



TITLE The host immune response to gastrointestinal nematode infection in sheep

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1 Review Article

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3 The host immune response to gastrointestinal nematode infection in sheep

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22

## 23 SUMMARY

24 Gastrointestinal nematode infection represents a major threat to the health, welfare and  
25 productivity of sheep populations worldwide. Infected lambs have a reduced ability to absorb  
26 nutrients from the gastrointestinal tract, resulting in morbidity and occasional mortality. The  
27 current chemo-dominant approach to nematode control is considered unsustainable due to the  
28 increasing incidence of anthelmintic resistance. In addition there is growing consumer  
29 demand for food products from animals not subjected chemical treatment. Future mechanisms  
30 of nematode control must rely on alternative, sustainable strategies such as vaccination or  
31 selective breeding of resistant animals. Such strategies take advantage of the host's natural  
32 immune response to nematodes. The ability to resist gastrointestinal nematode infection is  
33 considered to be dependent on the development of a protective acquired immune response;  
34 although the precise immune mechanisms involved in initiating this process remain to be fully  
35 elucidated. In this paper current knowledge on the innate and acquired host immune response  
36 to gastrointestinal nematode infection in sheep and the development of resistance is reviewed.

37

38 **Keywords:** gastrointestinal nematode, sheep, innate immunity, protective antibodies

## 39 INTRODUCTION

40 Gastrointestinal nematode (GIN) parasitism is a major constraint affecting sheep production  
41 systems. Naïve lambs are exposed to infection when grazing contaminated pasture.  
42 Consequently, infections are generally comprised of a mix of species, which infect both the  
43 abomasum and intestine. The species of infective larvae on pasture is dependent on a number  
44 of factors including temperature and moisture and therefore often displays a seasonal  
45 distribution (1). As GIN are highly aggregated within the host population, susceptible  
46 individuals can harbour thousands of worms, which in turn leads to increased pasture  
47 contamination. Current sheep production systems are highly dependent on the availability of  
48 efficacious anthelmintic products and are threatened by the increasing incidence of  
49 anthelmintic resistance. Resistance to all anthelmintic classes has now been reported, with the  
50 exception of derquantel, which first came to market in 2010 (2-5). The looming spectre of  
51 widespread anthelmintic resistance has led to renewed interest in alternative nematode control  
52 strategies such as vaccination, breeding for resistance and immunomodulatory anthelmintics.  
53 Many of these strategies exploit the natural host immune response to GIN. The major host  
54 defence mechanism against GIN is considered to be acquired immunity (6), which develops  
55 over time in response to challenge and is dependent on the age of the animal, nutritional status  
56 and genotype (7-9). A current challenge for sheep producers is to allow stock sufficient  
57 exposure to GIN in order to develop immunity without impairing production.

58

## 59 MANIFESTATIONS OF IMMUNITY

60 The development of immunity to GIN is complex and highly variable. The rate of  
61 development of immunity depends on the breed of sheep, the nematode species to which they  
62 are exposed and the intensity of infection. While lambs rapidly develop the ability to control  
63 GIN such as *Nematodirus battus* (10), resistance to other species, such as *Teladorsagia*

64 *circumcincta*, is much slower to develop (9). Immune competence can be observed through  
65 prevention of establishment of most incoming infective larvae, suppressed GIN growth (and  
66 therefore fecundity), the expulsion of adult worms, or a mixture of the above (6, 11, 12).  
67 Lambs start to demonstrate immune competence from 2 to 3 months of age (13), with regular  
68 exposure to larval challenge allowing the immune response to develop until a significant  
69 protective immune capability is developed by 10 to 12 months of age (1, 11). Adult sheep  
70 tend to remain relatively resistant to infection, harbouring only a few adult worms, although  
71 regular exposure to some level of infection is required to retain immunity (14). An alternative  
72 view is that immunity develops in two stages; suppression of worm growth precedes  
73 suppression of worm establishment and survival (15). Immunity to intestinal worms also  
74 develops more rapidly than immunity to abomasal worms (16).

75           Nutritional stress, ill-health and pregnancy can all influence an individual's immune  
76 status. It has been observed that the nutritional status of the host during GIN infection is  
77 important, with the provision of additional protein to growing sheep during infection resulting  
78 in enhanced immunity to GIN (17, 18). A relaxation in host immunity to GIN is observed in  
79 ewes during the periparturient period, from approximately 2 weeks before lambing to  
80 approximately 6 weeks post lambing, although this timing is very variable. It is largely due to  
81 nutritional stress in the ewe and can be prevented by supplementary feeding (19). The  
82 increase in faecal egg count (FEC) is known as the periparturient rise (20), and is a major  
83 contributor to pasture larval contamination encountered by lambs (21).

