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The sheep's immune response to nematode parasites and prospects for its exploitation

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ABSTRACT

The immune response of sheep to nematode parasites involves a cascade of events beginning with the recognition of nematode antigen, followed by stimulation of the immune system to elicit the appropriate cellular response at the gut mucosa and finally the release of products which promote the elimination of the parasite from the gut. These processes are examined and deficiencies in the knowledge are highlighted. The application of the knowledge of the sheep's immune system to the identification of a marker for resistance and opportunities for the control of nematodes through manipulation of the sheep's responsiveness are discussed.

The progress that has been achieved in the experimental breeding of sheep for nematode resistance is examined. The use of such valuable scientific resource flocks for studies which advance the understanding of the immunology of resistance is discussed.

Keywords Sheep; nematodes; resistance; resilience; immunity; immunology; markers; unresponsiveness.

INTRODUCTION

Disease caused by nematode parasites is a major constraint on sheep productivity. The intensification of monocultural grazing management systems over the last 25 - 30 years has increased exposure of sheep to larval nematodes on the pasture, and in consequence aggravated nematode induced disease. This intensification has been supported in large part by the high efficacy of the 'new' broad spectrum anthelmintics levamisole and the benzimidazole group of compounds, and their extraordinary cost effectiveness.

The current economic reality in New Zealand is that anthelmintic usage sustains about one third of all sheep production (1988 f.o.b. N.Z.\$2800 million) (Brunsdon, 1987). This production is now at risk from the emergence of strains of nematodes which are resistant to currently available drenches. The increasing number of farms on which 'drench failure' has been reported points to the growing magnitude of the problem (McKenna, 1989).

The relatively short time between the introduction of levamisole and the benzimidazole anthelmintics and the emergence of strains of nematodes resistant to

them (Kettle *et al.*, 1981;1982), and more recently, the emergence of ivermectin resistant strains of *Haemonchus* (Van Wyk *et al.*, 1987) suggests that nematodes are developing resistance faster than the pharmaceutical industry can develop new anthelmintics.

In addition, the growing worldwide concern about the long-term human health affects of residues of chemicals in primary products has exerted market pressure for a reduction in the use of anthelmintic chemicals for nematode control.

Methods for the control of nematodes in sheep which overcome the problems caused by anthelmintic resistant strains of nematodes and which reduce the dependence on anthelmintics are being sought.

New management approaches which are aimed at extending the useful life of the existing anthelmintics are being investigated. This may be achieved through devising methods for reducing the frequency of drenching thus reducing the rate of emergence of resistant nematodes.

It is likely that the control of nematodes in sheep will always require some use of anthelmintic, if only for the control of outbreaks of clinical disease. Unless strains of nematodes resistant to current anthelmintics

revert to susceptibility new classes of anthelmintics will be required for this purpose. Anthelmintic development is a very costly process, and it is considered that a new class is unlikely to be developed before the end of the decade.

MAF Technology is undertaking research which addresses two novel control options. At Wallaceville, the sheep's immune response to nematodes is being studied in order to identify a marker for resistance and to determine whether the responsiveness of sheep can be enhanced. Wallaceville is also collaborating with Ruakura on research to assess the feasibility and practical implications of breeding sheep for nematode resistance. These approaches are likely to be difficult to implement but appear to be the most attractive long term options for nematode control.

In this paper an attempt will be made to outline progress in the experimental breeding of nematode resistant sheep and the current state of knowledge of the immune response of the sheep to nematode parasites. The potential for application of this knowledge to improve the control of nematodes is addressed. The parasitological aspects of resistance of sheep will be examined first in order to provide a background for the discussion of the immune responses to nematodes.

Genetic variation in resistance of sheep to nematodes.

It has been recognised for many years that there is considerable variation both between and within breeds in nematode egg output in sheep faeces. Within any one flock the distribution of faecal egg counts (FECs) may change with time, parasite exposure, and nutritional status. In general, a higher proportion of the flock tends towards lower FECs as the flock gets older. There is however a strong tendency for lambs to retain the same FEC ranking within the flock on successive sampling occasions (Douch *et al.* 1984).

The number of nematode eggs passed in the faeces of the sheep has generally been regarded, at the flock level, as a fairly reliable indicator of nematode burden, and has been used extensively in epidemiological studies. At the level of the individual sheep, the FEC is less reliable as an indicator of the extant nematode burden. FEC can be regarded as the summation of several factors which are influenced by resistance.

