

# Immunological aspects of nematode parasite control in sheep<sup>1</sup>

J. E. Miller<sup>\*2</sup> and D. W. Horohov<sup>†</sup>

<sup>\*</sup>Department of Pathobiological Sciences, School of Veterinary Medicine, Departments of Veterinary Science and Animal Science, Louisiana State University, Baton Rouge 70803; and <sup>†</sup>Department of Veterinary Science, University of Kentucky, Lexington 40546

**ABSTRACT:** Gastrointestinal nematode parasitism is arguably the most serious constraint affecting sheep production worldwide. Economic losses are caused by decreased production, the costs of prophylaxis and treatment, and the death of the infected animals. The nematode of particular concern is *Haemonchus contortus*, which can cause severe blood loss resulting in anemia, anorexia, depression, loss of condition, and eventual death. The control of nematode parasites traditionally relies on anthelmintic treatment. The evolution of anthelmintic resistance in nematode populations threatens the success of drug treatment programs. Alternative strategies for control of nematode infections are being developed, and one approach is to take advantage of the host's natural or acquired immune responses, which can be used in selection programs to

increase the level of resistance in the population. Vaccination can also be used to stimulate or boost the host's acquired immunity. The induction of protective resistance is dependent on the pattern of cytokine gene expression induced during infection by two defined CD4<sup>+</sup> T-helper cell subsets, which have been designated as Th1 or Th2. Intracellular parasites most often invoke a Th1-type response, and helminth parasites a Th2-type response. Breeds of sheep resistant to infection have developed resistance over a much longer term of host-parasite relationship than genetically selected resistant lines. The immune components involved in these different responses and types of host-parasite relationships will be reviewed. The potential for using vaccines has been investigated, with variable results, for several decades. The few successes and potential new antigen candidates will also be reviewed.

**Key words:** immunity, nematode, control, sheep

©2006 American Society of Animal Science. All rights reserved. J. Anim. Sci. 2006. 84(E. Suppl.):E124–E132

## INTRODUCTION

Parasitism, and gastrointestinal nematode parasitism in particular, is arguably the most serious constraint affecting small ruminant production worldwide. Economic losses are caused by decreased production, cost of prevention, cost of treatment, and the death of infected animals (Barger, 1982; Donald and Waller, 1982). It is difficult by any form of major survey or other estimation to establish precise figures on losses incurred in production from infection and disease. Even minimal accuracy of loss estimates is difficult because production diseases or disorders may result from interaction with nutritional and environmental stresses, management methods, concurrent diseases, genetic predispositions, or other factors. Periodic reports on

such losses from governmental agencies and others, always range into millions of dollars per year and include all phases of production in the United States (Gibbs and Herd, 1986), New Zealand (Brunsdon, 1988), and Australia (McLeod, 1995).

Problems with nematode parasitism are often classified as production disease (i.e., chronic subclinical condition affecting productivity such as weight loss, reduced weight gain, reproductive inefficiency, etc.). Publications of the USDA-APHIS-VS provided some data on the magnitude of the problem. Sixty-two percent of 5,174 sheep producers surveyed in the United States identified stomach/intestinal nematodes as a major concern (NAHMS, 1996a). These losses were compounded in the southeastern region of the United States because climatic conditions are generally more conducive to the growth and establishment of large nematode parasite populations. Seventy-five percent of 467 sheep producers surveyed in this region identified stomach/intestinal nematodes as a major concern (NAHMS, 1996b).

The control of nematode parasites traditionally relies on grazing management, anthelmintic treatment, or both. However, grazing management schemes are often

<sup>1</sup>Invited review. Presented at the "Management of Gastrointestinal Nematodes in Sheep" symposium held at the American Society of Animal Science Annual Meeting, Cincinnati, OH, July 24–28, 2005.

<sup>2</sup>Corresponding author: jmille1@lsu.edu

Received August 11, 2005.

Accepted November 8, 2005.

impractical due to expense or to the hardiness of infective larvae on pasture. In addition, the evolution of anthelmintic resistance in nematode populations threatens the success of drug treatment programs (Craig, 1993; Prichard, 1994; Waller, 1994; Condor and Campbell, 1995; Sangster, 1999). Alternative strategies for control of nematode infections are needed. One approach is to take advantage of the host's immune system as an adjunct to current methods of control. The reduced need for drug treatment should slow the evolution of drug resistance in nematodes. Also, increasing consumer pressure makes it desirable to pursue methods of decreasing drug residues in meat and meat products. There is considerable evidence that at least part of the natural variation in resistance to nematode infection is under genetic control (Wakelin, 1978; Barger, 1989; Stear et al., 1999). Resistance is most likely based on inheritance of genes that play a primary role in expression of host immunity. Thus, the host's ability to respond to infection relies predominantly on the level of immune competence. Aspects of the immune status of the host will be discussed as it relates to control of infection in sheep.

### *Nematodes Involved*

Nematodes common in the abomasum are *Haemonchus contortus* (Barber pole worm) and *Teladorsagia circumcincta* (Brown stomach worm). Nematodes common in the small intestine are *Trichostrongylus colubriformis* (Bankrupt worm), *Cooperia* spp. (Cooper's worm), and *Nematodirus* spp. (Threadneck worm). The nematode common in the large intestine is *Oesophagostomum* spp. (Nodular worm). The nematode of particular concern worldwide especially in tropical and subtropical climatic regions is *H. contortus*. In the United States, this would include the Southeast and other areas where it is hot and humid during the summer. The tremendous egg-laying capacity of this nematode is maintained by adults feeding on blood. It should be noted that late-stage immature larvae also feed on blood and this combined with adult feeding can result in severe blood loss, leading to anemia, anorexia, depression, loss of condition, and eventual death. The nematode of particular concern in temperate climatic regions is *T. circumcincta*. In the United States, this would include most of the country outside the Southeast. The larvae of this nematode enter gastric glands of the abomasum, resulting in 1) decreased acid production due to loss of parietal cell function; 2) absence of proteolytic pepsin because, as stomach pH rises, pepsinogen is not activated; and 3) movement of serum protein, particularly albumin, and sodium from circulating blood into the lumen of the abomasum, thus leading to fluid dumping and diarrhea. Larvae and adults of other species contribute to pathology by causing mucosal irritation, which further interferes with nutrient absorption. In the absence of death, all nematodes together contribute to "production disease," which affects the animal's abil-

ity to increase or maintain its production (i.e., weight, reproduction, and so on).

