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Review paper

Ovine IgE and its role in immunological protection and disease

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ABSTRACT

The importance of internal and external parasites in limiting productivity and compromising the welfare of sheep has provided the impetus for extensive research on ovine IgE with the objectives of better understanding protective immunological responses and developing novel methods of control; particularly vaccination. The molecular structures of ovine IgE and its high affinity receptor have been determined and the former information has assisted the development of monoclonal antibodies (mAb) to ovine IgE by 2 of 3 groups who have produced these reagents. The availability of these mAbs has enabled the description of IgE responses following infections with a wide variety of parasites in sheep and in an ovine model of atopic asthma. While IgE responses are consistently associated with parasitic diseases of sheep, it has not been proven that this antibody isotype is involved in protection. The foundation of present knowledge and reagents, together with new emerging technologies, should allow the role of IgE in parasitic diseases of sheep to be determined.

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1. Introduction

Gastrointestinal (GI) nematode parasites and ectoparasites can impose substantial limitations on productivity of sheep and control of these parasites involves substantial cost to sheep farmers. Furthermore, the development of anthelmintic and insecticide resistance by the parasites, as well as increasing demands by consumers and regulators to reduce and restrict the use of chemicals, has led to research aimed at finding alternative ways to control parasite populations or reduce the consequences of infection. Because sheep, in many cases, are able to develop effective immunity to parasites, one approach has been to understand the mechanisms of protective immunity with a view to using this knowledge to develop novel control strategies. Elevated IgE in serum is typical following external and internal parasitic infections and is presumed to be involved in the protective responses. The hope that understanding the role of IgE would assist discovery of the mechanisms that damage parasites and identify protective antigens/allergens has driven research in this area. Further, the potential of the IgE response to contribute to the adverse consequences of parasitic disease for the host provides another important incentive to understand the role of this immunoglobulin isotype in parasitic diseases.

Allergy phenotypes directly equivalent to those in atopic humans and domestic animals have not been reported in sheep to our knowledge. However, the sheep has been used extensively as a model of atopic asthma and the possibility that the skin diseases observed with scab mite and louse infestation in sheep may be equivalent in many respects with atopic dermatitis has been suggested.

Further characterisation of ovine IgE responses, better understanding of the factors that promote IgE production and the development of methods for manipulating the IgE response in sheep will contribute further to understanding the immunological mechanisms operating in parasitic and allergic diseases.

2. Detection of ovine IgE

2.1. Early attempts

The presence of reaginic or homocytotropic antibody in sheep was first described in the late 1960s at a time when there was intense interest in this immunoglobulin class in a range of species and the designation of IgE was first made in human beings (Hogarth-Scott, 1969; Bennich et al., 1969). Curtin (1969) raised an anti-ovine 'IgG_{1a}' antiserum by immunising goats with ovine IgG and IgG₁ fractions possibly contaminated with IgE. This antiserum was shown by Hogarth-Scott (1969) to block the homocytotropic antibody in serum from sheep parasitized with

Ostertagia circumcincta (now *Teladorsagia circumcincta*) in passive cutaneous anaphylaxis (PCA) reactions using antigens from the parasite, and that the antiserum itself elicited immediate hypersensitivity responses in the skin. Hogarth-Scott (1969) additionally demonstrated by PCA that the homocytotropic antibody persisted in the skin for at least 14 days following injection and was labile when heated to 56 °C. In further studies, the accumulation of cells staining for this immunoglobulin in the abomasal and intestinal mucosa and regional lymph nodes of sheep infested with GI parasites was shown and increases in serum levels of the immunoglobulin together with increasing PCA titre specific for the parasite antigens were described following infection with *T. circumcincta* (Curtin and Anderson, 1971, 1972). All of these findings were consistent with 'IgG_{1a}' being IgE, however, this was not confirmed (Miller, 1984).

2.2. Polyclonal antibodies to ovine IgE

Further progress on characterising ovine IgE and developing reagents for its detection did not occur until the early 1990s. Yilmaz et al. (1993) immunised sheep by combined administration of *Ascaris suum* cuticle extract parentally and embryonated eggs orally. In PCA using the cuticle extract, the homocytotropic antibody produced showed substantial lability when heated at 56 °C for 2–4 h and a latency period following intradermal injection of up to 20 days providing evidence that it was IgE. The homocytotropic antibody was separated from the sera by size exclusion, ion exchange and *Ascaris* antigen affinity chromatography and the preparations obtained were used to raise polyclonal anti-ovine IgE antibodies in rabbits. On immunoblots following SDS-PAGE of ovine IgE fractions and sera, the polyclonal antibodies identified the ovine IgE heavy chain at a molecular weight (MW) of 70–72 kDa.

