

Studies on the immunoglobulin E responses to *Teladorsagia circumcincta* in sheep: purification of a major high molecular weight allergen

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SUMMARY

Studies on the immunoglobulin (Ig)E immune responses to the gastric nematode, Teladorsagia circumcincta, have demonstrated a major high molecular weight allergen (HMWTc). Cross reactive allergens of similar MW were demonstrated for Trichostrongylus colubriformis and Cooperia curticei, but not for Haemonchus contortus. Purification of HMWTc was achieved by gel-filtration chromatography, and nonreducing SDS-PAGE and Western blot analysis revealed two closely associated bands with a molecular weight of approximately 140–150 kDa. Reduction showed four IgE reactive bands of 120, 50, 45 and 30 kDa, and deglycosylation abrogated the immunoreactivity of the 120 and 30 kDa bands. Ultrastructural immunolocalization by electron microscopy revealed that the IgE reactivity was confined to the cuticular surface of the infective (L3) larvae. ELISA studies to determine the IgE anti-HMWTc responses in lambs during their first grazing season, demonstrated significantly higher IgE antibody in lambs with low accumulative faecal egg count (FEC) compared to animals with high accumulative FEC. These studies provide evidence for a protective function of IgE antibody in Teladorsagia infections in lambs.

Keywords *Teladorsagia circumcincta, immunity, IgE, allergen, nematode*

INTRODUCTION

A characteristic feature of gastrointestinal nematode parasite infections is a pronounced increase in both total and specific immunoglobulin (Ig)E antibody (1). The importance of this antibody in terms of protection remains unclear, although there is evidence that IgE may have a pivotal and protective function in some helminth parasites (2,3). However, only a few of the allergens that elicit these responses have been identified and characterized. These include the polyprotein allergens of *Ascaris* spp. (4) and *Toxocara canis* (5), the major membrane allergens of schistosomula (6), tropomyosin from *Onchocerca volvulus* (7), and the surface associated glycoproteins of *Brugia malayi* and *Wuchereria bancrofti* (8).

During our investigations on the immune response to *Teladorsagia circumcincta* in sheep, we have previously shown that infections are characterized by a pronounced IgE antibody response to the infective third stage larvae (L3), but not to the adult (L5) worms (9). This IgE reactivity appeared to be localized to the surface of the L3 and, in the present study, we describe continued work on the analysis of these allergens. Electron microscopy has been employed to further define the localization of the IgE reactivity. Isolation and purification of a major, high-molecular weight complex allergen from *Teladorsagia* L3 has been achieved, and the generation of specific polyclonal antibodies have been employed to confirm the location of this allergen to the outer surface cuticle of the L3. Purification has also enabled some physico-chemical characteristics of this antigen, including its reduction to four immunoreactive subunits and sensitivity to deglycosylation. Further work has investigated the IgE anti-allergen antibody response in lambs subjected to continuous challenge on pasture. Although preliminary, these results

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support the view that this antibody response is important in the immune regulation of *Teladorsagia* in lambs.

MATERIALS AND METHODS

Parasite material

Infective larvae (L3) were obtained from faeces collected from monospecifically infected donor lambs given anthelmintic susceptible isolates of *T. circumcincta*, *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Cooperia curticei* which have been maintained at Moredun Research Institute for several years.

Worm (L3) antigen

The soluble proteins from worms were extracted by grinding approximately 1 g of L3 in a pestle and mortar with sharp sand and PBS, pH 7.2, to form a smooth homogenous paste at 4°C. This was harvested by diluting in ice-cold PBS (final volume 5 ml), and centrifuged in an airfuge (Costar, Cambridge, MA, USA) at maximum speed for 10 min. The supernatant was collected and the protein concentration estimated by a commercial protein assay (Pierce BCA protein assay, Rockford, IL, USA). Antigen concentrations for each preparation were adjusted to 1 mg/ml, before being aliquoted (100 µl) and stored at -20°C.

