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Analysis of the protective immune response following intramuscular vaccination of calves against the intestinal parasite *Cooperia oncophora*

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ABSTRACT

Recently we reported the successful vaccination of calves against *Cooperia oncophora* with a double domain activation-associated secreted protein, purified from the excretory-secretory material of adult stage parasites. In an attempt to elucidate the immune mechanisms involved in protection, the humoral and cell-mediated immune responses following vaccination and infection were compared with non-vaccinated control animals. Antigen-specific IgG1, IgG2 and IgA levels were significantly increased in sera of vaccinated animals post vaccination, whereas no effect was observed for IgM. Antigen-specific intestinal IgG1 levels were significantly increased in the vaccinated animals, whereas no differences were observed for antigen-specific IgA, IgM and IgG2 levels. Upon re-stimulation in vitro with the vaccine antigen, a significant proliferation of both αβ- and γδ-T cells, and B cells, collected from mesenteric lymph nodes, was only observed in vaccinated animals. RNA-seq analysis of intestinal tissue yielded a list of 67 genes that were differentially expressed in vaccinated animals following challenge infection, amongst which were several cell adhesion molecules, lectins and glycosyl transferases. A correlation analysis between all immunological and parasitological parameters indicated that intestinal anti-double domain activation-associated secreted protein IgG1 levels correlated negatively with cumulative faecal egg counts and positively with the proportion of L4s and L5s. The proportion of immature stages was also positively correlated with the proliferation of αβ T cells. Worm length was negatively correlated with the transcript levels of several lectins and cell adhesion molecules. Overall, the results indicate that intramuscular administration of the vaccine resulted in an immune memory response particularly characterised by increased antigen-specific IgG1 levels in the intestinal mucosa.

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1. Introduction

The intestinal nematode *Cooperia oncophora* is one of the most prevalent cattle parasites in areas with a temperate climate and often occurs in co-infections with the abomasal parasite *Ostertagia ostertagi*. Mixed infections with these two species can result in significant economic losses (Charlier et al., 2014). Although *C. oncophora* is often regarded as a mild pathogen, its importance is increasing as it is a species in which anthelmintic resistance is developing and spreading rapidly. Isolates resistant to benzimidazoles (Jackson et al., 1987) and macrocyclic lactones (Demeler et al., 2009; Edmonds et al., 2010), as well as multidrug-resistant isolates (Mejia et al., 2003), have already been isolated in the field.

Non-chemical alternatives to anthelmintic drugs are thus desirable and vaccines are generally regarded as one of the more sustainable and environmentally safe options. In a recent study we reported on the successful evaluation of a double domain activation-associated secreted protein (dd-ASP) purified through size-exclusion chromatography from the excretory-secretory (ES) material of adult *C. oncophora* worms (Vlaminck et al., 2015). Under experimental conditions, the dd-ASP vaccine reduced the cumulative faecal egg output by 91% and increased the number of inhibited L4s present in the intestines of vaccinated animals

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compared with control animals. When evaluated under field conditions in first season grazing calves, the vaccine significantly decreased faecal egg counts of *C. oncophora* by 58.5% over the entire grazing period. Numbers of infective *C. oncophora* larvae were lower on plots grazed by vaccinated calves, with a significant reduction in mean pasture larval counts of 65% at housing. Finally, a significant reduction of 81.6% in total numbers of *C. oncophora* worms was shown in the vaccinated group compared with the control group (Vlaminck et al., 2015).

The immune mechanisms involved in this vaccine-induced protection are still unclear. Previous studies have shown that naturally acquired protection against *C. oncophora* was associated with a parasite-specific IgA and IgG1 response (Kanobana et al., 2001, 2003b) and an eosinophil influx at the site of infection, potentially regulated by CD4+ T-cells (Kanobana et al., 2002, 2003a). Furthermore, animals with genetic resistance to the closely related *Cooperia punctata* were shown to produce more Th2 cytokines (Bricarello et al., 2008) than susceptible animals. More recently, a number of reports were published in which transcriptomics were used to discover a number of genes potentially involved in the immune response against *C. oncophora* (Li and Gasbarre, 2009; Li et al., 2009, 2011a). Shortly after a single infection, genes associated with skeletal and muscular systems were impacted, suggesting smooth muscle hypercontractility may be initiated as a result of infection (Li and Schroeder, 2012). After a secondary infection, the Vitamin D receptor and inducible nitric oxide synthase seemed to be associated with the development of resistance (Li et al., 2011a). Finally, a study in which the host responses were monitored over a period of 42 days following a *C. oncophora* infection revealed the upregulation of the cell adhesion molecule CDH26 starting 2 weeks p.i., correlating with the infiltration of eosinophils (Li and Gasbarre, 2009).

