Immunological responses of sheep to *Haemonchus contortus*

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**Summary**

Infections with *Haemonchus contortus* are a major constraint on ruminant health world-wide. Young lambs are very sensitive to *Haemonchus* infection. Older lambs and sheep acquire immunity after a continuous or seasonal exposure to the parasite. The mechanisms underlying immunity are still not completely understood. Antibodies, in particular local IgA and IgE, certainly play a role. The role of IgG is less clear. Lymphocyte proliferation responses seem to correlate to immunity. Sheep that have high antigen-induced lymphocyte responses have a low susceptibility to infection. Furthermore, several studies have demonstrated that immunity against *H. contortus* is associated with mastocytosis and hyper-sensitivity reactions. More recently, increasing attention is being paid to the role of cytokines (interleukins and γ-interferon) in the activation of specific defence mechanisms. Reverse transcriptase–polymerase chain reaction (RT–PCR) assays to study cytokine mRNA expression have become available. The inability of young lambs to mount a significant Th2 response, which is normally characterized by high IgE levels, mastocytosis and eosinophilia, may account for the phenomenon of unresponsiveness in these animals.

Key words: *Haemonchus contortus*, sheep, immunity, antibody, cellular mechanisms.

**Introduction**

Infections with the blood-feeding nematode *Haemonchus contortus* are a major constraint on sheep and goat health and production in many parts of the world. The parasite mainly affects the abomasal mucosa of its host. Adult worms feed on blood and can cause severe anaemia, resulting in poor growth rate and weight loss, and heavy infections can result in death. The control of gastrointestinal nematodes in general is at present dependent on the repeated use of anthelmintics and, where possible, pasture management. However, clean pastures are not readily available under intensive grazing conditions and, perhaps more importantly, there is an increasing occurrence of parasites resistant to the action of anthelmintics (Jackson, 1993; Waller, 1994; Borgsteede et al. 1997; van Wyk, Malan & Randles 1997). Furthermore, there are concerns regarding drug residues in meat and the environment (Madsen et al. 1990; Lumaret et al. 1993).

The above-mentioned problems could be overcome by the development of immunological methods to control gastrointestinal helminths. A good knowledge of the mechanisms underlying protective immunity in sheep is a prerequisite for the development of such methods. The immune response against intestinal nematodes has been studied extensively in humans and rodent models (Miller, 1984, 1996; Cox & Liew, 1992; Sher & Coffman, 1992). Traditionally, it has been accepted that immunity against these parasites comprises the production of specific IgE antibodies, eosinophilia and mucosal mastocytosis (Miller, 1984, 1996; Urban, Madden & Svetic, 1992) and that it is dependent on the activation of T helper (Th) 2 cells (Finkelman et al. 1991).

As far as the ruminants are concerned, most studies have been mainly restricted to studying systemic and peripheral antibody responses. Recently, important advances have been made in obtaining tools to dissect the ruminant immune responses to nematode infections and our understanding of this response has increased considerably in the last years. For example, monoclonal antibodies specific for ovine IgE have become available (Shaw et al. 1996; Kooyman et al. 1997), several cytokines have been cloned, sequenced and expressed (Wood & Seow, 1996) and potential protective antigens have been identified (Newton & Munn, 1999).

The purpose of this brief review is to focus on some of the recent insights in the immune responses of sheep to gastrointestinal nematode infections in ruminants in general and of *Haemonchus* infections in sheep in particular. Furthermore, some recent observations regarding the possible cause of unresponsiveness in young lambs will be presented. Finally, the immune responses induced by vaccination of sheep against gastrointestinal nematodes will be discussed.

**Antibody**

**Ovine immunoglobulins**

The ovine immune system comprises IgG1, IgG2, IgM, IgA and IgE isotype antibodies. During the last decades many research groups have studied the possible role of these antibodies in immunity against gastrointestinal nematodes. Research has mainly
focused on both systemic and locally produced IgA and IgG (reviewed by Miller, 1996). The role of IgM is often considered of minor importance (Schallig, van Leeuwen & Hendriks, 1995). Recently, monoclonal antibodies against ovine IgE have also become available (Shaw et al. 1996; Kooyman et al. 1997) and evidence for the important role of this particular antibody in immunity against gastrointestinal nematodes is rapidly growing.