Gastric lymph

Gastric lymph was collected and stored, as described in an earlier study (9). Briefly, the common gastric lymph duct was cannulated in three sheep, which had been previously infected with *T. circumcincta*, and lymph collected 3–7 days after receiving a challenge of 50 000 *T. circumcincta* L3. The concentrations of IgE antibody to *Teladorsagia* L3 in this lymph reached optimal levels on day 5 post challenge (9), and a pool of day 5 lymph from the three sheep was prepared (termed 'immune' lymph), aliquoted in 100 µl amounts and stored at -20°C. Gastric lymph from noninfected or challenged animals was also collected (termed 'normal' lymph) (9), for control purposes.

SDS-PAGE and immunoblotting

Discontinuous SDS-PAGE was with 0.75 mm thick 7.5% slab gels (Mini Protean II; Biorad, Richmond, CA, USA), and samples were electrophoresed at a constant 200 V for 45 min. Samples of L3 antigen were treated with nonreducing or reducing buffer (10) and boiled for 3 min before they were loaded onto the gels at 5 µg/lane. Protein bands in the gel were detected using the method of Morrissey (11).

Proteins from SDS-PAGE gels were transferred onto nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) using a semidry transfer apparatus at 0.9 mA per cm² of gel for 1 h at room temperature. After transfer, the nitrocellulose membranes were incubated in PBS containing 0.5% Tween 80 and 0.5 M NaCl (PBS/T80) for 1 h to block nonspecific protein adsorption. The blots were then incubated for 1 h with immune gastric lymph (diluted 1 : 10 in PBS/T80) which was pretreated as described for sera for ELISA (see below). The membranes were washed six times for 5 min in PBS/T80 before incubation with monoclonal IE7 (diluted 1 : 10 in PBS/T80). Following a further six washes in PBS/T80, the membranes were incubated with goat antimouse IgG-Horse Radish Peroxidase (HRP) (diluted 1 : 1000 in PBS/T80) (Dako, Glostrup, Denmark). After a final six washes in PBS/T80, peroxidase activity was revealed by chemiluminescence detection, using ECLtm reagent (Amersham Life Sciences, Bucks, UK) as described by the manufacturer. Protein molecular weight markers were employed (SeeBluetm Plus2 Pre-Stained Standards, NovexTM, San Diego, CA, USA), to estimate the molecular weight of reactive bands.

Dot blots were also performed during these studies, to monitor the eluted fractions following liquid chromatography. Dots of sample (2 µl) were applied to a nitrocellulose membrane, allowed to dry, and allergen was detected as described above for Western blotting. Immunoreactions on Western blots were also performed using the polyclonal rabbit anti-allergen antibody. In essence the same procedure to that of the monoclonal antibody was used, except that a mouse anti-rabbit-HRP conjugate (1 : 1000 in PBS/T80) (Dako) was employed, and peroxidase activity was revealed with diaminobenzidine (DAB)/urea/H₂O₂ substrate (Sigma Ltd., Poole, UK).

Control blots were treated the same as those above, except that (a) the mab IE7 was replaced with diluent, and (b) immune lymph or rabbit serum was replaced with the same dilution of lymph or rabbit serum from noninfected or immunized animals.

Deglycosylation of allergen

N-linked deglycosylation was carried out by boiling samples of 10 µg of purified allergen in 200 µl of 20 mM sodium phosphate buffer, pH 7.2, containing 10 mM EDTA, 1% (v/v) 2-mercaptoethanol, 0.1% (w/v) SDS and 1% (v/v) Triton X100 (Sigma) for 5 min and allowing the mixture to cool before the addition of 1 U of glycopeptidase F (PNGase F; Boehringer Mannheim, Mannheim, Germany) and incubated for 24 h at 37°C. The immunoreactivity of the deglycosylated sample, and a sample of allergen

similarly treated except for the addition of glycopeptidase F, were analysed by SDS-PAGE and Western blotting.