These include total adult worm burdens acquired and their fecundity. In addition, environmental factors such as nutritional status of individual sheep within the flock, preferential grazing behaviour, and physiological factors influencing the microenvironment of the gut and larval establishment all influence FEC. Despite their limitations, FECs are currently the most practical parameter to indicate a lamb's relative resistance status.

A further trait of sheep which may be commercially useful is resilience, the ability of a sheep to grow well relative to its peers regardless of FEC. This characteristic may help to reduce selection pressures on the nematode to develop resistance to the sheep's immune system by permitting nematodes to cycle through susceptible sheep.

Prospects for breeding nematode resistant sheep.

Several research groups have been investigating the prospects for breeding nematode resistant sheep. In New Zealand, lines of Romney sheep selected for resistance or susceptibility have been established at Wallaceville and Ruakura. Exchanges of genetic material between the Wallaceville and Ruakura lines has allowed comparison of the performance of progeny under different environments and management systems (Baker *et al.*, 1990).

At Wallaceville, selection for lines of sheep showing divergence in FEC under field challenge was initiated in 1979 (Vlassoff, pers. comm.). At that stage, selection for both ewe and ram lambs for the lines was based on FEC and growth performance (Bisset, 1989; Vlassoff, 1989). More recently, in order to accelerate progress in the divergence of resistant and susceptible lines and build up experimental flocks for research purposes, progeny tested rams have been mated with performance selected ewes (Bisset *et al.*, 1989). Nematode larval challenge acquired during grazing is still used to provide the challenge for performance tests.

In Australia, a number of studies involving the CSIRO Divisions of Animal Health, Animal Production and Animal Genetics and the University of New England have selected resistant and susceptible lines of Merino sheep on the basis of faecal egg count following immunisation and artificial challenge of progeny groups with *Trichostrongylus colubriformis* or *Haemonchus contortus* (reviewed by Piper 1987).

The New Zealand approach appears to have some advantages over other methods, namely no immunizing or artificial challenge is required, and FEC is derived from all available species. However, for selection in a commercial situation other traits would need to be considered.

These studies, in which estimates of heritability of resistance were in the range 0.25 - 0.40 (Piper 1987; Baker *et al.*, 1990) demonstrate that breeding sheep for resistance to nematode parasites is possible, and that experimental flocks can be developed as a scientific resource.

The feasibility of using similar procedures in commercial flocks is currently under investigation. From a practical viewpoint, however, the performance testing of progeny groups requires artificial challenge or withdrawal of drench treatment and may result in unacceptable production losses. Nevertheless, this approach may be sufficiently attractive to ram breeders to enable some genetic improvement to be made. A further practical consideration is that because of the diversity of production traits and breed types in the national flock many separate selection programmes would be required.

Immunological observations in nematode resistant sheep

The study of fundamental biological processes has been advanced significantly through the development of selection lines of animals expressing the trait of interest, e.g. Booroola fecundity gene in sheep. The development of experimental lines of sheep highly resistant or susceptible to nematode parasites, and the development of embryo splitting technology to provide clonal groups of sheep which will permit transplant/transfer of tissues between individuals without causing histocompatibility problems (Smith *et al.*, 1986) should result in significant advances in our understanding of the immune system of the sheep.

The immune response to nematodes has been the subject of numerous papers and several recent reviews (e.g. Rothwell, 1989; Millar, 1984). This paper will address some of the outstanding questions pertaining to mechanisms of ovine immunity to nematodes. The major elements of the immune response to nematodes are represented schematically in Figure 1.

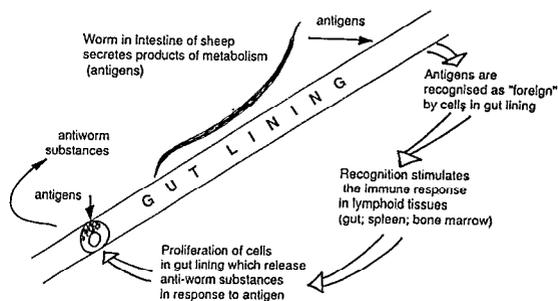


FIG.1 Schematic representation of the sheep's immune response to nematodes.

The worm-gut interface.

A logical starting place in the investigation of the response of sheep to nematode parasites is the gut-worm interface. After ingestion by the sheep, the infective third stage larva exsheaths and penetrates the gastrointestinal mucosa where it develops through the fourth stage to become an adult in a little more than two weeks. Products of the worm must be recognised as non-self by the sheep to initiate the immune response.

Evidence is accumulating that antigenic products of the larval stages of nematodes promote the immune response in sheep (Douch *et al.*, 1984). The nature of nematode antigens have been the subject of a number of investigations. Most studies have indicated that these antigens are mixtures of complex glycoproteins (Pritchard, 1986).