2.3. Monoclonal antibodies

Since Yilmaz's work several innovative methods have been used to generate monoclonal antibodies to ovine IgE. Purified mucosal mast cells (Shaw et al., 1996), *Escherichia coli* expressed recombinant peptides based on segments of the ovine IgE heavy chain constant region (Cε) gene sequence (Kooyman et al., 1997) and a chimeric IgE composed of the ovine heavy chain and mouse light chains (Clarke et al., 1997; Bendixsen et al., 2004) have all been used to successfully generate mAbs to ovine IgE.

2.3.1. XB6 and YD3

The first report of the production of mAbs to ovine IgE was by Shaw et al. (1996), who used mast cells isolated

from the intestinal mucosa of sheep parasitized with GI nematodes for the immunisation of mice. Two mAbs, designated XB6 and YD3, were identified by screening for antibodies reacting with cells in frozen sections of small intestine, isolated mucosal cells, and dot blots of lysed mast cell homogenate. The mAbs induced an immediate hypersensitivity reaction when injected into the skin of sheep and bound to mast cells and presumptive B-cells in frozen sections of mesenteric lymph node. The heavy chain of ovine IgE affinity-purified using the YD3 mAb showed a MW of 86 kDa on SDS-PAGE. Competitive binding assays showed that the XB6 and YD3 mAbs bound different epitopes on IgE. The utility of the mAbs was demonstrated for the isolation of mast cells by panning when coated on solid surfaces and immunomagnetic bead separation when attached to the beads, the affinity purification of serum IgE and for detecting IgE in an antigen-capture ELISA. Subsequently, the latter ELISA was developed to measure total IgE with YD3 as the capture mAb and biotinylated XB6 as the detecting mAb (Shaw et al., 1997). An ELISA to detect parasite-specific (*Trichostrongylus colubriformis*) IgE in sera using a 1:1 mixture of the two mAbs, YD3 and XB6, was reported a year later (Shaw et al., 1998). Saturated ammonium sulphate (SAS) precipitation of sera was found to increase the sensitivity of the latter ELISA presumably through reducing competition from other immunoglobulin isotypes specific for the antigens. Interestingly, Pernthaner et al. (2005) could only detect specific IgE responses in intestinal lymph after the affinity purification of IgE whereas SAS precipitation or the use Protein G to remove IgG failed to enhance assay sensitivity.

2.3.2. IE7

Kooyman et al. (1997) used the sequence for ovine IgE heavy chain (Engwerda et al., 1992) to amplify the Cε3–Cε4 domains of the constant region (nucleotides 1111–1575) which were then expressed in *E. coli* to produce the recombinant protein, reIgE1–2. The purified recombinant (20 kDa) was used to raise both rabbit polyclonal antibodies and a mouse mAb (IE7) to ovine IgE. Primary screening for identification of the mAb used an ELISA for reIgE1–2 and subsequent screening utilised Western blots of reIgE1–2 or IgE-enriched fractions of lymphocyte extracts. On Western blots IE7 recognised non-reduced IgE (200 kDa), a broad heavy-chain band (80 kDa) and deglycosylated heavy chain (65 kDa). Western blotting of serum fractions also demonstrated that IE7 was specific for the IgE in sheep and goats but did not react with IgE from a range of other species including cattle. While the rabbit anti-reIgE1–2 polyclonal serum appeared to cross-react with other ovine immunoglobulin classes, its use as capture antibody in combination with IE7 as detecting antibody produced an ELISA that was specific for IgE in sheep. IE7 was also shown to detect parasite-antigen-specific IgE in SAS treated fractions of sheep sera. For both immunoassays, it was necessary to heat sheep sera to 56 °C to increase binding of IE7 mAb to the IgE. The authors demonstrated the use of IE7 for immunohistochemical staining of mast cells and IgE-producing plasma cells in fixed tissues and developed ELISAs for total IgE and parasite-specific IgE in sheep infected with GI parasites.

Activity in causing degranulation of mast cells was not reported, presumably due to the need to denature IgE by heating to 56 °C before binding occurs.

2.3.3. Y41

The production of mAbs to ovine IgE by Clarke et al. was also reported in 1997 although the lead mAb was used earlier in studies of flystrike in sheep and was provided to Shaw et al. (1996) for comparative study (Colditz et al., 1994; MacDiarmid et al., 1995). This group cloned cDNA for the entire constant region (Cε) of ovine IgE into an expression vector which encoded a VDJ segment for anti-(4-hydroxy-3-iodo-5-nitrophenyl) acetic acid (anti-NIP). The vector was transfected into mouse hybridoma cells producing antibody light chain with anti-NIP specificity (Clarke and Beh, 1994). Transfected cells produced chimeric anti-NIP IgE containing ovine Cε and the antibody was affinity-purified on NIP-sepharose columns. The purified chimeric antibody was then used to immunise mice for mAb production (Clarke et al., 1997). The resultant hybridoma cultures were screened for reactivity with the purified chimeric antibody by ELISA and three mAbs, designated Y4, Y15 and Y41, were cloned. In ELISA with wells coated with purified ovine immunoglobulins, the mAbs showed cross-reactivity with IgG2, but not IgG1, however no evidence for cross-reactivity was observed in competitive ELISAs or on Western blots with the Y41 mAb. Intestinal mast cells from *T. colubriformis*-immunised sheep were stained with Y41 in indirect immunoperoxidase reactions and this mAb blocked release of sheep mast cell proteinase (SMCP) from these cells when challenged with *T. colubriformis* antigen *in vitro*. However, the mAb did not trigger release of SMCP *per se* indicating that it failed to crosslink IgE on the surface of mast cells. The mAb from the Y41 clone has since lost its original reactivity (Bendixsen et al., 2004).