Allergen purification

The IgE reactive antigen(s) were purified by gel-filtration chromatography on FPLC (Pharmacia, Uppsala, Sweden) and HPLC (Waters Ltd, Watford, UK). Following antigen extraction, 1 ml of *Teladorsagia* L3 antigen containing approximately 0.6 mg of protein was applied to a Sephacryl 300 (Pharmacia) column (1.5 cm by $\times$ 30 cm), equilibrated on FPLC in PBS, pH 7.2. Flow rate was maintained at 0.5 ml/min, and 1 ml fractions were collected. Fractions containing allergen, as assessed by immunodot blotting (described below), were pooled, concentrated to 80 μ l on Centricon 10 concentrators (Amicon, Beverly, MA, USA) and then applied to a Superose 6 gel-filtration column (Pharmacia) equilibrated on HPLC in PBS, pH 7.2. Flow rates were maintained at 0.2 ml/min, and 0.5 ml fractions were collected throughout the separation. Fractions were analysed by dot blots for allergen, and positive fractions pooled and concentrated on Centricon 10 concentrators. Following a protein estimation by a commercial protein assay (Pierce BCA protein assay), the purified allergen was aliquoted in 10 μ l amounts and stored at -20°C prior to analysis by SDS-PAGE and Western blotting.

Polyclonal anti-allergen antibody

Two rabbits each received approximately 200 μ l of purified allergen containing 10 μ g of protein in PBS pH 7.2 and 200 μ g of the adjuvant Quil A, by subcutaneous injection. The rabbits received two further similar injections at 4-week intervals. One week after the terminal injection, a blood sample was taken for antibody analysis by western blotting, and 3 days later the rabbits were euthatized and exsanguinated. Serum from a nonimmunized rabbit was collected for control purposes.

Enzyme-linked immunosorbent assay for IgE anti-allergen antibody

Sample selection and preparation

Sera were collected during a previous study (12), in which 72 Greyface $\times$ Suffolk lambs and their dams were turned out in April to graze pastures naturally contaminated with *T. circumcincta*. Lamb faecal egg counts (FEC) were performed on a weekly basis throughout the season using a modification of the sensitive flotation technique described by Jackson (13), and blood was taken weekly from each

animal. At the end of the grazing season (September), lambs were ranked according to their scores based on their cumulative FEC as previously described for goats (14), such that highest ranking 'responder' animals had the lowest cumulative FEC. Sera from 10 lambs with the lowest-ranking order (nonresponders) and 10 lambs with the highest-ranking order (responders) were selected for assay as described below. The selected sera were from lambs that had been bled at 3, 7, 13 and 18 weeks, with samples for each time point being collected on the same day.

Sera for analysis were prepared as described in a previous study, where it was shown that the optimal demonstration of IgE antibody required preheating samples at 56°C for 30 min, prior to sequential precipitation with 30% and 50% saturated ammonium sulphate (15).

ELISA

The assay was performed as previously described using the IE7 anti-IgE monoclonal (9), except that plates were coated with 1 μ g/ml of purified HMWt_c and a biotinylated secondary antibody (1 : 1000 dilution of goat antimouse-biotin; Dako) followed by a Streptavidin-Horse Radish Peroxidase (Dako, 1 : 5000 dilution) amplification step was employed. All dilutions and wash steps were performed with PBS, pH 7.2 containing 0.05% Tween 20. The assay for each time point was performed on a single plate to exclude between plate variation, and all samples were assayed in duplicate. The colour reaction was developed with ortho-phenylenediamine and H_2O_2 , and stopped by the addition of 25 μ l of 2.5 M H_2SO_4 (16). The OD 492 nm was measured in a Dynex MRX II plate reader (Dynex Technologies Inc., Ashford, UK). Controls consisted of the addition of all steps, except for the mab IE7 which was replaced with the wash diluent.

Ultra-structural localization

Third stage larvae of *T. circumcincta* species were prepared for ultrastructural electron immunocytochemical investigation.

The larvae were fixed in 2% double-distilled glutaraldehyde (DDG) in 0.1 M sodium cacodylate buffer (pH 7.2) containing 3% sucrose for 1 h at 4°C . The specimens were buffer washed, dehydrated through graded ethanol to propylene oxide and infiltrated and embedded in Agar 100 resin (Agar Scientific, Stansted, UK), and polymerized for 48 h at 60°C . Thin sections, approximately 70–80 nm in thickness, were cut on a Reichert Ultracut E ultra-microtome and mounted on bare 200-mesh nickel grids.