**IgA and IgG**

A possible relationship between IgA and IgG anti-parasite antibodies and resistance to Haemonchus has been described in many studies. In general, an increase in serum antibodies against larval and adult antigens after primary or secondary infection is observed (Smith, 1977; Duncan, Smith & Dargie, 1978; Smith & Christie, 1978; Charley-Poulain, Luffau & Perry, 1984; Gill, 1991; Schallig et al. 1994a, 1995; Gomez-Munoz et al. 1998). However, a direct relationship between the serum antibody levels and the immune status of sheep is questioned (Bowels, Brandon & Meeusen, 1995; Gomez-Munoz et al. 1999). This is probably due to the fact that *H. contortus* is confined to the surface of the abomasum and its mucosa and that the peripheral immune response is probably a poor reflection of the local mucosal response. For example, Schallig et al. (1994a, 1995) found only low levels of anti-*H. contortus* serum IgA in immune sheep. In contrast, several other studies demonstrated that IgA plays an important role in the mucosal response of immune sheep (Smith et al. 1983, 1984, 1986; Charley-Poulain et al. 1984; Gill, Husband & Watson, 1992a).

A problem with studying the local immune response is the in vivo accessibility of the abomasum for experimental sampling. The development of the technique to cannulate the gastric lymph duct which contains efferent lymph from the ovine stomachs made it possible to monitor local immune responses to abomasal nematodes (Smith, 1988). Gill et al. (1992a) used the technique of abomasal cannulation followed by serial biopsy to study the kinetics of local immune responses to *H. contortus* in sheep. The number of IgA-, IgG1-, IgG2- or IgM-containing cells in the abomasum of sheep before infection was low. Following infection, increased numbers of immunoglobulin-containing cells were observed in the abomasum and peak values were found 21 and 28 days after infection. IgA-containing cells were the most frequently observed cell types, followed by IgG1, suggesting an important role for IgA, and to a lesser extent for IgG1, in the immune response against haemonchosis.

The mechanisms by which IgG and IgA antibodies attribute to immunity against gastrointestinal nematodes is not completely clear. The antibodies could have a direct effect on the parasite. For instance, Gill et al. (1993a) suggested that anti-parasite IgA and IgG antibodies contribute to resistance by neutralising or inactivating vital metabolic enzymes of *H. contortus*. Smith et al. (1985) observed a negative correlation between the magnitude of the gastric lymph IgA response of Teladorsagia circumcincta-infected sheep and worm length suggesting that IgA antibodies could interfere with the worms’ ability to feed (Smith, 1988). Furthermore, it has also been shown that anti-parasite IgG suppresses Trichostrongylus colubriformis feeding in vitro (Bottjer, Klesius & Bone, 1985). A more general role for IgG and IgA antibodies by participating in hypersensitivity reactions has also been suggested (Gill et al. 1993a; Miller, 1996). Examples are the ability of secretory IgA to induce eosinophil degranulation (Abu-Ghazaleh et al. 1989), the binding of IgA/antigen immune complexes to inflammatory cells in the mucosa and the subsequent release of cytokines and inflammatory mediators (Dubucquoi et al. 1994) and the cytophilic properties of IgG1 for mast cells (Askenase, 1977).

**IgE**

An increase in total and parasite-specific IgE is generally regarded as an important factor in the host response to helminth infection (Jarrett & Miller, 1982; Hagan, 1993; Pritchard, 1993). The majority of studies on IgE responses to helminth infections have been in humans and rodents. In humans, high levels of serum IgE are thought to be associated with protection against gastrointestinal nematodes (Pritchard, Quinell & Walsh, 1995). Until recently, the role of IgE in protection against helminths in ruminants has been studied less extensively. However, specific antibodies against bovine (Thatcher & Gershwin, 1988) and ovine (Shaw et al. 1996; Kooyman et al. 1997) IgE have now become available.