2.3.4. 2F1

Bendixsen et al. (2004) produced new mAbs using the chimeric IgE essentially as done by Clarke et al. (1997). The mAb 2F1, which had the greatest anti-IgE activity, was selected for detailed study and compared with the IE7 mAb (Kooyman et al., 1997). In contrast to IE7, 2F1 recognised native ovine IgE without the need for denaturing or SAS treatments. 2F1 did not cross-react with ovine IgG1, IgG2, or secretory IgA in ELISA and in this regard was similar to the IE7 mAb. IE7, but not 2F1, reacted in Western blots of colostral IgE separated by SDS-PAGE under reducing conditions whereas both mAbs showed bands at 200 kDa when the IgE was not reduced. 2F1 bound to isolated intestinal mucosal mast cells from *T. colubriformis*-infected lambs and stained plasma cells and mast cells in frozen tissue sections with greater intensity than IE7. Activity in causing degranulation of mast cells was not reported. A quantitative ELISA for total IgE was developed using 2F1 to capture IgE, IgG from a rabbit immunised with the chimeric IgE and affinity-purified with the chimeric IgE for detection, and standards of known amounts of chimeric IgE. Ovine IgE in colostrum and intestinal tissue homogenates from *T. colubriformis*-infected lambs were measured. Detection of antigen-specific IgE in ELISA and

Western blotting was demonstrated using extracts of intestinal tissue from lambs infected with *T. colubriformis*. This mAb has also been used to detect goat IgE (Tarigan and Huntley, 2005).

3. Molecular characteristics of IgE in sheep

3.1. Characteristics of IgE heavy chain

Estimates of the MW of the native ovine IgE heavy chain range from 70 to 86 kDa as determined from SDS-PAGE under reducing conditions, while the complete IgE molecule under non-reduced conditions has a MW of approximately 200 kDa (Yilmaz et al., 1993; Shaw et al., 1996; Kooyman et al., 1997; Huntley et al., 1998a; Bendixsen et al., 2004). However, the heavy chain typically runs as a broad band which may reflect varying degrees of glycosylation of this typically heavily glycosylated immunoglobulin (Shaw et al., 1996). Clarke and Beh (1994) determined that the ovine chimeric IgE they produced contained 7.8% sugar residues by weight. In a further indication of abundant carbohydrate residues, deglycosylation of native ovine IgE led to a reduction of MW from 80 to 65 kDa with the latter regarded to be in agreement with the predicted MW of the unmodified polypeptide determined from the published sequence (Engwerda et al., 1992; Kooyman et al., 1997).

3.2. IgE heavy chain constant region (C ϵ) sequence

Although the C ϵ sequence is the least conserved of the Ig heavy chain genes, Clarke and Beh (1992, 1993) reasoned that the bovine and ovine genes would be similar. They used probes derived from a bovine C ϵ genomic clone to detect the equivalent gene in a cosmid library constructed from genomic DNA from the liver of a merino ram. This work indicated that the ovine and bovine C ϵ genes were closely related with many restriction enzyme sites in the same relative positions and the genes were located similar distances 3' of the IgA heavy chain constant gene. Restriction fragment length polymorphisms (RFLPs) in DNA from a Merino half sib family indicated allelic variation due to an insertion/deletion near the 5' end of the C ϵ gene which potentially could influence expression levels. In further work, the allelic variation was found to be due to a 36 bp deletion in a region of untranslated DNA 5' of the C ϵ gene that, in the larger allele, contained two (~87 bp) repeats each composed of two smaller (~42 bp) core repeats, plus an additional 23 bp fragment of a fifth core repeat (Clarke et al., 2001). Another, less common, allelic variant was also identified and was presumed to be due to a further deletion of a core repeat. Engwerda et al. (1996) also examined RFLPs in the C ϵ region using genomic DNA from lines of sheep resistant or susceptible to fleece rot and flystrike. A range of fragment sizes with some of the restriction enzymes also indicated allelic variation in or near this gene.