After drying, the sections were etched with 10% hydrogen peroxide (H_2O_2) for 10 min at room temperature

and rinsed thoroughly with 20 mM Tris/HCl buffer (pH 8.2) containing 0.1% bovine serum albumin (BSA) and Tween 20 (1 : 40 dilution) and were first incubated with normal goat serum (NGS) for 30 min at room temperature and then with immune or normal lymph (preheated at 56°C for 30 min) diluted to 1 : 5 with Tris/BSA buffer, for 1 h at room temperature. Following this incubation the sections were washed in buffer and transferred to monoclonal IE7, at a working dilution of 1 : 2 for 18 h at room temperature. Following this incubation, the sections were transferred to a 25- μ l droplet of 10-nm-size gold-conjugated goat anti-mouse IgG (International Biocell, Cardiff, UK) for 1 h, lightly fixed with 1% DDG (3 min) washed with buffer and rinsed with distilled water. Finally, the sections were double-stained with uranyl acetate (5 min) and lead citrate (8 min) and viewed in a transmission electron microscope. In a second experiment, an antibody raised to the isolated and purified *T. circumcincta* worm allergen was used to identify the location of the allergen in the worm, using immunogold labelling. As previously described, tissue sections were incubated first with NGS, and then with primary antibody (raised in rabbit) diluted to 1 : 15000 with Tris/BSA buffer, for 18 h at room temperature. Following this, the sections were transferred to a 25- μ l droplet of 10-nm-size gold-conjugated goat anti-rabbit IgG (International Biocell) for 1 h, lightly fixed with 1% DDG buffer washed, rinsed with distilled water and stained for viewing in the electron microscope.

Controls were prepared using (i) non immune lymph in place of immune lymph; (ii) immune lymph in the absence of anti-ovine IgE; (iii) anti-ovine IgE in the absence of immune lymph; and (iv) gold-labelled antiserum in the absence of immune lymph, anti-ovine IgE and allergen antibody. For rabbit antibody localization, the same controls were employed, except that normal rabbit serum replaced the rabbit anti-allergen.

Statistical analysis

ELISA results of the antibody levels from high vs. low responder groups were analysed at each time point using Students' two-tailed *t*-test.

RESULTS

Western blots of whole L3 antigen

Preliminary nonreducing SDS-PAGE and Western blot analysis with immune gastric lymph demonstrated that there were several IgE reactive antigens in homogenate preparations of *T. circumcincta* L3, although the most pronounced was a broad band(s) of high molecular weight

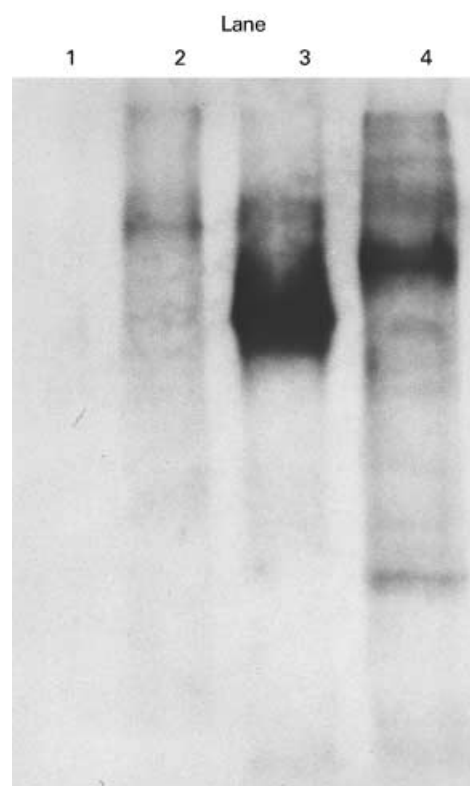


Figure 1 Nonreducing SDS-PAGE/Western blot, showing immunoreactivity of gastric lymph IgE antibody from *T. circumcincta* immune sheep. Lanes (5 μ g/track) contained L3 homogenates of *H. contortus* (lane 1), *C. curticei* (lane 2), *T. colubriformis* (lane 3) and *T. circumcincta* (lane 4). Note the cross reactive allergens in preparations of *T. colubriformis* and *C. curticei*, but not for *H. contortus*.

of approximately 140–150 kDa (Figure 1). Cross reactive allergens were also detected in L3 preparations of *T. colubriformis* and *C. curticei*, but no reaction was detected with *H. contortus* L3 antigen (Figure 1). No bands were detected when immune lymph was replaced with lymph from noninfected sheep.