Studies on the role of IgE in helminth-infected sheep were aided by the development of a specific monoclonal antibody against ovine IgE by Kooyman et al. (1997). Specific oligonucleotide primers were designed on the basis of the nucleotide and amino acid sequences of an ovine ε-chain cDNA (Engwerda et al. 1992) which allowed the amplification of a part of the C region (Cε3–Cε4, nucleotides 1111–1575) of ovine IgE. The amplification product was cloned into a suitable expression vector and the recombinant protein was purified by affinity chromatography. Specific polyclonal and monoclonal antibodies were produced. The monoclonal antibody, designated IE7 which is specific for sheep and goat IgE, was used to study IgE responses in *H. contortus* or Teladorsagia circumcincta infected sheep (Kooyman et al. 1997; Huntley et al. 1998a, b).
Kozyman et al. (1997) developed an IgE ELISA to monitor total and antigen-specific IgE serum levels during a controlled infection experiment. Infection of sheep with H. contortus resulted in increased total serum IgE levels at 2–4 weeks after infection. A negative correlation between worm counts and total IgE serum levels at necropsy was found in repeatedly infected sheep. This is in line with observations in Ostertagia ostertagi infected calves (Thatcher, Gershwin & Baker, 1989; Baker & Gershwin, 1993). In addition, significantly increased levels of excretory-secretory (ES) adult antigens specific IgE titres were found after infection. In contrast, no significant changes in third stage larvae (L₃) antigen-specific IgE levels in sera could be detected after infection. This indicates that L₃ antigens of H. contortus are probably less allergenic than ES antigens.

Huntley et al. (1998a) studied the IgE response of naive or primary infected sheep to a challenge with 50000 L₃ of T. circumcincta (for details of the design of this experiment see Stevenson et al. 1994) in serum and gastric lymph using a dot blot assay based on the monoclonal antibody IE7. In naive sheep, lymph and serum IgE concentrations increased from days 8 and 14 after infection, respectively. The IgE response of previously infected sheep to the challenge was more rapid, but not necessarily greater, than that following a primary infection. IgE concentrations were usually approximately four-fold higher in gastric lymph than in serum irrespective of whether the sheep were responding to a primary or a challenge infection. This indicates local production of IgE in the regional lymph nodes.

Serum and gastric lymph samples obtained in the experiment described above were also analysed using an ELISA to investigate the IgE antibody responses (Huntley et al. 1998b). During a primary response, IgE antibody to antigens obtained from L₃ and adult T. circumcincta were negligible, with low levels of IgE antibody detected in the gastric lymph and serum samples. There was, however, a marked IgE antibody response in 50% of the sheep to L₃ antigens during 2–8 days after challenge of primary infected sheep. In contrast, low levels of IgE antibody to adult antigens were found (Huntley et al. 1998b). This observation is in contrast to the findings of Kooyman et al. (1997), who demonstrated a clear response against adult ES antigens of H. contortus. In addition, Shaw et al. (1998) described the systemic IgE response in sheep to primary and challenge infections with Trichostrongylus colubriformis and demonstrated peak IgE responses to adult antigens between 20–27 days post infection and a secondary IgE response to both adult and L₃ ES antigens. Apparently there are differences in the ovine IgE responses to these species. Whether these relate to differences in the biology of the parasites is unclear. T. colubriformis dwells in the small intestine and, although Haemonchus and Teladorsagia both live in the abomasum, they have different habits. H. contortus feeds on blood whereas T. circumcincta browses the epithelium and mucosa, and may therefore induce different immune mechanisms. Alternatively, these differences may simply reflect differences in breed of sheep or infection regimes.

**Lymphocyte proliferation responses**

It is well known that the lymphocyte plays an important role in the generation of immune responses against helminth parasites. Sheep that are repeatedly infected with or immunised against H. contortus generally have lymphocytes that respond by proliferation in vitro to antigens from this parasite (Haig et al. 1989; Schallig, van Leeuwen & Hendrikx, 1994b; Schallig & van Leeuwen, 1997). Furthermore, Torgerson & Lloyd (1992, 1993) showed that lymphocytes from lambs, even animals totally naive to H. contortus, proliferate in response to soluble L₃ antigens. Riffkin & Dobson (1979) suggested that such lymphocytes might be important in the innate resistance of sheep to this parasite. Thus, naive sheep that had the highest antigen-induced lymphocyte responses, had a low susceptibility to experimental infection. In addition, it has been found that vaccinated sheep not responding with a protective immune response to H. contortus challenge infection had lower proliferation responses against the vaccine antigens compared to those that were protected (Schallig & van Leeuwen, 1997).

