excretory/secretory antigens (ESP) (Nam 2009). Protection against infection with *T. gondii*, obtained after vaccination trials using recombinant protein (Döşkaya et al., 2007) or DNA vaccine have been shown in mice (Scorza et al. 2003). Pigs vaccinated with GRA1-GRA7 DNA vaccine cocktail can be efficiently primed against *T. gondii* infection (Jongert et al., 2008a). Additionally immunogenicity and protection afforded by the recombinant GRA1 protein vaccine was higher than the DNA vaccine based on GRA1 sequence, in BALB/c mice infected with the RH strain of *T. gondii* (Döşkaya et al., 2007).

The aim of present study was to investigate whether the recombinant protein vaccines, based on SAG1, SAG2 and GRA1*T. gondii* antigens when administered covalently attached to non-toxic MDP were able to elicit humoral and cellular immune responses against *T. gondii,* in sheep.

Table 1
Composition of vaccines.

Group	First injection	Second injection (six weeks later)
1	rSAG1[a]	rSAG1[a]
2	rSAG2[a]	rSAG2[a]
3	rGRA1[a]	rGRA1[a]
4	rSAG1[b]+ rSAG2[b]+ rGRA1[b]	rSAG1[b]+ rSAG2[b]+ rGRA1[b]
5	PBS	PBS

[a] 300 ug.
[b] 100 ug.

2. Materials and methods

2.1. Sheep

Three-year-old non-pregnant Coopworth ewes, were tested for past *Toxoplasma* infection using the latex agglutination test kit at a serum dilution of 1:16 (Toxotest, Eiken Chemical Co, Japan), and 25 sero-negative animals were selected for the experiment. Animals were managed under conventional New Zealand farming conditions, where they grazed a mixture of ryegrass (*Lolium perene*) and white clover (*Trifolium repens*). At the end of the experiment all animals were culled using a schedule 1 method and death confirmed before necropsy (UK Animals in Scientific Procedures Act 1986).

All experimental procedures were carried out under the authority of the Lincoln University Animal Ethics Committee (AEC #914).

2.2. Experimental design

The 25 sheep selected for the experiment were randomly divided into 5 equal groups. Immunization was carried out as per the description in Table 1.

Control animals were injected with phosphate-buffered saline (PBS) (group 5). There were four treatment groups. Group 1 was immunized with 300 μg of recombinant protein vaccine rSAG1; group 2 with 300 μg of recombinant protein vaccine rSAG2; group 3 with 300 μg of recombinant protein vaccine rGRA1; and group 4 with 100 μg of each recombinant protein vaccine rSAG1 + rSAG2 + rGRA1.

All injections, as a total volume of 1 ml, were given intramuscularly in the dorsal neck region with the second injection given six weeks after the initial one. Blood samples were taken from the jugular vein every week until five weeks after the second injection. All the ewes were grazed as one flock for the duration of the trial.

2.3. Production of recombinant SAG1, SAG2 and GRA1 antigens

The production of recombinant SAG1 and GRA1 antigens for vaccine preparation, ELISA and IFN-γ test were performed as described in Hiszczyńska-Sawicka et al. (2003). The production of recombinant SAG2 antigen for vaccine preparation, ELISA and interferon-gamma (IFN-γ) test was performed as described in Hiszczyńska-Sawicka et al. (2005).

2.4. Preparation of native antigen

Tachyzoites of the S48 strain of *T. gondii* used for antigen preparation were supplied by University of Technology, Sydney, Australia. Tachyzoites were harvested from the peritoneal fluid of Swiss mice that had been intraperitoneally infected with 3×10^6 tachyzoites 3 days earlier. Parasites were harvested, washed in phosphate buffered saline (PBS) and centrifuged at 450*g* for 15 min.