Purification of allergen

Purification of the major high molecular weight allergen for *T. circumcincta* (HMWTc) was achieved using gel-filtration chromatography, by the sequential elution from Sephracryl S300 and Superose 6 columns. Fractionation of HMWTc was performed first on Sephracryl S300, and the fractions assayed for IgE reactivity by dot blotting with immune lymph. The fractions positive for HMWTc eluted in a broad peak, and these were collected, pooled and concentrated, prior to fractionation on Superose 6. The IgE immunoreactive fractions eluted between 70 ml and 80 ml, and represented a major eluted peak with an approximate molecular weight of 150 kDa. The fractions were pooled



Figure 2 Nonreducing SDS-PAGE (7.5%), following silver staining to demonstrate the immunoreactive proteins during the purification of HMW Tc . Lane 1, Sephacryl 300 active fraction; lane 2, final purification step on Superose 6.

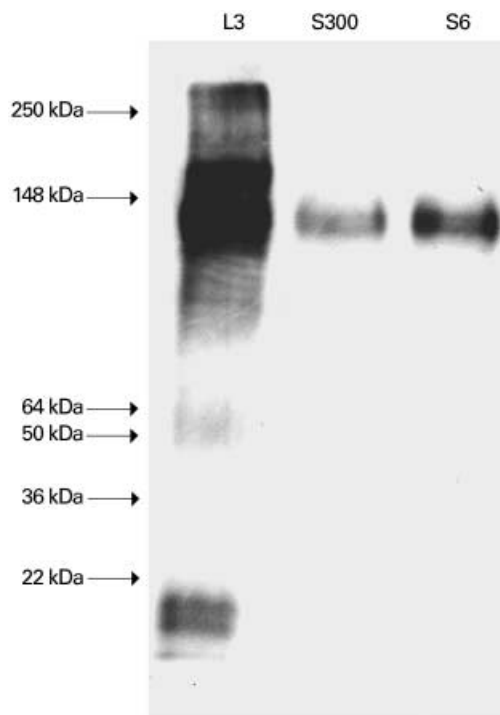


Figure 3 Nonreducing SDS-PAGE/Western blot, showing immunoreactivity of lymph IgE anti-*T. circumcincta* to *T. circumcincta* whole L3 homogenate (lane 1), Sephacryl 300 pooled IgE antibody reactive fraction (lane 2) and Superose 6 final purified fraction (lane 3). The purified product appears to consist of two, very closely, associated bands.

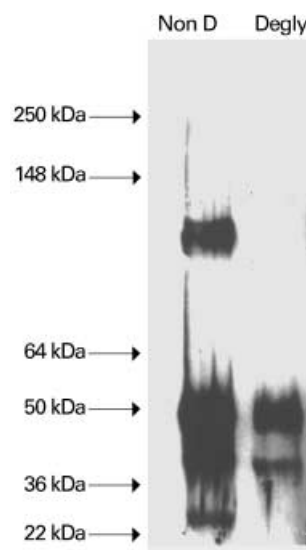


Figure 4 Reduced SDS-PAGE/Western blots for lymph IgE antibody (IgE), on nondeglycosylated (NonD) or *N*-glycan deglycosylated (Degly) purified HMW Tc allergen. Deglycosylation abrogated the IgE anti-HMW Tc antibody activity to the highest and lowest immunoreactive bands. The IgE blot was developed using chemiluminescence.

and concentrated, and analysed by SDS-PAGE and Western blots. In nonreducing conditions, a major silver-stained band with an approximate molecular weight of 150 kDa was observed, although some minor, faint staining bands were also detected at a relatively low mw of 16–18 kDa (Figure 2). On Western blots, the IgE reactivity was confined to the broad 140–150 kDa band, although in some blots this band appeared as two very closely associated bands (Figure 3). In reducing conditions, Western blot analysis of purified HMW Tc showed four immunoreactive bands of approximately 120 kDa, 50 kDa, 45 kDa and 30 kDa (Figure 4).