### *Host/Nematode Interaction*

To complete their life cycle, nematode parasites have to develop and lay eggs in the host. The host becomes infected by consuming infective third-stage larvae during grazing. After ingestion, the larvae lose their protective sheath and invade the mucosa of the abomasum, small intestine, or large intestine depending on which nematode is involved. While in the mucosa, larvae develop to the fourth larval stage and then return to the surface of the gut mucosa where they become adult worms. The major host defense mechanism is immunity. When infectious agents enter the body, the immune system reacts through a series of activities that mobilize various components (e.g., antibodies, lymphocytes, mast cells, eosinophils), which then attack and eliminate the invaders. These components act on the larval stages in the mucosa and adults in the lumen. The immune system matures with age; therefore, young animals are most susceptible to infection and become more resistant with age. Moreover, young animals usually harbor the heaviest infection levels and suffer the most severe consequences. Adult animals have developed stronger immunity and usually harbor lower infection levels.

One way in which infection level is measured is by quantifying the number of eggs being passed in the feces. Relatively high and low fecal egg counts (**FEC**) are usually seen in young and adult animals, respectively. Young animals are also more subject to clinical disease when signs of infection (diarrhea, rough hair coat, anemia, weight loss, bottle jaw, etc.) are seen. In older animals, infection becomes more subclinical in which the only subtle sign may be weight loss. However, under poor nutrition or stressful conditions, the immune system is compromised and cannot respond adequately. Therefore, older animals can become infected when signs of clinical disease are present.

The prepatent period (time from ingestion of infective larvae to mature egg-laying adults) of most nematodes is about 3 wk, but this period can be extended for those that have the capability to enter a period of delayed development called hypobiosis, where fourth-stage larvae stop development and remain in the mucosa for an extended period of 3 to 4 mo. There is no apparent host response to these hypobiotic larvae. Hypobiosis usually occurs when there is insufficient moisture in the environment or temperatures are too cold for pasture larval development and survival. This can be in summer or winter depending on the nematode and geographical location. What triggers induction of hypobiosis and emergence is not well understood and probably involves some immune and environmental cues.

Infection is normal in sheep and the number of nematodes found in individual animals varies with essentially all animals having some nematodes. It is rare to

find animals uninfected. The nematode population in a flock is generally overdispersed; that is, a majority of the nematodes are in a minority of the animals. So, there is a range of host response to infection that controls what happens to the nematodes during the host phase of their life cycle. Most infections result in minimal damage unless conditions change that alter the ability of the host to maintain control; and then damage can become more severe, possibly leading to death depending on the nematode species present.

A number of factors might alter this balance and all have some effect on immunity (innate and acquired), which is the predominant mechanism of host resistance to infection. Innate immunity is when infection is limited during the initial exposure to infection. There may be an immune component involved, but the mechanism is not well understood. Some breeds that have been managed by survival of the fittest without or with minimal chemical intervention seem to demonstrate resistance to infection during the neonatal period (Gamble and Zajac, 1992; Bahirathan et al., 1996) whereas more intensely managed domestic breeds do not. It may be that these resistant breeds are able to mount an effective immune response much faster, as immune competence is usually not acquired until >4 mo of age depending on the breed and nematode species (Barger, 1988).

Two well-recognized (and thought to be immune-mediated) host responses to infection are immune exclusion and the self-cure phenomenon. In immune exclusion, ingested larvae fail to establish in heavily infected animals (Miller et al., 1983; Newlands et al., 1990). If the infection is removed by deworming, then ingested larvae will become established. Immune mediation is suggested in that corticosteroid treatment will result in larval establishment in heavy infections, thus abrogating exclusion. The self-cure phenomenon occurs when established adult nematodes are spontaneously expelled when there is a massive larval invasion over a very short exposure period (Stewart, 1955; Adams, 1983; Hong et al., 1989). This is usually observed after heavy rain that liberates large numbers of sequestered infective larvae from feces. This larval invasion results in an increase in abomasal pH and thus nematode expulsion. It is also thought that immunoglobulin E (IgE)-mediated hypersensitivity may be involved.

The effects of nematode infection can be influenced by the nutritional status of the host (Knox et al., 2003). It is well known that well-fed animals can better withstand infection than animals on an inadequate diet. It is also true that nematodes interfere with the ability of the host to utilize nutrients efficiently. Therefore, it is important to understand this seesaw effect. The better an animal is fed, the better it is able to tolerate increasing infection levels, but eventually a point may be reached, depending on the nematode species and conditions involved, in which infection overwhelms the host's ability to function properly. To satisfy body demands, most nutrients are absorbed from the gut dur-

ing digestion and additional nutrients are available as needed from body reserves. The term "nutrient partitioning" refers to the process of directing the flow of nutrients to where they are most needed at the current time. Depending on the host's age and sex, season of the year, and exposure to various potential infectious (parasitic and otherwise) agents, nutrients are partitioned for growth, breeding, pregnancy, lactation, immunity, and so on. The ability of the host to maintain a proper balance of this partitioning ensures that nutrients are used appropriately. For example, as nematode infection increases, more damage is done to the mucosa, which will result in reduced absorption of nutrients, thus making the host utilize more stored body reserves. In addition, proteins are the building blocks of the host's immune system. As proteins are made less available, the host's immune function is compromised and it becomes more susceptible to subsequent infection. Overall, the net result of inadequate feeding, for the conditions encountered, will be loss of productivity unless the balance is restored.