**Eosinophils and mast cells**

**Mast cell responses**

One of the most marked features of a gastrointestinal nematode infection is the recruitment and hyperplasia of mucosal mast cells (MMCs). Mucosal mastocytosis, including the presence of intra-epithelial globule leucocytes, is invariably associated with gastrointestinal helminthiasis (Huntley et al. 1992), suggesting that type I immediate hypersensitivity reactions are important in worm expulsion (Miller, 1984).

Earlier studies on the kinetics of ovine mast cell responses were based on histochemical detection, toluidine blue staining, and counting of these cells. Recently, antibodies against sheep mast cell protease (SMCP) have become available. This enzyme is exclusively located in the MMCs and globule leucocytes (Huntley et al. 1986). The development of an ELISA for SMCP has made it possible to measure its concentration in gastrointestinal tissues. The concentration of SMCP is correlated with the number of MMCs and globule leucocytes present in the tissues (Huntley et al. 1992).

Huntley et al. (1995) studied the mucosal mast cell responses of sheep previously maintained on pasture
and treated with anthelmintic when housed and of worm-free lambs to a mixed challenge infection with *Trichostrongylus vitrinus* and *Teladorsagia circumcincta*. Eleven days after challenge, the ewes had significantly lower worm burdens than the naive lambs, but significantly higher tissue concentrations of SMCP. Toluidine blue staining revealed significant increased numbers of MMCs in sections of the abomasum and jejenum from ewes when compared with the lambs.

Numbers of MMCs in the abomasal tissue of non-, primary- or secondary-*H. contortus* infected sheep were also studied (Schallig, van Leeuwen & Cornelissen, 1997*a*). The numbers of MMCs in non-infected sheep were low, approximately 17.5/0.2 mm\(^2\). Primary infection resulted in a significant increase to around 40/mm\(^2\). The number of toluidine blue-stained cells were significantly increased after secondary infection, 52.1/mm\(^2\), compared to the counts after primary infection, suggesting a correlation between protection and the numbers of MMCs found in the abomasum after infection.

*Eosinophils*

Circulating and tissue eosinophilia is a common feature of helminthiasis. Eosinophils have been shown to be involved *in vivo* with helminth rejection by treating mice or guinea pigs with anti-eosinophil serum or anti-interleukin 5 (IL-5) monoclonal antibodies during infections with several helminths (Rainbird, MacMillan & Meeusen, 1998). Eosinophils accumulate around the tissue of invasive *L*. *contortus* of sheep gastrointestinal parasites, including *H. contortus*, *in vivo* (Rainbird *et al*. 1998). In addition, eosinophils obtained from mammary washes of sheep were shown to immobilise and kill *H. contortus* larvae *in vitro* in the presence of antibody specific against a *L*. *contortus* surface antigen (Rainbird *et al*. 1998). The level of larval immobilization in the presence of antibody was increased when complement and IL-5 were added. Ultrastructural analysis of the eosinophil–larvae interaction at 6 h of incubation showed degranulation of adhering eosinophils onto the surface of the larvae. By 24 h of incubation, many larvae showed signs of damage and most eosinophils had degenerated. This suggests that eosinophil-mediated killing may be an effector mechanism for the elimination of *L*. *contortus* in immune sheep (Rainbird *et al*. 1998).