84

## 85 THE INNATE IMMUNE RESPONSE

86 The immune system of vertebrates is composed of two arms, the innate (non-specific)  
87 immune response and the adaptive (specific) response, the various cellular and biochemical

88 components of which work together to protect vertebrates from a range of threats. The first  
89 line of defence against GIN is the innate immune system, which plays a role in sensing GIN,  
90 then initiating and driving the acquired immune response. Of particular relevance are innate  
91 physical barriers to the establishment and survival of GIN, and subsequently the process by  
92 which the host recognises the presence of GIN and activates an immune response.

### 93 *Physical barriers to the establishment and survival of GIN*

94 The inner surface of the gastrointestinal tract is covered with a layer of mucus, primarily  
95 produced by epithelial goblet cells (22). This is the front line of the innate defence against  
96 ingested food and pathogens in the gastrointestinal tract. The primary component of mucus is  
97 mucin, however it also contains an array of bioactive molecules such as defensins and trefoil  
98 factors (23). Many of these bioactive molecules have been shown to be anti-microbial, or to  
99 stimulate inflammation (24). Both increased mucus production and the presence of inhibitory  
100 substances in the mucus have consistently been observed during the development of immunity  
101 to GIN (25-27).

102 Enteric smooth muscle contractility has been shown to play an important role in  
103 mediating nematode resistance in mice, with changes in intestinal motility reported to be  
104 responsible for parasite expulsion (28). However, its role in GIN expulsion in sheep is less  
105 clear. An up-regulation of genes related to the structure and function of the enteric smooth  
106 muscle was observed in lambs selected for resistance to GIN when compared to their  
107 susceptible counterparts (29). Additionally, the concentration of bradykinin, a physiologically  
108 active peptide which can promote vasodilation and smooth muscle contraction was negatively  
109 correlated with the number of adult *7'. circumcincta* worms in immune sheep (30). Contrary to  
110 this, however, it has been reported that susceptible Suffolk lambs showed greater duodenal  
111 contractile force compared to resistant lambs in response to *7'. circumcincta* infection (31).

### 112 *Pattern recognition receptors (PRRs)*

113 Among the earliest systems for the detection of pathogens are the germline-encoded pattern  
114 recognition receptors (PRRs) such as C-type lectin receptors (CLRs) and toll-like receptors  
115 (TLRs). CLRs and TLRs are expressed by many cell types, including the cells of mucosal  
116 surfaces and tissue immune cells such as macrophages and dendritic cells, the major antigen  
117 presenting cells (APCs) (32, 33). PRR proteins identify both pathogen-associated molecular  
118 patterns (PAMPs; pathogen molecular structures not found in the host), and damage  
119 associated molecular patterns (DAMPs; molecules released from damaged or stressed cells).  
120 Both PAMPs and DAMPs can result in the initiation and perpetuation of the inflammatory  
121 response. As well as being the first line of defence, PRRs play an important role in the  
122 induction of cytokines and other signals responsible for the activation and manipulation of the  
123 adaptive immune system (34).

124           While viral, bacterial and fungal ligands which act as potent PAMPs and are  
125 recognised by mammalian PRRs are well described, less is known about the role of PRRs in  
126 the response to nematode infection. TLR genes (*TLR2*, *TLR4* and *TLR9*) have been found to  
127 be more abundantly expressed in the gut mucosa of genetically resistant sheep following GIN  
128 challenge (35). CLRs are also candidates for innate recognition of surface carbohydrate  
129 present on nematodes. The mannose receptor (a CLR) has been shown to bind to  
130 excretory/secretory proteins of the mouse nematode *Trichuris muris*, but was not essential for  
131 protective immunity (36).

132           Tissue phagocytic cells such as dendritic cells and macrophages play a critical role in  
133 innate immunity, but also help initiate acquired immunity through their ability to sample  
134 antigens, migrate to secondary lymphoid tissue and activate antigen-specific T cells within  
135 this tissue. M1 (classically activated) macrophages are activated through TLRs and interferon-  
136 gamma (IFN- $\gamma$ ), whereas M2 (alternatively activated) macrophages are stimulated by the  
137 interleukins (IL) IL-4 or IL-13. These states are not static however, with ovine M1 and M2

138 patterns capable of reverting from one to the other according to cytokine availability (37). M2  
139 macrophages have three main functions during helminth infection: regulation of the immune  
140 response, healing of damaged tissue, and resistance to parasite invasion (38). During a Th2-  
141 type response to nematode infection, M2 macrophages express chitinase and FIZZ family  
142 member proteins (ChaFFs), suggesting an effector or wound-repair role for the molecules at  
143 the site of nematode infection (39). Chitinases degrade chitin, a molecule present in the  
144 exoskeletal elements of some animals, including helminth larvae (40). A joint role for  
145 macrophages and neutrophils in preventing establishment of *H. contortus* larvae has also been  
146 suggested (41). Macrophage-like cells were also occasionally observed associated with  
147 completely destroyed *H. contortus* larvae from sensitized sheep (42).