### *Immune Response to Infection*

The induction of protective resistance or exacerbation of diseases associated with infectious agents is dependent on the pattern of cytokine gene expression induced during infection (Urban et al., 1991; Sher et al., 1992). In murine models the existence of two defined CD4<sup>+</sup> T-helper cell subsets has been based on 2 distinct patterns of cytokine gene expression. These have been designated Th1 or Th2 (Mossman and Coffman, 1989). Cytokines usually produced by the Th1 subset include IL-2, interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor, and by the Th2 subset include IL-4, IL-5, IL-9, IL-10, and IL-13 (Urban et al., 1996; Romagnani, 1999). Although both subsets of cells may be induced during infection, it has been shown by measuring cytokines in lymphocyte culture supernatant or cytokine mRNA as an indicator of gene expression, that one or the other population often dominates a given response. Intracellular parasites most often invoke a Th1-type response and helminth parasites a Th2-type response.

Although a Th2 pattern of cytokine responses is dominant in mice resistant to nematode infection, the roles of specific cytokines and effector mechanisms involved is less clear and results vary considerably with other host-parasite systems examined (Urban et al., 1992). The following examples, although incomplete, demonstrate the diversity of results reported from murine systems. Protective resistance of mice against *Heligmosomoides polygyrus* is abrogated by treatment with anti-IL-4 or anti-IL-4 receptor antibodies (Urban et al., 1991). However, antibodies to IL-3, IL-4, and IL-5, which eliminate mast cell hyperplasia, eosinophilia, and IgE have no effect on the primary course of infection of *Nippostrongylus brasiliensis* (Coffman et al., 1989; Madden et al., 1991; Finkelman et al., 1994). In other studies anti-IL-5 treatment, which abrogates eosino-

philia, does not alter acquired protective resistance to *Trichinella spiralis* (Herndon and Kayes, 1992) or *Toxocara canis* (Parsons et al., 1993) infections but does eliminate protection against migratory larvae of *Strongylus venezuelensis* (Korenaga et al., 1991) and *Angiostrongylus cantonensis* (Sasaki et al., 1993). In studies on vaccination against *Strongylus vulgaris* in ponies, Th2 cytokines IL-4 and IL-5 were elevated and Th1 cytokines IL-2 and IFN- $\gamma$  were decreased in peripheral blood mononuclear cells and cecal-colonic lymph node cells following challenge infections in immunized protected ponies suggesting that the Th2 response is important in protective immunity (Swiderski et al., 1999).

The role that immunity plays in primary and secondary challenge infection in sheep has been evaluated for the most part in within-breed model systems. Characteristic immune response to nematode infection is an accumulation of mucosal mast cells (MMC) and eosinophils in the gastrointestinal tract (Rothwell, 1989). In rodents, the MMC accumulation is antigen dependent and driven by the T-cell-derived IL-3 (Haig et al., 1982; Paramentier et al., 1982). These MMC undergo changes to globule leukocytes during infection (Huntley et al., 1984).

Mature sheep made resistant by repeated infection, sheep lines selected for resistance, and resistant breeds have demonstrated high numbers of globule leukocytes (Gregg et al., 1978; Gamble and Zajac, 1992; Gill et al., 1993). There is a temporal correlation between MMC hyperplasia and expulsion of nematodes in rodents, which might indicate a cause and effect relationship (Woodbury et al., 1984). The regulation of MMC is not well understood. Murine mast cell development is dependent on IL-3, IL-4, IL-9, and IL-10 (Thompson-Snipes et al., 1991). These factors are products of immune T-lymphocytes in mice (Mosmann and Coffman, 1989). A review by Cox and Liew (1992) indicated that IL-4 and IFN- $\gamma$  are the 2 cytokines that play a major role in antibody-mediated immunity dependent on Th2 cells. Local production of cytokines by these lymphocytes could account for differences in susceptibility to nematode parasitism if MMC activity plays a role in protection (Tepper et al., 1990).

It appears that the protective response generated by Th2 T cells involves many redundant interactive mechanisms, which are yet to be clearly defined. Nonetheless, factors that promote the generation of a Th2 response appear to be central to the induction of protective resistance against gastrointestinal nematodes in many studies. However, recent experiments have indicated that primary *Ostertagia ostertagi* infection in cattle does not subscribe totally to the classic Th1/Th2 paradigm (Almeria et al., 1997a,b; Canals et al., 1997). In these studies involving primary infection, mRNA level was decreased for IL-2 and increased for IL-4, IL-10, and IFN- $\gamma$ . In a subsequent study, primary infection followed by challenge infection showed that mRNA level was decreased for these cytokines, which indicated that animals exhibiting protection from reinfection with *O.*

*ostertagi* do not show a shift to greater percentages of immunoglobulin-positive cells characteristic of a primary infection (Almeria et al., 1998). It should be noted that these cattle studies did not address resistant vs. susceptible lines or breeds and only describe the response to *O. ostertagi* infection. No studies have compared resistant vs. susceptible lines or breeds of cattle.

Immune suppression with dexamethasone resulted in reversion of *H. contortus* resistance to susceptibility in a genetically selected resistant line of Merino sheep (Presson et al., 1988), and the conclusion was that such selected resistance was immunologically mediated. Anti-CD4<sup>+</sup> T-cell monoclonal antibodies abrogated resistance to *H. contortus* infection in that same line of sheep and anti-CD8<sup>+</sup> T-cell monoclonal antibodies had no effect (Gill et al., 1993). Thus, CD4<sup>+</sup> T cells were implicated as being important in that selected line of resistant sheep. Expression of mRNA coding for IL-2, IL-4, and IFN- $\gamma$  was observed in mesenteric lymph node cells from a mixed nematode infection (predominantly, small intestinal nematodes) in both a resistant and a susceptible line of lambs (Pernthaner et al., 1997). Mesenteric lymph node cells from the resistant lambs had greater mRNA expression of IL-2 and IFN- $\gamma$  after 1 d of culture and greater levels of IL-4 after 3 d than susceptible lambs. After depletion of CD4<sup>+</sup> T-cells, lambs vaccinated with gut antigens were not protected; therefore, the immune response to vaccination was CD4<sup>+</sup> T-cell dependent (Karanu et al., 1997). More recently, Gill et al. (2000) and Pernthaner et al. (2005) provided some further insight into the Th1/Th2 dichotomy in sheep. Gill et al. (2000), using *H. contortus* in a Merino within-breed model, showed that, for both abomasal and mesenteric lymph node lymphocytes, IFN- $\gamma$  production was decreased and IL-5 production was increased in the genetically selected line compared with the nonselected line at 5 and 28 d postinfection. Pernthaner et al. (2005), using *T. colubriformis* in a Romney within-breed model, showed that expression of IL-5, IL-13, and TNF- $\alpha$ , but not IL-4, IL-10, or IFN- $\gamma$ , genes increased in intestinal lymph cells of the genetically selected line compared with the nonselected line during a 21-d period after infection. These studies provide evidence that protection is driven predominantly by a Th2-type response.