Engwerda et al. (1992) used primers designed from conserved regions of known rodent (mouse and rat) and primate (human, chimpanzee and orangutan) C ϵ genes to amplify a fragment of the ovine gene from cDNA derived

from abomasal lymph nodes of sheep infected with *Haemonchus contortus*. This fragment was used to probe a cDNA library derived from the sheep lymph nodes and an 1802 bp cDNA was identified and cloned. This clone encoded the 4 constant domains (C ϵ 1–4) and the variable heavy (VH) chain region including precursor (S), variable (V), diversity (D) and joining (J) regions. The deduced AA sequence of the ovine heavy chain showed overall homology of 45–47% with the primate and rodent genes which was typical of IgE sequences between orders of animals. Cysteine and tryptophan residues, important in domain and molecular structure, are typically highly conserved and this was observed in the ovine IgE clone. Seven of the 8 tryptophan residues in the sheep gene matched those in the rodent and primate genes while all 13 cysteine residues were found in the primate genes and 10 of these were conserved in rodents. Additionally, 2 cysteine residues in the VH-region of the ovine cDNA clone occurred in the same position observed in this region in other species. Nine potential carbohydrate attachment sites were identified in the ovine C ϵ indicating ovine IgE potentially is highly glycosylated in agreement with observations in other species; however, the position of only one glycosylation site is highly conserved and this also was observed in the ovine gene. The nucleotide sequence determined from a cosmid clone of genomic DNA containing the ovine C ϵ gene (Clarke et al., 2001) was very similar to that obtained by Engwerda et al. (1992), and the deduced AA sequence was in agreement with regard to the conservation of cysteine and tryptophan residues and the number of carbohydrate attachment sites. Clarke et al. (2001) determined that the ovine C ϵ gene showed 96 and 92% similarity at the nucleotide and amino acid (AA) levels respectively with similarly derived bovine sequence. The 4 exons (CH1–4) in the two species had identical lengths and the same cysteine residues and putative carbohydrate attachment sites. Deduced AAs of the CH1 and CH2 were identical between the two species while CH3 and CH4 had 13 and 19 different AAs respectively. It was postulated that the latter divergence may explain the failure of mAbs raised to bovine IgE to recognise ovine IgE.

In comparisons of PCR amplified cDNAs of C ϵ 3/4, the goat sequence showed 95% nucleotide and 84% AA identity with those of sheep reflecting the relatively close relatedness of these species (Griot-Wenk et al., 2000). Phylogenetic analysis of the deduced AA sequences of the IgE heavy chain constant regions of sheep and 8 non-ruminant species including rodents, primates, dog, horse and pig showed that the sheep sequence was most closely related to that of the pig and least similar to the rodent sequences (Vernersson et al., 1997).

3.3. High affinity receptor for IgE

Fc ϵ RI is the high affinity receptor on the surface of mast cells and basophils responsible for activation and degradation following binding of IgE and crosslinking by allergens. This tetramer, transmembrane receptor is composed of an α , a β and 2 γ chains with the extracellular portion of the α chain responsible for binding the Fc region of IgE. The sequence of the ovine α chain has been

determined from cDNA derived from cultured bone marrow mast cells (McAleese et al., 2000). The mature protein was similar to those of other species being composed of 234 AA, having 4 conserved cysteine residues and 6 potential N-linked glycosylation sites. Sequence homology with horse, human and dog α chains was 58–60% but lower identity was observed with rodent sequences. The sequences of the β and γ chains have been determined in a similar manner (McAleese and Miller, 2003). The β chain encodes a protein of 242 AAs which showed 62% identity with the horse sequence. The γ chain sequence encoded 85AAs with the mature protein expected to be 65 AAs and typically showed greater than 90% homology with γ chains of other species as expected for this highly conserved protein. Both the β and γ chains contained the conserved tyrosine residues of the immunoreceptor tyrosine-based activation motifs in the cytoplasmic domains.

The presence of Fc ϵ RI on ovine mast cells has not been formally demonstrated although this is expected given the typical behavior of these cells in allergic conditions. Similarly, there is evidence for antigen-specific *in vitro* degranulation of ovine basophils indicating that these cells also express Fc ϵ RI (Pfeffer et al., 1997). It is presently unknown if Fc ϵ RI is present on other ovine cell types or whether lower affinity IgE receptors exist in sheep. Immunohistochemical staining using IgE-specific mAb provided no evidence that the epidermal, antigen-presenting Langerhans cells of sheep bound IgE suggesting that in this regard the sheep is like rodents rather than dogs and man (Shu et al., 2009).

4. Enumeration of IgE-bearing cells

The mAbs produced to ovine IgE have been shown to bind to IgE-bearing cells in tissues with similar patterns (Shaw et al., 1996; Kooyman et al., 1997; Bendixsen et al., 2004). Several studies have enumerated IgE positive cells in sheep following parasite infection. In 2-year-old sheep receiving weekly infections with *Trichostrongylus axei*, IgE positive cells in the abomasal mucosa, which are mainly mucosal mast cells, increased in number from 11 to 14 days after the initial infection (Pfeffer et al., 1996). These cells appeared in the mucosal approximately a week before mucosal mast cell derived globule leukocyte numbers increased. In sheep immunised by three 14-day truncated infections with *T. colubriformis* there were significantly more IgE positive cells in the lamina propria than in control sheep (Harrison et al., 1999). These cells were present before and up to 24 h after a challenge infection. In a study of ovine rectal mucosa, IgE positive cells, thought to be plasma cells due to intracellular staining, were identified in the lamina propria but were not observed in lymphoid follicles (Sedgmen et al., 2002). Sheep ranked as responsive to pasture trichostrongylid infection based on faecal egg count (FEC) had significantly more IgE-bearing cells in circulation than non-responsive animals at the mid-point of their first grazing season but not later or in a second season (Pettit et al., 2005). Flow cytometry analysis showed the circulating IgE-bearing cells were characteristic of small to medium sized lymphocytes, suggesting