The identification of specific components possessing protective immunogenic activity has been regarded as the first step towards the production of a nematode vaccine. However, this has not proved to be a simple task. Determination of the appropriate excreted/secreted nematode components requires an act of faith that the assay(s) used recognise the important constituents. The assays most frequently used have been serum antibody blotting of electrophoretically separated components (O'Donnell *et al.*, 1985), combined with determination of their mitogenic activity on lymphocytes derived from a variety of tissues (Windaon, pers. comm.). In addition, some studies have used a small animal model for determining the efficacy of the

antigen fraction to induce resistance to nematode challenge (O'Donnell *et al.*, 1989a,b).

For a vaccine based on nematode antigen to be effective, it must be capable of inducing the 'correct' immune response at the intestinal mucosa when given at a site distant from the gut. To be commercially viable, such a vaccine would need to be effective in young, susceptible lambs.

How does the nematode antigen stimulate an immune response? The major histocompatibility complex (MHC) is a group of closely linked genes whose products (antigens) play a major role in determining the host's ability to mount an immune response, particularly at the level of recognition of foreign products as non-self. In sheep, MHC class I antigens have been identified serologically and studies have indicated an association between possession of the serotypes SY 1a and SY 1b and resistance to nematodes (Outeridge *et al.*, 1986; Douch and Outeridge, 1989). However, such associations are not strong, and may not be functional. More recently, the sheep MHC class II has been studied at the gene level and the possession of particular RFLP fragments appears to be strongly associated with susceptibility to infection with nematodes (Hulme *et al.*, 1989).

Nematode antigen recognition is not likely to be the only factor limiting the immune response. It is also necessary that antigen recognition be translated into the appropriate effector response.

Effector mechanisms

The manner by which the sheep actually prevents the establishment of the larval worm and promotes the elimination of the adult worm remain important unanswered questions.

The products of the sheep that may be involved have been the subject of much research over the years. These processes appear to be complex and could be mediated through host products which have not yet been characterised.

However, several host-derived products have been implicated:

- * antibodies secreted into the gut lumen
- * secretion of mucus components having increased stickiness

- * products of immediate or delayed-type hypersensitivity reactions (including biogenic amines, eicosanoids, tumour necrosis factor)
- * a combined affect of all or some of these.

While studies have demonstrated elevated levels of many of these substances in sheep which are resisting nematode challenge (e.g. Douch *et al.*, 1984) few studies have demonstrated an effect of host product on the nematode.

Our approach has been to examine the intestinal mucus of resistant and susceptible Romney sheep for a direct antiparasite action using a bioassay based on the ability of exsheathed nematode larvae to migrate from agar gels. In this assay, mucus from resistant sheep markedly inhibited larval migration whereas mucus from susceptible sheep did not (Figure 2) (Douch *et al.*, 1983; 1984). Similar results were obtained by Kimambo and MacRae (1988) for Suffolk x Finn Dorset sheep.

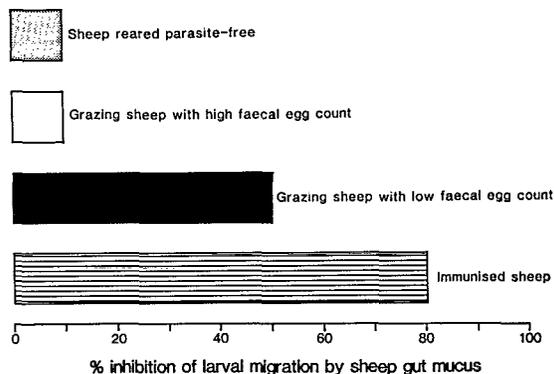


FIG.2 Antiparasite activity of mucus from sheep.

A number of putative mediators of nematode rejection when tested in this bioassay failed to inhibit larval migration, even at concentrations well in excess of normal physiological levels (Table 1). More detailed analysis of the mucus substances indicated that they had properties similar to the leukotrienes. Definitive analysis of these inhibitory mediators is required to ascertain their precise structure. The cellular origin of these products also remains to be determined.

