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Successful immunization against a parasitic nematode by vaccination with recombinant proteins☆

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ABSTRACT

Infection of humans and livestock with parasitic nematodes can have devastating effects on health and production, affecting food security in both developed and developing regions. Despite decades of research, the development of recombinant sub-unit vaccines against these pathogens has been largely unsuccessful. We have developed a strategy to identify protective antigens from *Teladorsagia circumcincta*, the major pathogen causing parasitic gastroenteritis in small ruminants in temperate regions, by studying IgA responses directed at proteins specific to post-infective larvae. Antigens were also selected on the basis of their potential immunomodulatory role at the host/parasite interface. Recombinant versions of eight molecules identified by immunoproteomics, homology with vaccine candidates in other nematodes and/or with potential immunoregulatory activities, were therefore administered to sheep in a single vaccine formulation. The vaccine was administered three times with Quil A adjuvant and the animals subsequently subjected to a repeated challenge infection designed to mimic field conditions. Levels of protection in the vaccinates were compared to those obtained in sheep administered with Quil A alone. The trial was performed on two occasions. In both trials, vaccinates had significantly lower mean fecal worm egg counts (FWECs) over the sampling period, with a mean reduction in egg output of 70% (Trial 1) and 58% (Trial 2). During the period of peak worm egg shedding, vaccinates shed 92% and 73% fewer eggs than did controls in Trials 1 and 2, respectively. At post mortem, vaccinates had 75% (Trial 1) and 56% (Trial 2) lower adult nematode burdens than the controls. These levels of protection are the highest observed in any system using a nematode recombinant sub-unit vaccine in the definitive ruminant host and indicate that control of parasitic helminths via vaccination with recombinant subunit vaccine cocktails is indeed an alternative option in the face of multi-drug resistance.

1. Introduction

Infection of livestock and humans with parasitic nematodes has devastating effects on health and production, affecting food security worldwide [1,2]. Despite decades of research, the development of recombinant vaccines against these pathogens has been unsuccessful [3,4]. The primary cause of parasitic gastroenteritis (PGE) in small ruminants in temperate regions worldwide is the nematode *Teladorsagia circumcincta*. Infection is acquired via ingestion of third stage larvae (L3) from pasture. Thereafter, immature (xL3 and L4) and adult worms reside in the host's abomasum causing substantial production losses [5]. Currently, *T. circumcincta* is controlled using anthelmintics; however, multi-drug resistance is widespread [6,7] and development of a vaccine is now a priority. Protective immunity against *T. circumcincta* develops after prolonged continual exposure [8] and the degree of immunity acquired depends on the level of parasite challenge, animal age and genotype [9]. Immunity is associated with decreased establishment and development rate of nematode larvae in the abomasal glands and reduced egg output from adults [8,10–13]. The mechanisms responsible for these effects are complex: immediate hypersensitivity reactions, cellular effectors and humoral responses have all been indicated [12,14–22]. The successful adoptive transfer of immunity, using gastric lymph of previously-infected lambs, to

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Table 1
Recombinant proteins used in *Teladorsagia circumcincta* vaccine trial.

Name	Accession number	Function[a]	Expression system/solubility	Reference
Tci-SAA-1	CAQ43040	L3-enriched surface associated antigen	pET22b(+) *E. coli* BL21 (DE3)-RIL Soluble	[26]
Tci-MIF-1	CBI68362	L3-enriched macrophage migration inhibitory factor	pET22b(+) *E. coli* BL21 (DE3)-RIL Soluble	[29]
Tci-ASP-1	CBJ15404	L4-enriched activation-associated secretory protein	pET SUMO *E. coli* BL21 (DE3)-RIL Soluble	[31]
Tci-TGH-2	ACR27078	Transforming growth protein 2-like protein	pET SUMO *E. coli* BL21 (DE3)-RIL Soluble	[28]
Tci-CF-1	ABA01328[b]	L4-enriched Secreted cathepsin F	pPICZαC *Pichia pastoris* X33 strain Soluble	[23]
Tci-ES20	HF677587	Excretory/secretory (ES) protein	pPICZαC *Pichia pastoris* X33 strain Soluble	[21]
Tci-MEP-1	HF677586	Astacin-like ES metalloproteinase	pET SUMO *E. coli* BL21 (DE3)-RIL Insoluble	[21]
Tci-APY-1	CBW38507	L4-enriched ES calcium-activated apyrase	pSUMO *E. coli* BL21 (DE3)-RIL Soluble	[30]

[a] Putative or inferred function.
[b] Tci-CF-1 is highly polymorphic, the clone used for vaccine production had the following amino acid substitutions compared to published sequence. In each case the amino acid in the published sequence is in italics, that in the vaccine isoform sequence is in normal font and the amino acid position in the published sequence is in subscript: $I_{44} \Rightarrow T_{44}$, $N_{101} \Rightarrow D_{101}$, $T_{129} \Rightarrow A_{129}$, $R_{137} \Rightarrow Q_{137}$, $R_{305} \Rightarrow K_{305}$, $L_{306} \Rightarrow P_{306}$, $S_{307} \Rightarrow Y_{307}$.

helminth-free monozygotic twins highlighted the importance of local immune responses in protection against re-infection [12] and larval antigen-specific IgA in gastric secretions has been implicated as playing a crucial role [12,14–16,19,21].