Polyclonal antibody

Polyclonal antibody was generated in rabbits, and initial screening by immuno-dot blots indicated that one rabbit sera produced a relatively high (< 1 : 500) titre against the immunizing antigen. The serum from this rabbit was used for subsequent immuno-localization studies. The staining characteristics on Western blots of L3 antigen in nonreducing and reducing conditions was similar to that of the IgE antibody in lymph. However, high nonspecific background was obtained when using chemiluminescence to demonstrate the reaction product, although the relatively less sensitive method with DAB produced weak but visible reactive bands (not shown). No reactive bands were

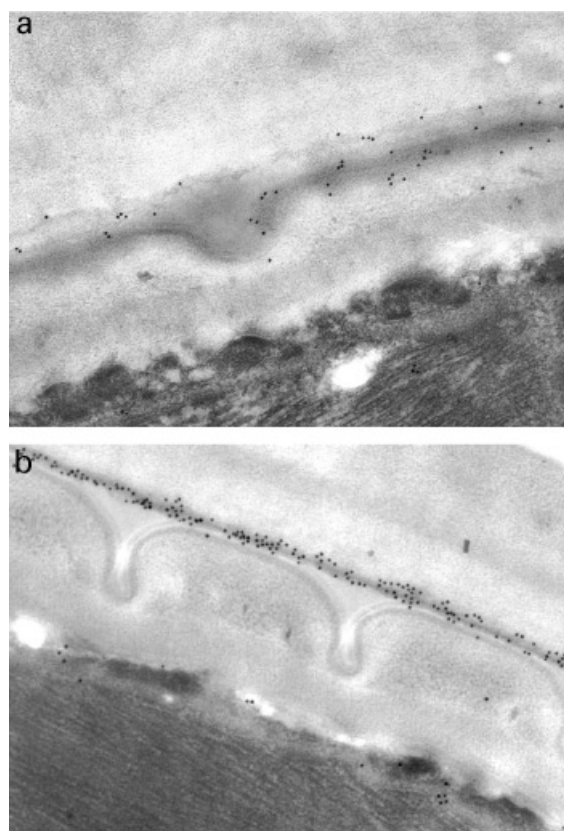


Figure 5 Electron micrographs demonstrating the surface localization of (a) IgE antibody in gastric lymph immune to *T. circumcincta* and (b), rabbit anti-HMWTC. Note the similarity for the localization of the 10 nm immunogold particles for both antibodies, to the outer cortical layer of the *T. circumcincta* (L3) larvae ($\times 52\,000$).

detected when immune serum was replaced with the same dilution of rabbit serum from a nonimmunized animal.

Deglycosylation

Deglycosylation abrogated the IgE reactivity of the 120 kDa and 30 kDa bands, and reduced the intensity of the reactive 50 kDa and 45 kDa bands (Figure 4). A similar effect was observed when the blots were incubated with rabbit antibody (not shown), although the visibility of the bands with the DAB substrate was less than those detected for IgE with chemiluminescence.

Ultrastructural localization

Immunogold localization of the IgE antibody in gastric lymph, revealed a distinctive and discrete pattern of particles adhering to the external cortical layer (Figure 5a). Very few gold particles adhered to the internal organs of the L3 larvae, and almost all of these appeared to be located

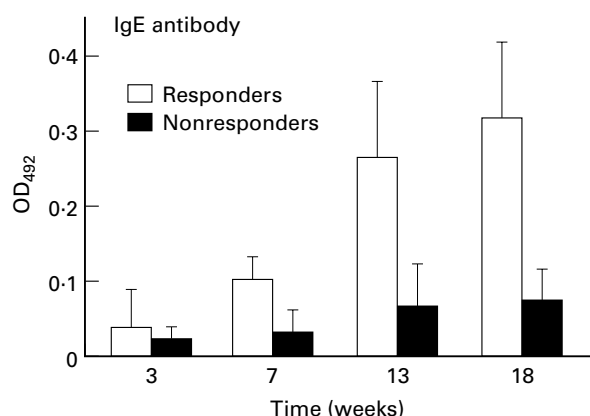


Figure 6 Graph showing IgE anti-HMWTC levels (mean $\pm$ SE) in sera from two groups of lambs ($n = 10$ per group), selected on the basis of cumulative FEC. Animals with low cumulative FEC ('responders') had significantly higher systemic levels (Student's *t*-test, $P < 0.05$) of antibody when compared to 'nonresponders' as early as 7 weeks after being placed on a contaminated pasture. Sera assayed from lambs on pasture for 13 and 18 weeks demonstrated an even greater significant difference between the two groups (Student's *t*-test, $P < 0.01$).