In contrast, little or no difference was observed in the number of circulating or tissue eosinophils in non-infected, primary or secondary infected sheep in the studies by Huntley *et al*. (1995) and Schallig *et al*. (1997*a*). This indicated that eosinophils per se do not have a direct effector function against gastrointestinal parasites, although an indirect role cannot be discounted as shown above.

cytokines

**Th\(_1\)/Th\(_2\) responses**

The immune response in sheep to gastrointestinal nematodes is thought to be mediated by CD4 + T cells generated in the mesenteric lymph nodes (Gill, Watson & Brandon, 1992*b*). Many experimental, mainly murine, models demonstrated that CD4 + T cells can often be classified into two subsets, T helper type 1 (Th\(_1\)) and Th\(_2\), based on the cytokines that they secrete (Mosmann *et al*. 1986; Mosmann & Coffman, 1989). Th\(_1\) cells produce a number of cytokines most notably interleukin 2 (IL-2), interferon-\(\gamma\) (IFN-\(\gamma\)) and tumour necrosis factor \(\beta\) (TNF-\(\beta\)) resulting in a cell mediated immune response. Th\(_2\) cells are defined by the production of IL-4, IL-5, IL-6 and IL-10 among others. A typical Th\(_2\) response is characterized by increased immunoglobulin secretion by B cells, in particular IgG\(_1\) and IgE, and proliferation of eosinophils and mast cells.

In the last 5 years significant progress has been made in the cloning and characterization of ovine cytokines (Wood & Seow, 1996), but their role in the ruminant immune response is still largely unknown. However, reverse transcriptase–polymerase chain reaction (RT–PCR) assays to study the cytokine mRNA expression have now been developed by several research groups and information on cytokine responses in the ruminant system is now becoming available.

Cytokines in nematode-infected ruminants

Canals *et al*. (1997) studied the cytokine profile induced by a primary non-protective infection with *Ostertagia ostertagi* in cattle. This infection resulted in decreased levels of IL-2 mRNA expression and increases in IL-4 and IL-10 transcription. Furthermore, a reduction in the percentages of T cells and an increase in B cells was observed. These observations are consistent with a Th\(_2\) response, but did not protect the calves against the *O. ostertagi* infection.

The cytokine mRNA expression in nematode-resistant and -susceptible line lambs artificially infected with *Trichostrongylus colubriformis* was studied by Pernthaner *et al*. (1997). Four weeks after infection mesenteric lymph node cells (MLNC) from both lines expressed high levels of mRNA coding for IL-2, IL-4 and IFN-\(\gamma\). MLNC from resistant lambs, when stimulated for 1 day with excretory/secretory antigen from adult *T. colubriformis*, had higher mRNA expression of IL-2 and IFN-\(\gamma\) and after 3 days of culture had higher levels of IL-4 mRNA than MLNC from susceptible-line lambs. This suggests, according to Pernthaner *et al*. (1997), that after an initial enhanced IFN-\(\gamma\)-mediated inflammatory response, regulatory Th\(_2\)-like
were designed using published sequences (Table 1). which served as a control in all the RT–PCR assays, aldehyde-3-phosphate dehydrogenase (GAPDH), primers for IL-2, IL-4, IL-5 and IFN- \( \gamma \) infected sheep. cDNA-specific oligo-nucleotide results obtained suggested that the primary infected the RT–PCR are summarized in Table 3. The secondary infection with cytokine expression of MLNC after a primary or used in the preliminary study presented below. summarized the optimal PCR conditions that were to confirm the identity and sequence of the amplification products. PCR conditions were optimized to facilitate the cloning of the mRNA fragments after the RT–PCR. The cloned fragments were sequenced to confirm the identity and sequence of the amplification products. PCR conditions were optimized using genomic DNA extracted from peripheral blood mononuclear cells of non-infected sheep. Table 2 summarizes the optimal PCR conditions that were used in the preliminary study presented below. The RT–PCR assays were used to analyse the cytokine expression of MLNC after a primary or secondary infection with \( H. \) contortus. The results of the RT–PCR are summarized in Table 3. The results obtained suggested that the primary infected sheep responded with a non-protective Th\(_1\) response, characterized by high levels of IL-2 and IFN-\( \gamma \) expression. In contrast, 3 out of 4 of the secondary infected sheep responded with a protective Th\(_2\) response, with no expression of IL-2 and IFN-\( \gamma \). The one animal in this group that did show expression of IL-2 and IFN-\( \gamma \) showed signs of another infection at necropsy. The fact that all animals had high levels of IL-4 and low to moderate IL-5 levels is at present difficult to explain. The preliminary data obtained in the study described above suggest that not the quantity of each individual cytokine but the ratio of the different cytokines is of importance to the final outcome of the Th response. Further studies of cytokine profiles induced by infection and/or vaccination either in the form of quantitative RT–PCR assays or cytokine ELISAs are required.