Naturally selected resistant breeds of sheep have developed resistance over a much longer host-parasite relationship term (decades and perhaps centuries) than short-term (few years) genetically selected resistant lines within breed. As addressed above (Presson et al., 1988; Gill et al., 1993, 2000), resistance in genetically selected resistant lines within breed appears to have an acquired immune component; however, the question of whether naturally selected breed resistance has acquired or innate (nonimmune) components has yet to be fully elucidated. The suggestion of an immunologically mediated component of resistance in Gulf Coast Native lambs has been reported (Peña, 2001; Peña et al., 2004). The effect of dexamethasone suppression was evaluated

on immune response and infection level for both newborn lambs and lambs at 4 mo of age. Both groups were vaccinated with *Brucella abortus* antigen to evaluate immune response to an antigen not seen before. The neonatal lambs grazed to acquire infection and the 4-mo-old lambs were dewormed after weaning and given experimental trickle infections. In both studies, the immune response was suppressed as indicated by significant reduction of lymphocytes and essentially no immunoglobulin response to the *B. abortus* antigen and a significant reduction in response to an *H. contortus* whole-worm antigen. Fecal egg counts and nematode counts were significantly increased in suppressed lambs compared with normal lambs, indicating that they became more susceptible. To further support that an immunological component is involved in resistance, Gulf Coast Native lambs were depleted of their circulating CD4<sup>+</sup> T-lymphocyte population using anti-CD4<sup>+</sup> monoclonal antibodies (our unpublished data). Subsequent to CD4<sup>+</sup> T-lymphocyte depletion, worm-free 6-mo-old lambs were experimentally challenged with *H. contortus*. Depleted lambs had significantly greater FEC and nematode counts. Therefore, an immunological component appeared to be involved in resistance. Both dexamethasone suppression and CD4<sup>+</sup> T-lymphocyte depletion have indicated that resistance can be abrogated in Gulf Coast Native sheep, suggesting that there is an acquired immune component.

Gamble and Zajac (1992), comparing St. Croix (resistant) and Dorset (susceptible) breeds of sheep, demonstrated that resistance appeared in St. Croix lambs early in life (8 wk of age) during challenge infection after priming infection but not during the priming infection, but they did not evaluate any immune response. St. Croix lambs maintained their resistance throughout the summer grazing season and immune responses were evaluated under this natural infection condition. There were no consistent differences between breeds for most of the immune response parameters measured (lymphoproliferation on peripheral blood mononuclear cells using phorbol and concanavalin A, *H. contortus* antigen-specific serologic ELISA, larval migration inhibition using abomasal mucus, and abomasal mucosal mast cells and eosinophils), but the trend was for St. Croix lambs to exhibit greater (sometimes significantly) responses than Dorset lambs. One consistent and significant difference was greater levels of abomasal mucosa globule leukocytes in St. Croix lambs.

Another model system compared resistant Santa Ines sheep with more susceptible Ile de France and Suffolk sheep (Amarante et al., 2005). No clear significant differences in mast cells, globule leukocytes, eosinophils, or immunoglobulin A between breeds were observed, further complicating what components may be involved.

Some immunologically induced local response mechanisms may affect rejection of nematodes. These responses are acquired because they occur after exposure. Intestinal mucus has been proposed to trap infective

larvae preventing their establishment (Rothwell, 1989). An increase in goblet cells occurs with immunity to *T. colubriformis*, and these cells secrete mucus. This may be part of immune exclusion. Nematodes (adult and larvae) may also be moved along the gut more rapidly with increased peristalsis due to local smooth muscle hypertrophy and hypercontractility, which appears to occur in immune animals (Tremain and Emery, 1994). Immune sheep may lose small intestinal epithelium during infection, helping to dislodge and expel larvae, which do not embed readily (McClure et al., 1992). Finally, immune mediators such as tissue mast cell protease damage cells leading to increased permeability and fluid dumping into the lumen (Miller, 1996). This may expose nematodes to host protective elements and excess fluid may act to facilitate worm expulsion along with peristalsis.

The ultimate objective of establishing mechanisms of host immunity (resistance) is to help find ways to augment control of infection in light of the anthelmintic resistance problem occurring worldwide. What is learned may help in development of improved interventions such as genetic marker-assisted selection and vaccines.

### Marker-Assisted Selection

Although replacement of a susceptible breed with a resistant breed is one method of genetically improving resistance to nematode parasites, selection for resistant animals within a breed is also viable (Gasbarre and Miller, 2000). Heritabilities for infection parameters such as FEC range from 0.22 to 0.63, which indicate that selection for resistance or selection against susceptibility using some measurement of FEC for nematode burden will be moderately successful. Likewise, the search for genetic markers can be concentrated in regions of the genome where immune components identified as being involved with resistance are located. Because nematode resistance is a quantitative trait, QTL methods are necessary for detection of these markers. In the QTL approach, markers spanning the genome are systematically tested for association with the trait of interest, in this case nematode resistance. Large pedigrees segregating for nematode burden are needed and marker-assisted selection is not possible until QTL are precisely mapped. Microsatellite markers are usually used for these analyses because of their extensive genetic variability. In these studies, reference families are genotyped with the markers and linkage between the markers and the trait locus is evaluated within families. Significant linkage between the markers and the trait positions the QTL onto a specific region within the genome. Refinement of the region continues with the addition of new markers or animals. Positional candidates for the trait of interest (e.g., nematode resistance) can be inferred based on their proximity to the linked markers. In addition, selection for animals with superior nematode resistance can be performed using