We developed a tripartite approach to identifying antigens for a *T. circumcincta* vaccine. First, we selected proteins [cathepsin F-1 (Tci-CF-1), astacin-like metalloproteinase-1 (Tci-MEP-1), a 20 kDa protein of unknown function (Tci-ES20) and activation-associated secretory protein-1 (Tci-ASP-1)] [21,23,31] by examining larval antigens that are targets of IgA in immune sheep [21,23], focusing on proteins excreted or secreted during the critical phase of worm growth and development [24]. We also selected Tci-SAA-1, an immunogenic homologue of a protective antigen from canine hookworm, *Ancylostoma caninum* (*Ac*-SAA-1 [25,26]) by bioinformatic analysis of *T. circumcincta* transcripts [26,27]. Finally, we identified potentially immunosuppressive molecules released by the larvae [macrophage migration inhibitory factor-1 (Tci-MIF-1), calcium-dependent apyrase-1 (Tci-APY-1) and a TGFβ homologue (Tci-TGH-2)] [28–30]. Here, we combined these antigens into a vaccine which could, in theory, nullify the immunomodulatory functions of parasite-derived molecules and concurrently allow enhanced immune responses against the immunostimulatory components. We tested the vaccine in two independent trials in which sheep were administered a sustained larval challenge after vaccination.

2. Materials and methods

2.1. Recombinant protein production

Eight recombinant proteins (Table 1) were produced. Production of recombinant Tci-SAA-1, Tci-MIF-1 and Tci-APY-1 has been described previously [26,29,30]. Tci-MEP-1 cDNA was amplified from *T. circumcincta* RNA (L4, prepared as in [27]) using the SMART™ RACE kit (Clontech) and sub-cloned into pET SUMO (Invitrogen) (omitting bases 1–48 encoding the signal peptide). The coding sequences (CDS) of *Tci-tgh-2* and *Tci-asp-1* (omitting signal peptide bases [31]) were each sub-cloned into pET SUMO (Invitrogen). Expression of recombinant proteins (Tci-SAA-1, Tci-MIF-1, Tci-APY-1, Tci-MEP-1, Tci-TGH-2 and Tci-ASP-1) in *Escherichia coli* BL21-CodonPlus® (DE3)-RIL competent cells (Stratagene) was induced using 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The CDS of *Tci-cf-1*, (omitting signal peptide bases 1–42) and the CDS of *Tci-ES20* (PCR-amplified from a *T. circumcincta* L4 SMART™ cDNA library), were each sub-cloned into pPICZαC (Invitrogen) and used to transform *P. pastoris* [X-33 (Mut$^+$) (Invitrogen)]. Tci-CF-1 and Tci-ES20 protein expression was induced with 0.5% methanol [32]. Soluble recombinant proteins were purified from cell lysates (*E. coli*) or culture supernatant (*P. pastoris*) using HisTrap™ HP columns (GE Healthcare), then dialysed against 20 mM phosphate buffer, 0.5 M NaCl, pH 7.6. Insoluble recombinant Tci-MEP-1 was purified via nickel column affinity chromatography in the presence of 8 M urea, then dialysed against 2 M urea in 20 mM phosphate buffer, 0.5 M NaCl, pH 7.6. Protein concentrations were determined using the Pierce BCA™ (bicinchoninic acid) assay (Thermo Scientific) with bovine serum albumin (BSA) standards and the integrity of each recombinant protein monitored via SDS-PAGE.

2.2. Immunization experiments

2.2.1. Trial 1

Fourteen Texel crossbred lambs, 204–206 days old, reared under conditions to exclude helminth infection (confirmed by fecal worm egg counts (FWECs), [33]), were housed in two groups of 7 animals in separate pens. Sheep in Group 1 were injected subcutaneously with 400 μg recombinant protein mix (incorporating 50 μg each of Tci-ASP-1; Tci-MIF-1; Tci-TGH-2; Tci-APY-1; Tci-SAA-1; Tci-CF-1; Tci-ES20; Tci-MEP-1) plus 10 mg Quil A (Brenntag Biosector). PBS-soluble proteins were administered as a mixture in a single injection with 5 mg Quil A in PBS. Tci-MEP-1 was formulated with 2 M urea in PBS plus 5 mg Quil A. The preparations were injected separately at two sites on the neck. Three immunizations were administered, 3 weeks apart. Sheep in Group 2 received three immunizations with urea/PBS/10 mg Quil A, at the same time as Group 1. Following the final immunization each sheep was administered orally with 2000 *T. circumcincta* L3, three times per week for 4 weeks. FWECs were performed three times per week from 14 days after the start of the L3 challenge period until the end of the experiment. Cumulative FWEC values were estimated using the trapezoidal method for calculation [34]). Abomasal nematode burdens were classified and enumerated following standard techniques [20,24,35]. Blood samples were taken prior to each immunization and weekly from the third immunization. Abomasal swab samples were collected at post-mortem [21] to determine levels of antigen-specific IgA and IgG in abomasal mucus.

2.2.2. Trial 2

Twenty-eight Texel crossbred lambs, 172–178 days old, were raised as described for Trial 1 and housed in four groups of 7 animals. Groups 1 and 3 were immunized with recombinant protein mix as described for Trial 1. Sheep in Groups 2 and 4 received immunizations with urea/PBS/Quil A, at the same time as Groups 1 and 3. At the final immunization, oral L3 challenge commenced in all Groups for 4 weeks. All biological samples were obtained as described above. Sheep in Groups 1 and 2 were euthanized 7 weeks after the start of the infection period and those in Groups 3 and 4 were euthanized 4 weeks later. Trials were performed under the regulations of a UK Home Office Project Licence; experimental design was ratified by MRI's Experiments and Ethics Committee.