**The possible cause of unresponsiveness**

*Unresponsiveness of young lambs against infectious diseases*

Young lambs under 6 months are generally more susceptible to infectious diseases than mature sheep. This phenomenon, called unresponsiveness or hypo-responsiveness has been described for some viral diseases and various bacterial infections (Blood & Henderson, 1974; Weis, Chanana & Joel, 1986), but it is most evident for infections with gastrointestinal nematodes (Manton *et al.* 1962; Urquhart *et al.* 1966; Neilson, 1975; Dineen, Gregg & Lascelles, 1978). The lower resistance of lambs against infectious diseases in general appears to be due largely to immunological hypo-responsiveness; it is not simply a consequence of underexposure to pathogens and antigens to develop active immunity or immunosuppressive activities of the micro-organisms (Watson *et al.* 1994). The most important component of the immunological unresponsiveness of lambs seems to be constitutive. The immune system appears to progress through a maturation process

### Table 1. Oligonucleotide sequence of forward (F) and reverse (R) primers used for RT–PCR

<table>
<thead>
<tr>
<th>Product</th>
<th>Primer</th>
<th>Expected fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>F: CCGGATCC-GCTGCTGGATTTACAGTTGC</td>
<td>370</td>
</tr>
<tr>
<td></td>
<td>R: GGGGTACC-GCTGCTGGATTTACAGTTGC</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>F: GCAAGGATCC-GCCCCAAAGAACACAACTGAGAAG</td>
<td>194</td>
</tr>
<tr>
<td></td>
<td>R: GATCAGGATCC-CTTTCAGAGGAGTCTTTCAGAGTA</td>
<td></td>
</tr>
<tr>
<td>IL-5</td>
<td>F: CCGGATCC-CTCATCGAACTCTGCTGATAG</td>
<td>262</td>
</tr>
<tr>
<td></td>
<td>R: GGGGTACC-ATGCCAGTGAGCTTCCCGT</td>
<td></td>
</tr>
<tr>
<td>IFN-( \gamma )</td>
<td>F: CCGGATCC-TGGCCAGGGCCCATTTTTAAAG</td>
<td>413</td>
</tr>
<tr>
<td></td>
<td>R: GGGGTACC-ATGCCAGTGAGCTTCCCGT</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: CCGGATCC-ATGCCAGTGAGCTTCCCGT</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>R: GGGGTACC-ATGCCAGTGAGCTTCCCGT</td>
<td></td>
</tr>
</tbody>
</table>

IL, interleukin; IFN-\( \gamma \), interferon-gamma; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; oligonucleotide sequence before ‘-‘, restriction site (BamHI or KpnI in ‘F’ or ‘R’ respectively).

### Table 2. Optimal [MgCl\(_2\)] and annealing temperatures used for amplification of different cytokines

<table>
<thead>
<tr>
<th>Primer set</th>
<th>[MgCl(_2)] in mM</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>1.8</td>
<td>55</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.2</td>
<td>55</td>
</tr>
<tr>
<td>IL-5</td>
<td>2.1</td>
<td>60</td>
</tr>
<tr>
<td>IFN-( \gamma )</td>
<td>1.5</td>
<td>55</td>
</tr>
<tr>
<td>GAPDH</td>
<td>2.1</td>
<td>60</td>
</tr>
</tbody>
</table>

cytokines such as IL-4 become predominant leading to a typical Th\(_2\)-like response.