markers mapped to the appropriate genetic region. Because of the significant economic importance of nematode resistance in sheep, identification of QTL and subsequent development of markers suitable for genetic selection is generating a strong research effort. To date, 4 populations suitable for these QTL searches have been constructed. The Commonwealth Scientific and Industrial Research Organization (Australia) developed lines of Merino sheep selected over several generations for high and low resistance to *T. colubriformis* and *H. contortus* (Beh and Maddox, 1996). A genome-wide scan was conducted by genotyping animals in the pedigrees with microsatellite markers and testing for linkage between the markers and FEC. Quantitative trait loci associated with *T. colubriformis* resistance were reported on chromosomes 1, 3, 6, 22, and 23, and QTL associated with resistance to *H. contortus* were identified on chromosomes 1, 3, 6, 14, and 23 (Beh et al., 1998). AgResearch (New Zealand) has established a selection line of Romney sheep for resistance to natural field challenge measured by FEC (Crawford et al., 1997). Preliminary results are suggestive of QTL on ovine chromosomes 1 and 3 (Crawford and McEwan, 1998). A putative QTL of large effect was identified on the long (q) arm of chromosome 3, similar to the results of Beh et al. (1998). The most likely candidate gene in this region is IFN- $\gamma$ , which has been mapped to the region 3q23 by in situ hybridization (Goldhammer et al., 1996) and is a cytokine that affects the immune response to nematode infection (Wakelin, 1996). Based on these results, a more detailed scan of the long arm of chromosome 3 was conducted using markers spaced at approximately 4-cM intervals. Four markers located within 2 cM of the IFN- $\gamma$  gene, including a microsatellite located in the first intron, showed the strongest association with resistance (A. M. Crawford, University of Otago, Dunedin, New Zealand, personal communication). A provisional patent describing these findings has been filed in New Zealand (Crawford and McEwan, 1998). The association between the IFN region and nematode resistance was confirmed in feral Soay sheep (Coltman et al., 2001). Fecal egg count was significantly associated with the 126-bp allele of the *o*(IFN)- $\gamma$  microsatellite but not with alleles at the flanking markers, suggesting that the QTL for reduced FEC is segregating near the IFN- $\gamma$  gene in these animals. These results independently verify the results of Crawford and McEwan (1998). A reference family population for QTL mapping of parasite resistance has been generated at Louisiana State University using Gulf Coast Native (resistant) and Suffolk (susceptible) sheep as parental animals for subsequent F<sub>1</sub> and F<sub>2</sub> generations. Infection, based on FEC, segregated nicely and subsequent genotyping and genetic linkage analysis demonstrated preliminary QTL on chromosomes 1, 3, and 19 (Cockett et al., 2005) with the most significant results on chromosomes 1 and 19. It is expected that these efforts will eventually identify specific genetic markers that can be used for marker-assisted selection of resistant animals.

## Vaccines

Because of anthelmintic resistance, efforts have increased in recent years to develop functional vaccines. This has been made possible by newer technologies in gene discovery and antigen identification, characterization, and production. Successful vaccines have been developed for lungworms in cattle and tapeworms in sheep. Also, irradiated larval vaccines have been shown to be quite effective in older sheep but not that effective in younger lambs (Urquhart et al., 1966; Smith and Angus, 1980). The most promising vaccine for *H. contortus* involves "hidden gut" antigens (Munn et al., 1993; Smith, 1999; Knox et al., 2003). These antigens are derived from the gut of the nematode and when administered to the animal, antibodies are made. These antibodies are ingested along with blood during feeding. Ingested antibodies bind to target proteins on intestinal cells, thus disrupting digestive processes, which lead to starvation and weakness. The worm is then removed from the gut by peristalsis. This vaccine has been tested successfully in sheep under experimental conditions and has had limited success under field conditions (Kabagambe et al., 2000; Smith et al., 2001). Reasons for this are unclear. Effect of this vaccine on *H. contortus* in goats has not been evaluated. The one drawback to this vaccine is that the antigens are normally hidden from the host and a number of vaccinations may be required to maintain antibody levels high enough to combat infection, which may be quite expensive. In addition, large numbers of adult nematodes are necessary to extract limited amounts of natural antigen; therefore, this will only be practical when methods are derived to artificially make the antigen through recombinant technology so that it can be mass-produced at a lower cost. Vaccines for other nematodes that do not feed on blood have largely focused on using antigens found in worm somatic tissue and secretory/excretory products (Griffiths and Prichard, 1994; Schallig and Van Leeuwen, 1997; Emery et al., 1999; Alunda et al., 2003). These antigens are usually recognized by the host after infection; thus, vaccine immunity should be obtained by natural exposure. The problem of reproducing any of these protective effects with recombinant versions of the antigens remains the single largest obstacle to progress.

## IMPLICATIONS

For the last few decades, nematode parasites have been controlled almost exclusively with anthelmintic agents. The fact that some of these nematodes have progressively developed resistance now severely limits the ability of anthelmintic agents to control infection; thus, animal productivity is compromised. Therefore, investigating all possible control methods, including integrated approaches, is a continuing effort. The genetics of resistance, which has an underlying immune compo-

ment, might be useful in finding and using genetic markers for marker-assisted selection, and thus, could be used in programs that breed for resistance. On the other hand, the more that is known about the host's specific immune responses, the better we will be able to explore potential vaccines.