they may be B-cells or possibly a population of mucosal mast cell precursors. A small portion of the IgE-bearing cells were granulocytes, most likely basophils.

5. IgE responses to parasitic nematodes

The association of helminth parasite infection and elevated IgE responses is well documented. With the development of reliable specific monoclonal antibodies to ovine IgE many studies have been published detailing IgE responses in serum and lymph following experimental and natural parasitic nematode infection of sheep.

5.1. Serum and mucosal IgE responses to parasitic nematodes

Both primary and challenge experimental infections of lambs with *H. contortus* (Kooyman et al., 1997), *T. circumcincta* (Huntley et al., 1998a) and *T. colubriformis* (Shaw et al., 1998; Harrison et al., 1999) have been shown to induce elevated serum total IgE responses. Trickle infection of adult sheep with *T. axei* resulted in elevated total IgE which was shown to closely follow increases in numbers of IgE positive cells in abomasal mucosa (Shaw et al., 1997). In a recent study using presumably a commercial RIA kit total serum IgE was shown to be elevated and related to levels of total eosinophils and total serum proteins in parasitized but not nonparasitized sheep and goats (Kataria et al., 2007).

Specific IgE responses in serum to larval and adult antigens of *T. circumcincta* and *T. colubriformis* were negligible after primary infection (Huntley et al., 1998b; Shaw et al., 1998). Challenge infections with *T. colubriformis* induced strong IgE responses to both larval and adult antigens while challenge infection with *T. circumcincta* resulted in only elevated IgE to third stage larval (L3) antigens. Sheep immunised by three 14-day long infections with *T. colubriformis* had significantly elevated specific IgE to *T. colubriformis* L3-excretory/secretory products (ES) 2 weeks after a third infection (Harrison et al., 1999). A significant rise in antigen-specific IgE was observed in intestinal homogenates of sheep after trickle infection with *T. colubriformis* (Bendixsen et al., 2004). In contrast, IgE to adult but not larval *H. contortus* antigens were elevated after both primary and challenge infections (Kooyman et al., 1997). While it would be convenient to speculate that these differences in IgE responses reflect parasite or host variables they could also be attributed to differences in challenge regime, breed of sheep, sensitivity of assay (Huntley et al., 1998b) or the preparation and stability of the antigens used.

The genetics of IgE responses from natural or field challenged sheep have been determined in progeny test and high and low FEC selection line lambs (Shaw et al., 1999). Levels of specific IgE to *T. colubriformis* L3-ES was heritable ($h^2 = 0.36$), showed negative genetic correlation with FEC (averaging -0.31) but was positively correlated with breech soiling in lambs.

There is considerable evidence that the level of nutrition and the manifestation of immunity to GI parasites are linked. Using restriction of protein in the diet of periparturient and non-pregnant ewes it was shown

that globule leukocyte counts and plasma IgE levels specific for *T. circumcincta* L3-ES were increased in protein supplemented ewes (Houdijk et al., 2005). FEC was negatively correlated with plasma IgE levels although IgE levels were not correlated with total worm burden. Deficiency in readily available carbohydrate also resulted in significantly reduced parasite-specific antibody including IgE during secondary parasite infection (McClure, 2009a). Diets low in carbohydrate additionally impaired development of both humoral and cellular immunological responses.

In a breed comparison experiment Texel and Suffock lambs were co-grazed with their dams on pasture until necropsy at 17 weeks of age (Sayers et al., 2008). A significant negative correlation was identified between mucosal IgE levels to *T. circumcincta* L3 antigen and faecal egg counts and worm burden in Texel but not in Suffock lambs. The authors suggest that parasite susceptibility in Suffocks may be associated with deficiencies in the IgE or mast cell response in these sheep.