TABLE 1 Effect of various substances on migration of *T.colubriformis* larvae

Substance	Percentage inhibition of larval migration		
	Final concentration ($\mu\text{g/ml}$)		
	100	50	25
None	0	0	0
Histamine	24	10	0
Tryptamine	22	16	0
Tyramine	18	11	0
Dopamine	30	7	0
Dopa	8	0	0
Adrenalin	12	0	0
Noradrenalin	0	0	0
Serotonin	0	0	0
Prostaglandin E ₁	0	0	0
Prostaglandin E ₂	0	0	0
Acetylcholine	0	0	0
Levamisole	100	100	99

Gut mucosal histology

Resistance of sheep to nematodes is characterised by an inflammatory response in the gut mucosa involving an increase in the numbers of several cell types. Comparison of the gut mucosal tissue of susceptible and resistant sheep in various studies reveals a consistent, strong association between resistance and the numbers of granulocytes in the mucosa (Douch *et al.*, 1986; 1988). This association is particularly marked in respect to mucosal mast cells and globule leukocytes. The numbers of these cells are well correlated with the larval migration inhibitory activity of mucus ($r=0.63-0.82$) and it is possible that the globule leukocyte is the cell type which produces mucous antiparasite substances.

The secretion of biogenic amines and other substances from granulocytes (eosinophils, mast cells) is stimulated when antigen crosslinks antigen-specific immunoglobulin bound to surface Fc receptors. It is likely that this type of reaction is the basis for the observed species specific stimulation of the sheep's immune response to nematodes (Douch, 1989b).

The globule leukocyte may be derived from the mucosal mast cell (Huntley *et al.*, 1984). This view is based on histological evidence which shows the presence of cells having intermediate staining characteristics, and evidence showing that globule leukocytes and

mucosal mast cells both contain ovine mucosal mast cell protease (Huntley *et al.*, 1986). Mast cell origins and their development have been the subject of much discussion and have been the subject of a recent review (Galli, 1990). Mucosal mast cells can be generated from bone marrow derived haemopoietic stem cells when cultured in the presence of a conditioned medium prepared from activated lymphocytes taken from nematode infected sheep (Haig *et al.*, 1988; Grimmett, Stankiewicz and Douch, unpublished data). However, there appears to be no published experimental evidence showing that mucosal mast cells will transform to globule leukocytes in culture.

It is not known how mucosal mast cells or globule leukocytes arise in the intestinal mucosa. After nematode antigen has been recognised, a chemical signal, possibly an interleukin, may initiate the recruitment of their progenitor cells to the mucosa. Further signals may promote proliferation and differentiation. The cells of the sheep intestinal mucosa which recognise nematode antigens and subsequently produce the interleukins have not been identified, but may be one or more of several acknowledged antigen recognising cell types (e.g. macrophage, M cell, dendritic cell) or possibly one of the lymphocyte types. Lymphocytes are known to produce a range of interleukins which function as chemical messengers and exert control over the recruitment, proliferation and differentiation of stem cells.

Immunohistological studies of the lymphocyte subsets in sheep gut mucosa have generally failed to show any strong relationship between numbers of lymphocytes or ratios of lymphocyte subsets and either resistance or susceptibility (Gorrell *et al.* 1988; Shaw and Douch, 1989). However, the function of such cells may not be related to their numbers.

Observations that lymphocytes within the epithelial cell layer express CD8 as a predominant phenotype may indicate a role for this cell in antigen recognition or surveillance processes.

The role of antibodies in resistance.

Numerous studies have been undertaken to examine the classes and levels of antibodies in sheep that are resistant to nematodes. Generally, the levels of circulating antibodies are elevated in nematode resistant sheep,

perhaps reflecting the greater cellular responsiveness (B lymphocyte production) of the resistant animal.

Antibodies of the various classes are secreted into the intestinal lumen and have been postulated to have a role in parasite damage (binding to functional structures in the worm's gut thus impairing its ability to absorb nutrients, promoting cell lysis through the action of complement). They may also enhance the 'stickiness' of the mucus components to the worm, thereby causing entrapment and removal from the intestine through normal flow of contents along the tract. Antibodies are also implicated in the 'arming' of effector cells e.g. mast cells, which then secrete agents damaging to the worm when they bind the appropriate antigen. They may also function to 'mop up' any stray antigenic material that may leak into the host tissues.

Unresponsiveness in young sheep

Young sheep are generally regarded as being more susceptible to nematode infection than older sheep (Douch *et al.*, 1984). However, examination of the distribution of FEC within a flock of young lambs reveals that a significant proportion do resist nematode infection (Figure 3).