## LITERATURE CITED

- Adams, D. B. 1983. Observations on the self-cure reaction and other forms of immunological responsiveness against *Haemonchus contortus* in sheep. *Int. J. Parasitol.* 13:571–578.
- Almeria, S., A. Canals, M. T. Gomez-Munoz, D. S. Zarlenga, and L. C. Gasbarre. 1998. Characterization of protective immune responses in local lymphoid tissues after drug-attenuated infections with *Ostertagia ostertagi* in calves. *Vet. Parasitol.* 80:53–64.
- Almeria, S., A. Canals, D. S. Zarlenga, and L. C. Gasbarre. 1997a. Isolation and phenotypic characterization of abomasal mucosal lymphocytes in the course of a primary *Ostertagia ostertagi* infection in calves. *Vet. Immunol. Immunopathol.* 57:87–98.
- Almeria, S., A. Canals, D. S. Zarlenga, and L. C. Gasbarre. 1997b. Quantification of cytokine gene expression in lamina propria lymphocytes of cattle following infection with *Ostertagia ostertagi*. *J. Parasitol.* 83:1051–1055.
- Alunda, J. M., F. Angulo-Cubillan, and M. Cuquerrella. 2003. Immunization against ovine haemonchosis with three low molecular weight somatic antigens of adult *Haemonchus contortus*. *J. Vet. Med. B Infect. Dis. Vet. Public Health* 50:70–74.
- Amarante, A. F. T., P. A. Bricarello, J. F. Huntley, L. P. Mazzolin, and J. C. Gomes. 2005. Relationship of abomasal histology and parasite-specific immunoglobulin A with the resistance to *Haemonchus contortus* infection in three breeds of sheep. *Vet. Parasitol.* 128:99–107.
- Bahirathan, M., J. E. Miller, S. R. Barras, and M. T. Kearney. 1996. Susceptibility of Suffolk and Gulf Coast Native suckling lambs to naturally acquired strongylate nematode infections. *Vet. Parasitol.* 65:259–268.
- Barger, I. A. 1982. Helminth parasites and animal production. Page 133 in *Biology and Control of Endoparasites*. L. E. A. Symons, A. D. Donald, and J. K. Dineen, ed. Academic Press, New York, NY.
- Barger, I. A. 1988. Resistance of young lambs to *Haemonchus contortus* infections and its loss following anthelmintic treatment. *Int. J. Parasitol.* 18:1107–1109.
- Barger, I. A. 1989. Genetic resistance of hosts and its influence on epidemiology. *Vet. Parasitol.* 32:21–35.
- Beh, K. J., M. J. Callaghan, D. J. Hulme, Z. Leish, K. DiIenno, and L. Lenane. 1998. A search for genes affecting gastrointestinal parasite resistance in sheep. Pages 102–103 in *Proc. 26th Int. Conf. Anim. Genet. Int. Soc. Anim. Genet.*, Auckland, New Zealand.
- Beh, K. J., and J. F. Maddox. 1996. Prospects for development of genetic markers for resistance to gastrointestinal parasite infection in sheep. *Int. J. Parasitol.* 26:879–897.
- Brunsdon, R. V. 1988. The economic impact of nematode infection in sheep: Implications for future research and control. Page 4 in *The Economic Importance of Parasites of Livestock in New Zealand*. A. C. G. Heath, ed. N.Z. Soc. Parasitol.
- Canals, A., D. S. Zarlenga, S. Almeria, and L. C. Gasbarre. 1997. Cytokine profile induced by a primary infection with *Ostertagia ostertagi* in cattle. *Vet. Immunol. Immunopathol.* 58:63–75.
- Cockett, N., S. Bishop, G. Davies, T. Hadfield, S. Eng, and J. Miller. 2005. Use of QTL to determine parasite resistance in sheep. *J. Anim. Sci.* 83(Suppl. 1):128. (Abstr.)
- Coffman, R. L., B. W. P. Seymour, S. Hudak, J. Jackson, and D. Rennick. 1989. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245:308–310.
- Coltman, D. W., K. Wilson, J. G. Pilkington, M. J. Stear, and J. M. Pemberton. 2001. A microsatellite polymorphism in the gamma interferon gene is associated with resistance to gastrointestinal nematodes in a naturally parasitized population of Soay sheep. *Parasitology* 122:571–582.
- Condor, G. A., and W. C. Campbell. 1995. Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. *Adv. Parasitol.* 35:1–84.
- Cox, F. E. G., and F. Y. Liew. 1992. T-cell subsets and cytokines in parasitic infections. *Parasitol. Today* 8:371–374.
- Craig, T. M. 1993. Anthelmintic resistance. *Vet. Parasitol.* 46:121–131.
- Crawford, A. M., and J. C. McEwan. 1998. Identification of animals resistant to nematode parasite infection. New Zealand Provisional Patent 330201, Ovita, New Zealand.
- Crawford, A. M., S. H. Phua, J. C. McEwan, K. G. Dodds, C. S. Wright, C. A. Morris, S. A. Bisset, and R. S. Green. 1997. Finding disease resistance QTL in sheep. *Anim. Biotechnol.* 8:13–22.
- Donald, A. D., and P. J. Waller. 1982. Problems and prospects in the control of helminthiasis in sheep. Page 157 in *Biology and Control of Endoparasites*. L. E. A. Symons, A. D. Donald, and J. K. Dineen, ed. Academic Press, New York, NY.
- Emery, D. L., S. J. McClure, R. J. Davey, and T. Bendissen. 1999. Induction of protective immunity to *Trichostrongylus colubriformis* in neonatal Merino lambs. *Int. J. Parasitol.* 29:1037–1046.
- Finkelman, F. D., K. B. Madden, A. W. Cheever, I. M. Kotona, S. C. Morris, M. K. Gately, B. R. Hubbard, W. C. Gause, and J. F. Urban. 1994. Effects of IL-12 on immune responses and host protection in mice infected with intestinal nematode parasites. *J. Exp. Med.* 179:1563–1572.
- Gamble, H. R., and A. M. Zajac. 1992. Resistance of St. Croix lambs to *Haemonchus contortus* in experimentally and naturally acquired infections. *Vet. Parasitol.* 41:211–225.
- Gasbarre, L. C., and J. E. Miller. 2000. Genetics of helminth resistance. Page 129 in *Breeding for Disease Resistance in Farm Animals*. 2nd ed. R. F. E. Axford, S. C. Bishop, F. W. Nicholas, J. B. Owen, ed. CABI Publishing, New York, NY.
- Gibbs, H. C., and R. P. Herd. 1986. Nematodiasis in cattle: Importance, species involved, immunity, and resistance. *Vet. Clin. North Am. Food Anim. Pract.* 2:211–224.
- Gill, H. S., K. Altmann, M. L. Cross, and A. J. Husband. 2000. Induction of T helper 1- and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology* 99:458–463.
- Gill, H. S., D. L. Watson, and M. R. Brandon. 1993. Monoclonal antibody to CD4+ T-cells abrogates genetic resistance to *Haemonchus contortus* in sheep. *Immunology* 78:43–49.
- Goldhammer, T., R. M. Brunner, P. Schmidt, and M. Schwerin. 1996. Mapping of the interferon gamma gene (IFNG) to chromosomes 3 in sheep and 5 in goat by FISH. *Mamm. Genome* 7:470–471.
- Gregg, P., J. K. Dineen, T. L. W. Rothwell, and J. D. Kelly. 1978. The effect of age on the response of sheep to vaccination with irradiated *Trichostrongylus colubriformis* larvae. *Vet. Parasitol.* 4:35–48.
- Griffiths, G., and D. I. Prichard. 1994. Vaccination against gastrointestinal nematodes of sheep using purified secretory acetylcholinesterase from *Trichostrongylus colubriformis* – An initial pilot study. *Parasite Immunol.* 16:507–510.
- Haig, D. M., T. A. McKee, E. E. Jarrett, R. Woodbury, and H. R. P. Miller. 1982. Generation of mucosal mast cells is stimulated in vitro by factors derived from T-cells of helminth infected rats. *Nature* 300:188–190.
- Herndon, F. J., and S. G. Kayes. 1992. Depletion of eosinophils by anti-IL-5 monoclonal antibody treatment of mice infected with *Trichinella spiralis* does not alter parasite burden or immunologic resistance to reinfection. *J. Immunol.* 149:3642–3647.
- Hong, C., J. F. Michel, and M. B. Lancaster. 1989. Development of protection and the self-cure in lambs infected daily with *Ostertagia circumcincta*. *Vet. Parasitol.* 31:125–131.