5.2. IgE response in lymph

Several studies have focused on measuring IgE responses in lymph draining the GI tract of parasitized sheep. Elevated IgE concentrations in efferent lymph when compared to serum of sheep infected with the abomasal nematode parasite *T. circumcincta* led to the conclusion that IgE is produced either in the mucosa or the lymph nodes (Huntley et al., 1998a,b). Using nematode high and low FEC selection line sheep, Pernthaner et al. (2005) showed that concentrations of total IgE were consistently higher in afferent intestinal lymph than efferent intestinal lymph or serum suggesting that the intestinal mucosa is the main contributor to the polyclonal IgE production. In contrast, they showed that antigen-specific IgE was found at higher concentrations in efferent than afferent lymph which provides strong evidence that the regional lymph nodes following nematode infection are the main source of specific IgE production. Following *T. colubriformis* challenge, immune animals had higher levels of total IgE in lymph than non-immune sheep, low FEC sheep had higher concentrations of total IgE than high FEC sheep, and field-primed animals had higher total IgE levels than nematode naïve animals. There is a strong indication of stage-specific induction of IgA and IgE responses in lymph after infection with *T. colubriformis* (Pernthaner et al., 2006).

5.3. IgE binding antigens

The identification of individual IgE binding nematode antigens (allergens) may lead to the identification of new antigens for use in vaccination studies or as markers of protective immunity. Huntley et al. (1998b) using Western blots, detected IgE binding to several bands of *T. circumcincta* L3 antigens with considerably weaker binding to adult antigens. IgE binding was demonstrated to *H. contortus* adult antigens of around 35- and 40–50 kDa (Kooyman et al., 2000). A major high MW *T. circumcincta* L3 allergen has been isolated using chromatography techniques (Huntley et al., 2001). Under reducing conditions this

allergen produced 4 immunoreactive bands ranging from 30 to 120 kDa. At least 2 of these bands were shown to have IgE binding glycosylation.

Serum IgE obtained from high and low FEC selection line lambs bound to Western blotted *T. colubriformis* L3 antigens of 40, 31 and 21 kDa (Shaw et al., 2003). Using a proteomic approach the 31 kDa antigen was identified as nematode aspartyl protease inhibitor (Aspin). The entire coding sequence for Aspin was obtained and the mature protein expressed in *E. coli* (NCBI Accession No. AY189824). The 21 kDa antigen identified on Western blots was identified as a truncated form of Aspin. The 40 kDa antigen was identified as a galectin (Shaw, unpublished). Serum IgE responses to recombinant Aspin showed significant phenotypic and genotypic correlations with reduced FEC in high and low FEC selection and control lines without being correlated with increased breech soiling. However subsequent analysis on a number of commercial Romney lamb flocks failed to show significant correlations between IgE to Aspin and reduced FEC (Shaw, unpublished). Using IgE from intestinal homogenates of sheep bred for increased immunological responsiveness, Bendixsen et al. (2004) observed IgE binding on Western blots of *T. colubriformis* L3 antigens similar to that of Shaw et al. (2003).

5.4. Vaccination induced IgE responses

In a study of age related immune responses to vaccination with *H. contortus* antigens using a Th-1 stimulating adjuvant, ES-specific IgE was detected in sheep of 9 months of age but not at 3 or 6 months old at the start of the vaccination regime (Kooyman et al., 2000). The peak IgE responses of individual sheep were shown to vary considerably. In a further report of the same study *H. contortus* ES-specific IgG1 and IgA but not IgE were elevated after immunisation using a Th-1 stimulating adjuvant (Vervelde et al., 2001). Following parasite challenge, specific IgE was elevated in vaccinated groups but the levels were very low. In a subsequent study using recombinant *H. contortus* antigens, total IgE and antigen-specific IgE titres were low, while IgA responses were high and negatively correlated with worm numbers (Vervelde et al., 2002). When lambs were vaccinated with native *H. contortus* adult ES antigen in a strong Th-2 type inducing aluminium adjuvant, specific IgG1 and to a lesser degree specific and total IgE responses in serum occurred after booster immunisations and parasite challenge (Vervelde et al., 2003). The antibody responses appeared to be directed primarily at glycan epitopes on the *H. contortus* ES molecules. In a further study using fractionated *H. contortus* adult ES and aluminium adjuvant, ES-specific IgE levels in serum increased after immunisation and after challenge infection (Bakker et al., 2004). However no correlation was found between protection from challenge and antibody levels in serum or mucus. In a study of mucosal delivery of native and recombinant *T. colubriformis* proteins, McClure (2009b) induced significantly elevated serum IgG, IgA and IgE after delivery of parasite antigens to the epithelium overlying rectal Peyer's patches. Of importance is that these immune responses were generated without the use of adjuvants.

6. IgE responses to ectoparasites

The more significant ectoparasites of sheep include scab mite (*Psoroptes ovis*), body lice (*Bovicola ovis*) and facultative parasitic flies causing cutaneous myiasis (alternatively called flystrike, *Lucilia cuprina* and other strike flies), while a number of other mites and flies, sucking lice and ticks also affect sheep. The IgE responses of sheep to the scab mite and body louse have been examined and allergens have been identified from these parasites. Little information on IgE responses in respect of infestation with other ectoparasites of sheep is presently available.