The aim of this study was to investigate whether any of these immune mechanisms are also active in vaccinated animals, by analysing both the humoral and cell-mediated immune responses in cattle following vaccination and subsequent infection.

2. Materials and methods

2.1. Preparation of C. oncophora protein material

Collection of adult *C. oncophora* worms, preparation of the ES material and purification of the dd-ASP protein fraction were carried out as described previously (Borloo et al., 2013). Total protein homogenate of adult stage *C. oncophora* was obtained by incubating ground frozen worm tissue in PBS for 1 h at 4 °C while shaking, followed by centrifugation to remove solid parts.

2.2. Animal experiments and sample collection

All animal experiments were performed in accordance with the European Union Animal Welfare Directives and VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products) Guidelines for Good Clinical Practice. Ethical approval to conduct these studies was obtained from the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC2011/147). Detailed information concerning the vaccination experiment, with experimental infection, can be found in Vlaminck et al. (2015). In short, 14 helminth-naïve male Holstein–Friesian calves were randomised over two groups of seven animals. The animals from the vaccinated group were immunised three times i.m. with 30 μg of dd-ASP and 750 μg of QuilA adjuvant (Superfos Biosector, Denmark) at 3 week intervals. Calves from the control group were injected three times with 750 μg of QuilA. After the third and final immunisation, all calves were challenged five times per week with 1,000 *C. oncophora* L3s for a total of 25 days. Starting from 3 weeks after the first challenge infection, faecal egg counts (FECs) were determined from all calves three times per week for a period of 5 weeks. Eight weeks after the final immunisation, all calves were euthanised. Small intestines were washed and the number of *C. oncophora* counted. Adult worm lengths were measured (n = 50 per animal) in five animals randomly chosen from each group. The results of this vaccine trial are summarised in Table 1. Jugular blood samples were collected just prior to the first immunisation and 1 week after the second immunisation. Blood samples were centrifuged at 4000*g* for 10 min and sera were collected. Intestinal tissue samples (complete intestinal wall) were collected from the jejunum 3 m from the pylorus and used for gene transcription analyses, histology and protein extraction. Mesenteric lymph nodes were collected for the isolation of mononuclear cells. For gene transcription analyses, tissue samples were snap frozen in liquid nitrogen and stored at −80 °C until RNA was extracted. For histological analyses, the samples were stored in Carnoy's solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) and in 10% formaldehyde in PBS, both freshly made. Intestinal protein homogenate, used for measuring antibody responses, was obtained by incubating ground frozen tissue in PBS for 1 h at 4 °C while shaking, followed by centrifugation to remove solid parts.

2.3. Antibody responses

ELISA assays were used to determine serum and intestinal tissue-associated immunoglobulin IgG1, IgG2, IgA and IgM levels against the dd-ASP antigen. The antigen was coated at 5 μg/mL (intestinal antibody response) or 0.5 μg/mL (serum antibody response) overnight in carbonate buffer (0.025 M, pH 9.6). Plates were subsequently blocked in PBS solution with 0.5% Tween20 and 2% BSA for 1 h at room temperature. Thereafter, the plates were incubated for 1 h with either 100 μl of intestinal homogenate (200 μg/mL) or 100 μl of a 1:200 dilution of the serum in PBS with 0.5% Tween20 to respectively measure intestinal tissue-associated or serum antibody levels. All ELISA plates were subsequently incubated with sheep anti-bovine IgG1 (AbD Serotec, United Kingdom, AAI21P), IgG2 (AbD Serotec, AAI22P), IgA (AbD Serotec, AAI20P) and IgM (AbD Serotec, AAI19P) coupled to horseradish peroxidase as conjugates (diluted 1:100 in PBS with 0.2% Tween80 and 2% BSA in case of measurement of intestinal tissue-associated antibody levels and 1:500 in case of serum antibody levels), with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as the substrate. O.D. was measured at 405 nm with the 492 nm signal serving as a blank. A conjugate control was included in each measurement.

Table 1
Overview of the parasitological parameters obtained for the vaccination experiment with an experimental *Cooperia oncophora* challenge infection.

Group	*n*	*Cooperia* EPG	Worm count	% L4	Worm length (mm)
Control	7	7110 (1100–13,200)	6600 (550–12,200)	1.68 (0–8.43)	F: 12.5 (10.7–13.2) M: 9.50 (8.52–10.1)
Vaccinated	7	656[b] (0–1380)	3370 (200–6050)	29.4[b] (6.61–100)	F: 10.4 (9.66–12.4) [a] M: 8.40 (7.50–9.68) [a]

(Statistically significant, [a]$P < 0.05$; [b]$P < 0.01$).

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