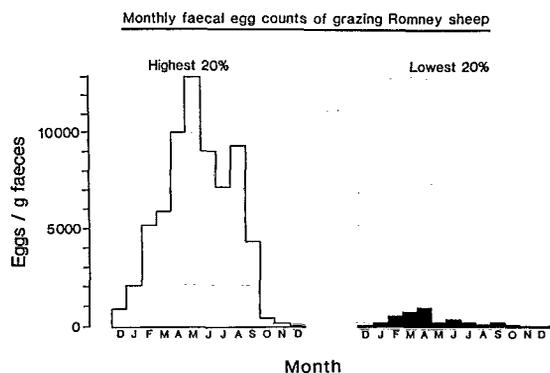


FIG 3 Monthly FECs of 'responsive' and 'unresponsive' sheep.

Studies in which Romney lambs aged two, three or four months were immunised and challenged with *Trichostrongylus colubriformis* showed that about half the lambs in each age group resisted the challenge, these

sheep had elevated numbers of globule leukocytes in their intestinal mucosae, those sheep which did not resist the challenge had few of these cells present (Douch, 1989a). Similar results were obtained by Gregg *et al.*, (1978) in young Merino sheep.

Young susceptible sheep may pass large numbers of nematode eggs until they are about 8 - 12 months old when they undergo a self-cure response marked by rapidly reducing FEC. This response is associated with the appearance of elevated numbers of globule leukocytes in the intestinal mucosa and increased antiparasite activity in the gut mucus (Douch *et al.* 1986). These are similar to the responses that occur in the more resistant lambs, but are taking place later in the life of the sheep.

Resistance and susceptibility, therefore, can be regarded as the two extremes of a continuum of responsiveness that breeding studies have shown to be under genetic control.

An effective immune response to nematodes depends on all the components of the immune cascade of the sheep being present and functional. The observations that susceptible young Romney sheep do eventually mount an effective immune response, suggests that unresponsiveness is associated with the regulation or control of the immune system, rather than the lack of any structural component (or genes coding for them).

The immune response may, in part, be regulated through the ability of cells to produce and respond to cytokines such as the interleukins. The action of cytokines is mediated through cell surface receptors, and it is possible that the number or 'reactivity' of such receptors may differ in responsive and unresponsive lambs. It is also possible that the 'maturation' of the immune system of sheep is under neurohormonal control (Jankovic, 1989), and like the onset of puberty, vary considerably between individuals.

Although the basis of (un)responsiveness of sheep to nematode parasites is not understood, it is likely that new opportunities for manipulation of the response will arise from its study. One could speculate that these might include the development of immunopotentiating agents which enhance interleukin production, and the development of vaccines against cell surface receptors.

Markers of resistance

Currently the only practical method of identifying nematode resistance in live sheep is FEC and this parameter has been useful for identification of genetically resistant or susceptible rams. FEC has many disadvantages as a marker for nematode resistance, as outlined above. A more reliable means of identifying genetically resistant sheep is required for progress to be made in the development of nematode resistant commercial flocks.

A number of parameters which show promise as prospective markers for resistance have been identified from comparative studies of the immune response of resistant and susceptible sheep. These include parameters which are based on physiological or immunological responses, e.g. circulating eosinophil numbers (Dawkins *et al.*, 1989; Buddle, Jowett and Thomson, unpublished data) and circulating antibody levels (Green, McParland and Douch, unpublished data). These parameters, like FEC, reflect the sheep's response to challenge and may not offer many advantages. They do have potential for use as a selection criterion, perhaps in addition to FEC, if they do not interfere with normal farm management practices.

Potentially the most useful marker will be one that is genetically based and reflects the inherent ability of the sheep to respond, regardless of the parasite challenge, e.g. MHC gene analysis, ovine lymphocyte antigen serotypes. Markers of the latter type have been evaluated in Wallaceville's selected resistant and susceptible flocks (Douch and Outerridge, 1989).

To obtain a true genetic marker for resistance, e.g. a DNA probe that binds to a gene which controls responsiveness, it will be necessary to unravel the immunological processes involved in resistance.

CONCLUSIONS

During the last ten years it has become abundantly clear that the sheep industry can not depend solely on chemical treatment to control parasite induced disease. The emergence of multiple anthelmintic-resistant strains of nematodes and the consequent failure to control nematodes has the potential to cripple the economic viability of the industry.

Considerable progress has been made in un-

derstanding the genetics of the sheep's resistance to nematode parasites. The development of experimental flocks of resistant sheep signals that the application of this approach to commercial flocks has the potential to reduce dependence on anthelmintics and avoid the problems of anthelmintic-resistance in nematodes.

Resistance of sheep to nematodes is an immunologically-based phenomenon. Progress has been made in understanding the ovine immune response but a much greater knowledge is needed both for the identification of markers for resistance that can be used in breeding programmes and for assessing whether the responsiveness of sheep can be enhanced through manipulation of the immune system.

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