6.1. *P. ovis*

P. ovis feeds on the surface of the skin and infestation typically leads to exudative and crusted lesions with many features of allergic dermatitis (van den Broek and Huntley, 2003). The first direct indication that sheep produce IgE to *P. ovis* was provided by Matthes et al. (1996) who demonstrated positive bands on Western blots of the crude mite antigen probed with sera from infested sheep and anti-ovine IgE mAb. *Psoroptes* spp. (*P. caniculi*)-specific serum IgE was shown to increase from 6 to 7 weeks and peak at 13 weeks following primary *P. ovis* infestations of sheep and showed a rapid amnestic response following a second infestation when the reduced lesion size indicated a degree of protection (van den Broek et al., 2000). While significant increases in IgG and IgM were detected following primary infestation in a subsequent study, in contrast to the amnestic IgE response, no significant differences in the responses of these isotypes were detected between primary and challenge infections (van den Broek et al., 2003b). Intradermal skin testing confirmed immediate hypersensitivity and late phase reactions in infested sheep consistent with IgE-mediated Type-1 hypersensitivity together with an eosinophil-rich delayed-type hypersensitivity response that the authors suggest may be cell, rather than IgE, mediated (van den Broek et al., 2003a). Antigen challenge of skin sites in naive sheep previously injected with serum from infested sheep (Prausnitz–Kustner tests) led to immediate reactions only. In an interesting parallel with human atopic dermatitis, 50% of induced *P. ovis* lesions on sheep were found to be colonised by *Staphylococcus aureus* and this colonisation appeared to be associated with earlier development of an IgE response to the mite (Oliveira et al., 2006).

In parallel with observations on *P. ovis* in sheep, goats subjected to a series of 3 challenge infestations with the superficially burrowing mite *Sarcoptes scabiei* showed progressively rapid and increased responses of mite-specific IgE in serum with each challenge while levels of specific IgG attained did not increase (Tarigan and Huntley, 2005). Lesion development was markedly reduced following the second challenge indicating a protective response. It was further shown that vaccination with soluble antigens from the mite led to robust specific serum IgG responses but failed to elicit a serum IgE response or protection.

Western blots of crude *P. ovis* antigen probed for reactivity with IgE from mite-infested sheep have shown

numerous (up to 13) positive bands ranging from <10 to 180 kDa while >20 IgE reactive spots were identified on blots derived from two-dimensional SDS-PAGE (Matthes et al., 1996; van den Broek et al., 2003b; Huntley et al., 2004; Oliveira et al., 2006). Nine potential allergens from *P. ovis* have been identified through sequence homologies with known allergens (Lee et al., 2002a,b; Temeyer et al., 2002; Kenyon et al., 2003; Nisbet et al., 2006, 2007). Presently, native *P. ovis* homologues of Der p 10 (tropomyosin), Der p 11 (paramyosin) and Der p 14 (apolipoprotein/vitellogenin) and the recombinant homologue of Der p 1 (cysteine proteases) have shown reactivity with IgE from infested sheep while the recombinant homologues of tropomyosin, paramyosin and GST were not reactive (Lee et al., 2002a,b; Huntley et al., 2004; Nisbet et al., 2006).

The association of IgE responses with curtailment of lesion size suggests a role for this isotype and the accompanying immediate hypersensitivity in protective immunity to these mites (van den Broek et al., 2000; Tarigan and Huntley, 2005). However, it may also be possible that the responses are beneficial to the mites, for example, leading to improved nutrition by increasing transudation of proteins across the skin (van den Broek et al., 2000).

6.2. *B. ovis*

The louse, *B. ovis*, like *P. ovis*, is regarded as surface feeding and infestation of sheep leads to irritation, fleece damage and a skin disease known as cockle. The morphological changes and cytokine gene expression profiles in skin and the immunological responses of sheep to infestation indicate a highly skewed Th-2 response leading to allergic hypersensitivity to this ectoparasite (Pfeffer et al., 2007; Shu et al., 2009). Presently there is little evidence that immunological responses are protective against this ectoparasite. In two bloodlines of Australian Merino sheep, *B. ovis*-specific and total serum IgE measured by ELISAs were shown to be higher in the line that was more susceptible to gastrointestinal nematode parasites and tended to have higher *B. ovis* infestation (James et al., 2002). However, unlike *B. ovis*-specific total serum immunoglobulins, specific and total IgE levels were not significantly correlated with louse scores. Additionally, in New Zealand Romney lambs, levels of IgE specific for crude *B. ovis* Ag were significantly higher in louse-infested than in naive lambs (Pfeffer et al., 2008). In a comparison with louse-naive lambs, infested lambs also show significantly higher levels of IgE⁺ cells and diffuse staining for IgE within the dermis in response to natural infestation and following intradermal *B. ovis* antigen challenge (Shu et al., 2009). These latter findings suggest greater involvement of IgE in the response at the local level than indicated by results obtained with serum IgE.

Purified ovine IgE from a louse-infested lamb was used to identify allergens from crude preparations of whole *B. ovis* and its faeces and a major allergen designated Bov o 1 has been isolated and characterised (Pfeffer et al., 2008, NCBI Accession No. BD404785). The Bov o 1 sequence shows weak homology with several lipid-binding proteins.