The cytokine expression profiles in sheep harbouring \( H. \) contortus infection are still mainly unknown. Réchards, van Leeuwen and Schallig (unpublished results obtained at the laboratory for Parasitology and Tropical Veterinary Medicine, Utrecht University, The Netherlands) have set up RT–PCR to study cytokine expression profiles in \( Haemonchus \) infected sheep. cDNA-specific oligo-nucleotide primers for IL-2, IL-4, IL-5 and IFN-\( \gamma \) glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which served as a control in all the RT–PCR assays, were designed using published sequences (Table 1). A BamHI or a KpnI restriction site was added to the forward or the reversed primer, respectively, to facilitate the cloning of the mRNA fragments after the RT–PCR. The cloned fragments were sequenced to confirm the identity and sequence of the amplification products. PCR conditions were optimized using genomic DNA extracted from peripheral blood mononuclear cells of non-infected sheep. Table 2 summarizes the optimal PCR conditions that were used in the preliminary study presented below.

The RT–PCR assays were used to analyse the cytokine expression of MLNC after a primary or secondary infection with \( H. \) contortus. The results of the RT–PCR are summarized in Table 3. The results obtained suggested that the primary infected
which starts during foetal life and continues during the first 12 months of life (Watson & Gill, 1991). Lambs have significantly lower proportions of CD4+ and CD8+ lymphocytes and greater numbers of γδ T cells in blood and lymph (Hein & Mackay, 1991; Watson et al. 1994). In addition, blood lymphocytes from lambs produce less γ interferon in vitro and young sheep mount, in general, smaller antibody responses than do mature animals (Colditz et al. 1996). These findings may explain, in part, why lambs are in general more susceptible to infectious diseases than adult sheep.

**Unresponsiveness against gastrointestinal nematodes**

In the case of gastrointestinal nematode infections unresponsiveness is thought to be associated also with lower numbers of CD4+ and CD8+ cells in blood and skin and lower levels of specific antibodies (Watson et al. 1994). However, in a pilot study Schallig and co-workers (unpublished results obtained at the laboratory for Parasitology and Tropical Veterinary Medicine, Utrecht University, The Netherlands) could not find significant lower levels of IgG, IgA or IgM antibodies in serum of infected lambs compared to adult sheep. In addition, Kooymann and Schallig (preliminary unpublished results obtained at the laboratory for Parasitology and Tropical Veterinary Medicine, Utrecht University, The Netherlands) compared the immune responses of lambs (3 or 6 months of age) and adult sheep (9 months of age) following vaccination with an experimental adult excretory/secretory (ES) vaccine (Schallig et al. 1997a) and subsequent challenge with *H. contortus*. The 9 or 6 month-old sheep were protected after vaccination against the challenge infection, whereas the young lambs became heavily infected. Serum antibody levels of the animals were measured and the number of eosinophils (both local and circulating) and mast cells in the mucosa of the abomasum were determined. No significant differences in antibody responses were observed between the three age groups. In contrast, peripheral blood eosinophils and mast cell counts in the abomasum were significant higher in the 9 month-old sheep compared to the young lambs. These data suggest that young lambs lack a Th2 response which is characterised by eosinophilia and mastocytosis (Finkelman et al. 1991; Urban et al. 1992; Miller, 1996). This may be due to the relatively low numbers of CD4+ in the abomasum of young lambs (Hein & Mackay, 1991; Watson et al. 1994), resulting in a low or insufficient production of IL-4, the cytokine which is probably crucial for the Th2 response. Other studies have also indicated that a reduction in CD4+ cells results in a reduction in immunity against *H. contortus* (Gill, Watson & Brandon, 1993b; Karanu et al. 1997). In addition, the abomasum of young lambs contains relatively high numbers of γδ T cells (Hein & Mackay, 1991). However, the full cytokine repertoire of these cells has not been determined. Recent studies using flow cytometry have shown that γδ T cells from mice infected with *Listeria monocytogenes* produce IFN-γ. In contrast, γδ T cells of mice infected with *Nippostrongylus brasiliensis* produce IL-4 (Ferrick et al. 1995). Furthermore, it has been reported that bovine γδ T cells stimulated with concanavalin A can express IL-2, IFN-γ and TNF-α (Wood & Seow, 1996). If these same cytokines are expressed by the γδ T cells in young lambs, a typical Th1 response would be induced which may not be sufficient to protect against gastrointestinal nematodes. This, however, remains to be supported with experimental data.