- Huntley, J. F., G. Newlands, and H. R. P. Miller. 1984. The isolation and characterization of globule leukocytes: Their derivation from mucosal mast cells in parasitized sheep. *Parasite Immunol.* 6:371–390.
- Kabagambe, E. K., S. R. Barras, Y. Li, M. T. Pena, W. D. Smith, and J. E. Miller. 2000. Attempts to control haemonchosis in grazing ewes by vaccination with gut membrane proteins of the parasite. *Vet. Parasitol.* 92:15–23.
- Karanu, F. N., T. McGuire, W. C. Davis, T. E. Besser, and D. P. Jasmer. 1997. CD4+ T lymphocytes contribute to protective immunity induced in sheep and goats by *Haemonchus contortus* guts antigens. *Parasite Immunol.* 19:435–445.
- Knox, D. P., D. L. Redmond, G. F. Newlands, P. J. Skuce, D. Pettit, and W. D. Smith. 2003. The nature and prospects for gut membrane proteins as vaccine candidates for *Haemonchus contortus* and other ruminant trichostrongyloids. *Int. J. Parasitol.* 33:1129–1137.
- Knox, M. R., J. W. Steel, C. A. Anderson, and L. L. Muir, ed. 2003. Nutrition-parasite interactions in sheep. *Aust. J. Exp. Agric. (Special Issue)* 43:1383–1488.
- Korenaga, M., Y. Hitoshi, N. Yamagouchi, Y. Sato, K. Fakatsu, and I. Tada. 1991. The role of IL-5 in protective immunity to *Strongyloides venezuelensis* infection in mice. *Immunology* 72:502–507.
- Madden, K. B., J. F. Urban, J. H. J. Ziltener, J. W. Schrader, F. D. Finkelman, and I. M. Kotona. 1991. Antibodies to IL-3 and IL-4 suppress helminth induced intestinal mastocytes. *J. Immunol.* 147:1387–1391.
- McClure, S. J., D. L. Emery, B. M. Wagland, and W. O. Jones. 1992. A serial study of rejection of *Trichostrongylus colubriformis* by immune sheep. *Int. J. Parasitol.* 22:227–234.
- McLeod, R. S. 1995. Costs of major parasites to the Australian livestock industries. *Int. J. Parasitol.* 25:1363–1367.
- Miller, H. R., F. Jackson, G. Newlands, and W. T. Appleyard. 1983. Immune exclusion, a mechanism of protection against the ovine nematode *Haemonchus contortus*. *Res. Vet. Sci.* 35:357–363.
- Miller, H. R. P. 1996. Mucosal mast cells and the allergic response against nematode parasites. *Vet. Immunol. Immunoparasitol.* 54:331–336.
- Mosmann, T. R., and R. L. Coffman. 1989. TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145–173.
- Munn, E. A., T. S. Smith, M. Graham, A. S. Tavernor, and C. A. Greenwood. 1993. The potential value of integral membrane proteins in the vaccination of lambs against *Haemonchus contortus*. *Int. J. Parasitol.* 23:261–269.
- NAHMS. 1996a. Reference of 1996 U.S. sheep health and management practices. USDA APHIS VS, Fort Collins, CO.
- NAHMS. 1996b. Reference of U.S. regional sheep health and management practices. USDA APHIS VS, Fort Collins, CO.
- Newlands, G. F., H. R. Miller, and F. Jackson. 1990. Immune exclusion of *Haemonchus contortus* larvae in the sheep: Effects on gastric mucin of immunization, larval challenge and treatment with dexamethasone. *J. Comp. Pathol.* 102:433–442.
- Paramentier, H. K., E. J. Ruitenbergh, and A. Elgersma. 1982. Thymus dependence of the adoptive transfer of intestinal mastocytopenia in *Trichinella spiralis* infected mice. *Int. Arch. Allergy Appl. Immunol.* 68:260–267.
- Parsons, J. C., R. L. Coffman, and R. R. Grieve. 1993. Antibody to IL-5 prevents blood and tissue eosinophilia but not liver trapping in murine larvae toxocanosis. *Parasite Immunol.* 15:501–508.
- Peña, M. T. 2001. The role of immunity in resistance of Gulf Coast Native sheep to *Haemonchus contortus* infection. Ph.D. Diss., Louisiana State University, Baton Rouge.
- Peña, M. T., J. E. Miller, and D. W. Horohov. 2004. Effect of dexamethasone treatment on the immune response of Gulf Coast Native lambs to *Haemonchus contortus* infection. *Vet. Parasitol.* 119:223–235.
- Pernthaner, A., S. Cole, L. Morrison, and W. R. Hein. 2005. Increased expression of interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha genes in intestinal lymph cells of sheep selected for enhanced resistance to remutodes during infection with *Trichostrongylus colubriformis*. *Infect. Immun.* 73:2121–2183.
- Pernthaner, A., A. Vlassoff, P. G. C. Douch, and D. R. Maass. 1997. Cytokine mRNA expression and IFN-gamma production in nematode resistant and susceptible line lambs artificially infected with gastro-intestinal nematodes. *Acta Parasitol.* 42:55–61.
- Presson, B. L., G. D. Gray, and S. K. Burgess. 1988. The effect of immunosuppression with dexamethasone on *Haemonchus contortus* infection in genetically resistant Merino sheep. *Parasite Immunol.* 10:675–680.
- Prichard, R. K. 1994. Anthelmintic resistance. *Int. J. Parasitol.* 54:259–268.
- Romagnani, S. 1999. Th1/Th2 cells. *Inflamm. Bowel Dis.* 5:285–294.
- Rothwell, T. L. W. 1989. Immune exclusion of parasitic nematodes from the alimentary tract. *Int. J. Parasitol.* 19:139–168.
- Sangster, N. C. 1999. Anthelmintic resistance: Past, present and future. *Int. J. Parasitol.* 29:115–124.
- Sasaki, O., H. Susaya, K. Ishida, and K. Yoshimura. 1993. Ablation of eosinophils with anti-IL-3 antibody enhances the survival of intracranial worms of *Angiostrongylus cantonensis* in mice. *Parasite Immunol.* 15:349–354.
- Schallig, H. D., and M. A. Van Leeuwen. 1997. Protective immunity to the blood-feeding nematode *Haemonchus contortus* induced by vaccination with parasite low molecular weight antigens. *Parasitology* 114:293–299.
- Sher, A., R. T. Gazzinelli, I. P. Oswald, M. Clerici, M. Kullberg, E. J. Pearce, J. A. Berzofsky, T. R. Mosmann, S. L. James, H. C. Morse, and G. M. Shearer. 1992. Role of T-cell derived cytokines in the down regulation of immune responses in parasitic and retroviral infection. *Immunol. Rev.* 127:183–204.
- Smith, W. D. 1999. Prospects for vaccines of helminth parasites of grazing ruminants. *Int. J. Parasitol.* 29:17–24.
- Smith, W. D., and K. W. Angus. 1980. *Haemonchus contortus*: Attempts to immunise lambs with irradiated larvae. *Res. Vet. Sci.* 29:45–50.
- Smith, W. D., J. A. van Wyk, and M. F. van Strijp. 2001. Preliminary observations on the potential of gut membrane proteins of *Haemonchus contortus* as candidate vaccine antigens in sheep on naturally infected pasture. *Vet. Parasitol.* 98:285–297.
- Stear, M. J., S. Strain, and S. C. Bishop. 1999. Mechanisms underlying resistance to nematode infection. *Int. J. Parasitol.* 29:51–56.
- Stewart, D. F. 1955. Self-cure in nematode infestations of sheep. *Nature* 176:1273–1274.
- Swiderski, C. E., T. R. Klei, R. W. Folsom, S. S. Pourciau, A. Chapman, M. R. Chapman, R. M. Moore, J. R. McClure, H. W. Taylor, and D. W. Horohov. 1999. Vaccination against *Strongylus vulgaris* in ponies: Comparison of the humoral and cytokine responses of vaccinates and nonvaccinates. *Adv. Vet. Med. Vet. Vacc. Diagn.* 41:389–404.
- Tepper, R. I., D. A. Levinson, B. Z. Stanger, J. Campos-Torres, A. K. Abbas, and P. Leader. 1990. IL-4 induces allergic-like inflammatory disease and alters T-cell development in transgenic mice. *Cell* 62:457–467.
- Thompson-Snipe, L., V. Dhar, M. W. Bond, T. R. Mosmann, K. W. Moore, and D. M. Rennick. 1991. Interleukin 10: A novel stimulatory factor for mast cells and their progenitors. *J. Exp. Med.* 173:507–510.
- Tremain, S. A., and D. L. Emery. 1994. Myoelectric activity of the small intestine during parasitic rejection. Page 43 in *Proc. 30th Annu. Sci. Meet. Aust. Soc. Parasitol.*, Nelson Bay, New South Wales, Australia.
- Urban, J. F., R. Fayer, C. Sullivan, J. Goldhill, T. Shea-Donahue, K. Madden, S. C. Morris, I. Katona, W. Gause, M. Ruff, L. S. Mansfield, and F. D. Finkelman. 1996. Local TH1 and TH2 responses to parasitic infection in the intestine: Regulation by