Elevated serum IgE specific for Bov o 1 was detected in 50% of louse-infested lambs and intradermal injection in infested lambs, but not naive lambs, evoked immediate and late phase responses typical of IgE-mediated hypersensitivity.

6.3. Flystrike

Colditz et al. (1994) demonstrated that IgE⁺ cells, presumed to be mast cells, were more common in the skin of sheep genetically selected for or showing phenotypic resistance to fleece rot and fly strike. In a subsequent study, there was no evidence for an IgE response in either serum or wound exudate following natural or induced myiasis however IgE⁺ cells were more numerous in the skin of one sheep that was most resistant to experimental infestation with *L. cuprina* (MacDiarmid et al., 1995). A further suggestion for a link between IgE and flystrike came from the observation that some RFLPs of the ϵ chain showed significantly higher prevalences in the susceptible than in the resistant sheep in the fleece rot/flystrike selection lines (Engwerda et al., 1996). Further work on the possible involvement of IgE in the fleece rot/flystrike disease complex has not been reported to our knowledge.

7. Colostrum

Bendixsen et al. (2004) provided the first direct evidence of IgE in ovine colostrum demonstrating levels of total IgE ranging from 0.55 to 11.05 ng/ml in Merino ewes at lambing. The level of total IgE in colostrum from New Zealand Romney ewes was much higher with an estimated mean of 93 μ g/ml, however only trace amounts were found in milk 30 days after parturition (Pfeffer et al., 2005). In the latter study, the mean level of total IgE in colostrum was three times higher than that in parturient ewe plasma suggesting that this immunoglobulin may be selectively concentrated in colostrum. The transfer of IgE to the lambs led to plasma levels at 2 days after birth comparable to those of the ewes but little remained in circulation by 30 days. The presence of specific IgE to *T. colubriformis* L3-ES was also examined in these animals. In this case, levels in colostrum were not significantly higher than in ewe parturient plasma however levels comparable to the ewes were detected in plasma of 2-day-old lambs and had virtually disappeared by 30 days. Further studies are required to confirm these results and to examine the potential effects of the transfer of IgE to the neonatal lamb on the development of immunity to parasites.

8. Allergy model

Atopy is characterised by increased levels of IgE, a key mediator of the 'early-phase' response in allergic asthma and widely used as a marker of disease both in humans and in animal models. In sheep, a proportion of animals that display skin reactivity to extract of the parasite *A. suum* respond with immediate bronchoconstriction following exposure to aerosolised antigen (Wanner et al., 1979). Although IgE levels have not been directly assessed in the *Ascaris* model, this early-phase response has been linked to

mast cell degranulation and histamine release and the pharmacological use of various anti-mast cell agents has been shown to significantly attenuate the early response to airway antigen challenge (Abraham, 2008).

In contrast, the more recent sheep model of allergic asthma based on house dust mite (HDM) allergen uses direct measures of IgE to assess the degree of sensitisation to allergen (Bischof et al., 2003). Systemic immunisation with HDM at an optimal dose results in the development of allergic sensitivity in 50–60% of animals. The sensitised animals are referred to as high-IgE responders or atopic sheep, and display high levels of allergen (HDM)-specific serum IgE (Bischof et al., 2003), typically reaching maximal levels in serum 14 days after the final immunisation (Bischof, unpublished). Atopic sheep respond to HDM lung challenges with a persistent recruitment of high numbers of eosinophils into the bronchoalveolar lavage (BAL) fluid and airway tissues (Bischof et al., 2003). Immunolocalisation of IgE within the airway tissues is strongly correlated with airway mast cells, which are seen to increase following chronic airway HDM exposure in atopic sheep (Snibson et al., 2005). IgE assessed in BAL fluid following allergen challenges has been found to be similar to baseline levels (Bischof et al., 2008), while serum IgE levels that remain elevated after multiple aerosolised HDM challenges can be suppressed following treatment with inhaled corticosteroids (Bischof, unpublished).

9. Concluding remarks

Despite a decade of endeavour, the function of IgE in immune responses to a variety of parasitic infections is still not clear. Studies have confirmed that nematode and ectoparasite infections typically induce a T-helper 2 response which includes elevated IgE responses in many cases. Questions of whether particular IgE responses directly prevent establishment or cause elimination of infection or may in fact be beneficial to some parasites still need to be answered. A further consideration will be determining the cost of such responses to the host in terms of tissue damage, nutrient consumption and loss and overall effects on productivity. In recent years there have been major developments in technologies which should assist in clarifying the role of IgE and other effector mechanisms in sheep immune responses. The use of biomic technologies and genetic manipulations coupled with novel experimental design using perhaps immunisation with different parasite stages, and variances in nutrition and breeds of sheep may help to untangle the relationships between IgE-mediated hypersensitivities and protective and pathological immunity in sheep.

Conflict of interest statement

None.

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