**Immunity induced by vaccination**

The increasing occurrence of anthelmintic resistance (Jackson, 1993; Waller, 1994; Borgsteede et al. 1997; van Wyk et al. 1997) has prompted the need for the development of alternative methods, vaccines, to control gastrointestinal nematodes. At present, there are several research groups trying to develop a vaccine against *H. contortus*. Such a vaccine must meet several criteria before it will be commercially
viable. First, it must be safe and efficacious, especially in young lambs. Second, it must be easy and cheap to produce. The current academic research at this moment is mainly concerned with the first item.

There are predominantly mainly two types of antigen preparations currently being evaluated: (1) natural antigens and (2) hidden antigens. Both types of antigen preparations afford varying degrees of immunity to lambs and sheep, but they also have their limitations.

**Natural antigens**

Numerous attempts have been made to induce immunity to *H. contortus* with irradiated larvae, somatic extracts of different life stages of the parasite and E/S preparations of larvae or adults all with variable results. It is out of the scope of this review to extensively discuss all these efforts in detail. Two recent examples with promising outcome are the following. Firstly, vaccination with a 70–83 kDa surface antigen of exsheathed L4 generated protection in five-month-old sheep (Jacobs *et al.* 1999). The protection induced with this natural antigen is dependent on the induction of a Th2 response (Jacobs *et al.* 1999). Secondly, Schallig *et al.* (1997a) demonstrated that immunisation with purified 15 and 24 kDa E/S products afforded significant protection against a challenge infection in older lambs and sheep. The immune mechanisms induced by vaccination with the 15/24 kDa E/S products is Th2 related and characterized by mastocytosis (Schallig *et al.* 1997a). Unfortunately, the 15/24 kDa E/S products did not provide protection to young lambs.

Both antigen preparations described above are at present obtained from living parasites. Harvesting these antigens is labour intensive and thus commercially not attractive. Recombinant expression of the putative protective antigens may facilitate their large-scale production. However, both antigen preparations contain glycosylated proteins (Ashman *et al.* 1995; Schallig *et al.* 1997b) which may hamper their production by conventional recombinant DNA technology.

**Hidden antigens**

Considerable effort has been applied to developing vaccines based on so-called hidden antigens, especially gut molecules. These antigens are normally hidden from the immune system and an immune response is not induced against these molecules during an infection. Vaccination with hidden antigen preparations has resulted in good protection against *H. contortus* infections in young lambs, older lambs, sheep and pregnant ewes (extensively reviewed by Newton & Munn, 1999). The protection induced by this type of vaccination is based on the induction of antibodies directed against these hidden antigens (Newton & Munn, 1999). The antibodies are taken up during a blood meal and probably affect the gut of the parasite (Smith, 1993). However, the level of protection obtained by this approach is dependent on the maintenance of high antibody levels over a rather long period. This must be achieved by vaccination alone, since natural boosting does not occur.

**Some concluding remarks**

The ovine immune response against *Haemonchus contortus* can be in general characterized as a typical Th2 response with high levels of serum IgE and mastocytosis. In addition, local IgA and, to a lesser extent, IgG are probably also important in protection. Furthermore, *in vitro* lymphocyte proliferation responses seem to correlate to immunity. Young lambs cannot mount a protective Th2 response, as reflected by the reduced number of mast cells and eosinophils in the abomasal mucosa. This may be due to the fact that young lambs have relatively low numbers of CD4+ cells and relatively high numbers of γδ T cells in their abomasum, resulting in a cytokine profile that is typical for a Th1 response, which is probably not sufficient to protect against gastrointestinal nematodes. In this light, studies on cytokine expression profiles in ruminants have become increasingly relevant because they may provide more insight in the possible application of (recombinant) cytokines as adjuvanting agents in vaccination experiments.

**Acknowledgements**

I would like to thank my former colleagues, in particular Marianne van Leeuwen, Frans Kooymann, Wim van der Aar and Maarten Eysker, at Utrecht University (Utrecht, The Netherlands), Dr John Huntley, Dr David Smith, Dr Dave Knox and their colleagues at Moredun Research Institute (Edinburgh, Scotland) and Dr Dante Zarlenga and his colleagues at USDA ARS (Beltsville, U.S.A.) for their valuable contributions to parts of the research described in this paper.

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