within the layer of hypodermal cells adjacent to the muscle cell region. Similar localization for rabbit anti-HMWTC was demonstrated, although more particles were observed in the hypodermal region (Figure 5b). Few, if any, gold particles were observed in the control sections incubated without primary or secondary antibodies.

IgE anti-allergen response

The results of the ELISA are shown in Figure 6. These demonstrate that lamb sera from highly ranked animals with relatively low accumulative FEC, had significantly higher systemic levels of IgE anti-HMWTC (Student's *t*-test, $P < 0.05$), as early as 7 weeks after being placed on a contaminated pasture. Sera assayed from lambs on pasture for 13 and 18 weeks demonstrated an even greater significant difference (Student's *t*-test, $P < 0.01$) between the two groups.

DISCUSSION

A major IgE reactive antigen has been identified on the surface of infective larvae of the sheep gastric nematode, *T. circumcincta*. IgE antibody responses to this allergen during the latter part of the grazing season were markedly higher in 'responder' lambs that had relatively low cumulative FEC, supporting the view that IgE antibody may be an important factor in enabling an effective immune response against *Teladorsagia*.

Enhanced IgE responses are a characteristic feature of helminth infections, although the importance of this isotype in the generation of immunity remains unclear. Numerous studies have identified antigenic determinants that may be important in the development of immunity to nematode parasites, and many of these are glycoconjugates which are secreted and/or bound to the surface of the parasite (17). For example, immunodominant surface antigens have been demonstrated on the larvae of *Dictyocaulus viviparus* (18), *Toxocara canis* (19) and *H. contortus* (20,21). However, fewer studies have investigated specifically the IgE responses to surface glycoproteins. The cuticular and muscle located protein, tropomyosin, has been shown to be associated with the development of immunity to the *Onchocerca volvulus* microfilariae in mice and humans, which may involve both IgE and IgG antibodies (7). A surface glycoprotein from two nematode species predominantly responsible for lymphatic filariasis in humans, namely *Brugia malayi* and *Wuchereria bancrofti*, induced both an IgG4 and IgE isotype antibody response, although the relative roles of these antibodies in the immune response and associated pathology is not clear (8,22). Similarly, anti-IgG4 and IgE antibodies to a surface serine protease inhibitor (serpin) have been implicated in immunity and/or pathology during studies on schistosomiasis (23).

Recent studies with *Ascaris lumbricoides* involving the secreted allergen ABA-1 have indicated that natural immunity to this nematode are associated with IgE, rather than IgG antibody (24). Earlier immunohistochemical evidence that the IgE reactivity was confined to the surface of *Teladorsagia* L3 was confirmed in the present ultrastructural study (9), in which immunogold particles were almost exclusively confined to the cuticular surface of the parasite. However, the identification and function of the isolated IgE reactive HMWTC complex is not known. It is possible that the complex is an integral component of the outer cuticular structure, such as the complex collagen-like proteins identified in structural analysis of the nematode *Caenorhabditis elegans* (25,26). However, it is more likely that the allergen(s) represent complexes secreted onto the surface of the worms, many of which have been shown to be lipids or glycoproteins (27,28). Studies with *Haemonchus contortus* have identified a 70–90-kDa glycoprotein present on the surface of L3 larvae, which is a recognized by the generation of antibodies during infection (20,29). Although the functions of these secreted surface antigens are unknown, it is clear from several studies that they are a major source of antigenic stimulation, and may be candidate antigens for a vaccine (30,31). Indeed, recent studies with the purified antigen from the surface of *Haemonchus contortus* L3 larvae have showed that

vaccination with this glycoprotein induced significant protection, and clearly demonstrates the potential of surface larval antigens as vaccine candidates (32). However, these latter studies were unable to correlate antibody levels or isotype, with protection. Whether the *Teladorsagia* glycoprotein described in the current study is similar to the *Haemonchus* L3 surface glycoprotein, either in terms of function or vaccine potential, is not known.