- IFN-gamma and IL-4. *Vet. Immunol. Immunopathol.* 54:337–344.
- Urban, J. F., I. M. Kotona, W. E. Paul, and F. D. Finkelman. 1991. Interleukin 4 is important in protective immunity to gastrointestinal nematode infection in mice. *Proc. Natl. Acad. Sci. USA* 88:5513–5517.
- Urban, J. F., K. B. Madden, A. Svetic, A. Cheever, P. P. Trotta, W. C. Gause, I. M. Katona, and F. D. Finkelman. 1992. Importance of Th2 cytokines on protective immunity to nematodes. *Immunol. Rev.* 127:205–220.
- Urquhart, G. M., W. F. Jarrett, F. W. Jennings, W. I. McIntyre, W. Mulligan, and N. C. Sharp. 1966. Immunity to *Haemonchus contortus* infection. Failure of x-irradiated larvae to immunize young lambs. *Am. J. Vet. Res.* 27:1641–1643.
- Wakelin, D. 1978. Genetic control of susceptibility and resistance to parasite infections. *Adv. Parasitol.* 16:219–308.
- Wakelin, D. 1996. *Immunity to parasites: How parasitic infections are controlled.* Cambridge University Press, Cambridge, UK.
- Waller, P. J. 1994. The development of anthelmintic resistance in ruminant livestock. *Acta Trop.* 56:233–243.
- Woodbury, R. G., H. R. P. Miller, J. F. Huntley, G. R. J. Newlands, A. Palliser, and D. Wakelin. 1984. Mucosal mast cells are functionally active during spontaneous expulsion of intestinal nematode infections in the rat. *Nature* 312:450–452.