These surface antigens may also play a significant role in immune evasion, through stage-specific antigen expression. For example, developmental variation in the glycoprotein content of surface structures has been demonstrated for *Ostertagia* spp. (33), and for several other nematodes including *H. contortus* (30,34), and *Oesophagostomum dentatum*²⁸. In this respect, it may be relevant that in our earlier work we have shown that IgE responses to *T. circumcincta* were predominantly to the L3 infective larvae, with only low responses detected against the adult worms (9).

Although yields of purified protein precluded a detailed analysis of the purified allergen termed HMWTC, preliminary studies on the glycoprotein nature were undertaken. Deglycosylation revealed that N-terminal glycans were present, and that these carbohydrate residues contributed substantially to the antigenic properties of the allergen. Reduction and deglycosylation of the 150 kDa complex (HMWTC) resulted in losing the IgE reactivity of two out of the four reduced bands entirely, with reduced reactivity of the remaining two bands. However, it is not known whether this reduced reactivity is due to incomplete deglycosylation, or to peptide recognition and further studies are in hand to determine the extent and nature of the glycosylation. It is increasingly apparent that carbohydrate residues appear to be associated with the 'allergenicity' of some Schistosome proteins (35), and recent studies have shown that IgE antibodies from *Schistosoma mansoni*-infected mice and *H. contortus*-infected sheep recognize complex-type N-glycans (36). This latter study showed that in sheep, a major proportion of the parasite specific IgE in serum of *H. contortus* infected animals, appeared to be reactive to a core N-glycan, α 1 to 3-fucose. This N-glycan is a common feature of several allergens, although whether similar glycoconjugates are involved in the IgE anti-HMWTC responses observed in the present study remains unknown. Cross reactivity was observed with two out of the three other species of nematode tested (*T. colubriformis* and *C. curticei* L3), indicating a similarity in their surface antigen epitopes. Further biochemical studies are required to identify these IgE reactive antigens.

There are a number of effector mechanisms that regulate populations of ruminant gastrointestinal nematodes (37) and, in responsive animals, acquired immune responses are

known to affect parasite establishment, growth, fecundity and persistence. An intriguing finding in the present study was the demonstration of significantly higher levels of IgE antibodies to HMW T_c in 6-month-old 'responder' lambs which had relatively low cumulative FEC scores, when compared to those with high FEC. This provides preliminary evidence that the IgE response is important in the mechanism of the immune regulation of worm burdens and/or fecundity in animals of this age. Despite numerous studies, the precise mechanism of worm regulation remains unclear, although IgE has been strongly implicated in the immune rejection of incoming L3 larvae in some host/nematode models, through the activation of mast cells and subsequent hypersensitivity response (38). However, studies with *Teladorsagia* have also indicated an antiparasite function for IgA antibody, determined by the statistical association of low FEC, worm length and IgA antibody to adult worm antigens (39). These authors have suggested that the principal mechanism for the immune control of *Teladorsagia* worms in lambs in their first grazing season is mediated via IgA antibody which affects both fecundity and growth of established worms, through an as yet unidentified mechanism (40). The present results would indicate, though, that an effective IgE mediated response is also operating in Greyface $\times$ Suffolk lambs grazed in their first season. Immune-mediated control of *Teladorsagia* therefore appears to be a complex process involving at least two mechanisms; on the one hand, an IgA mediated response affecting growth and fecundity and, on the other, an IgE mediated hypersensitivity reaction largely affecting incoming infective larvae. The relative importance of the two responses may vary depending on several factors including age, genotype, and the duration and level of worm exposure of these lambs.

In conclusion, our studies have purified a major surface allergen of *T. circumcincta* L3, and shown that relatively high IgE antibodies to HMW T_c are associated with low FEC. Further studies are in hand to confirm this association, and to define the HMW T_c complex.

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