#### 148 *Cytotoxic and proinflammatory cells*

149 At the site of infection in the gastrointestinal tract mast cells are recruited by the release of  
150 chemokines and other inflammatory mediators by innate immune cells. Although best known  
151 for their role in the allergic response, increased numbers of tissue mast cells have also been  
152 observed during helminth infection. Mast cells are inflammatory cells that can both respond  
153 directly to pathogens and send signals to other tissues to modulate both the innate and  
154 adaptive immune responses (43). Two subsets of mast cells have been described based on  
155 their location: connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs) (44).  
156 Mast cells appear uniformly scattered in tissue and activation of mast cells occurs primarily  
157 through antigen induced stimulation of the high-affinity immunoglobulin E (IgE) receptor  
158 (FcεR1s) expressed at the mast-cell surface (45). Mast cells can also be activated by directly  
159 interacting with PAMPs through PRRs (43). Mast cells store a number of inflammatory  
160 mediators (including histamine, leukotrienes and proteases) that are released upon  
161 degranulation into the surrounding tissues (46, 47). The effects of these chemical mediators  
162 are characteristic of type 1 hypersensitivity, and include smooth muscle contraction, increased  
163 vascular permeability and local blood flow, and enhanced mucus secretion. In response to

164 GIN infection, mast cells also produce Th2 cytokines such as IL-13, IL-4 and IL-5 in addition  
165 to chemotactic factors which contribute to the recruitment of multiple inflammatory cells  
166 including eosinophils, natural killer (NK) cells, and neutrophils (43). In sheep, nematode-  
167 induced activation of mast cells has been associated with the acquired immune response (48,  
168 49). An important mechanism controlling the number of adult *7'. circumcincta* in previously  
169 sensitised animals appears to be IgE-dependent mast cell degranulation (12), with sheep mast  
170 cell proteinase systemically released during nematode infections (50).

171 In addition to an increase in the numbers of mast cells, an increase in eosinophils is  
172 also characteristic of infection with nematode parasites. Eosinophils develop in the bone  
173 marrow from haematopoietic stem cells (51) in response to the Th2 cytokines IL-3, IL-5, and  
174 GM-CSF (52). Following infection, eosinophils proliferate in the blood in a process known as  
175 eosinophilia. Mature eosinophils are activated and migrate to the site of infection in response  
176 to various chemoattractants, such as IL-5 and members of the eotaxin family of chemokines  
177 CCL11, CCL24 and CCL26 (53). In tissue, eosinophils can show directional migration  
178 toward a parasite target (54). Following activation, the effector functions of eosinophils  
179 include immune regulation, resistance to parasitic invasion through degranulation and the  
180 release of eosinophil secondary granule proteins (EPGPs) and healing damaged tissue. The  
181 effector functions result in the damage and killing of larval stages of many helminth parasites  
182 (42, 55, 56).

183 Eosinophils have been shown to play a significant role in the development of  
184 resistance to multiple species of GIN in sheep (42, 57-59). A reduction in peripheral blood  
185 eosinophilia has been observed during primary infection with *7'. circumcincta*, which was  
186 hypothesised to be a result of recruitment of cells into the intestinal epithelium (60). However,  
187 the relationship between peripheral blood eosinophilia and tissue eosinophilia is reasonably

188 weak, with only a proportion of circulating eosinophils moving into the abomasal mucosa in  
189 response to GIN infection (58). Increases in tissue eosinophils have been observed during  
190 *Haemonchus contortus* infection of both naïve (61) and previously sensitised (42, 62) sheep,  
191 resistant Romney selection line animals with a naturally acquired mixed infection (63) and  
192 Suffolk and Texel lambs infected with *T. circumcincta* (64).

193

## 194 THE ADAPTIVE IMMUNE RESPONSE

195 On encountering a foreign antigen, antigen presenting cells (APCs) such as activated dendritic  
196 cells and macrophages migrate to the regional lymph nodes via the afferent lymphatic system  
197 where they display the antigens to their cognate T cell receptor via MHC class I or II carrier  
198 molecules. The activation of the naïve T cell by APCs initiates the adaptive immune response  
199 and results in the release of cytokines, leading to both T cell differentiation and the  
200 proliferation of further T cells.

### 201 *Antigen processing and presentation*

202 Thymus-derived T cells play a central role in the cell-mediated immune response. T cells are  
203 differentiated from other lymphocytes by the presence of a T cell receptor (TCR) on the cell  
204 surface. There are several types of T cell, including cytotoxic, helper and regulatory T cells.  
205 Cytotoxic T cells (T<sub>c</sub>) kill cells that are infected with viruses or other intracellular pathogens  
206 or damaged cells. They are also known as CD8 T cells as they express the CD8 glycoprotein  
207 at their surface. T helper cells (T<sub>h</sub>) express the surface protein CD4, and provide essential  
208 additional signals to activate maturation of B cells, T<sub>c</sub> cells, and macrophages. T<sub>h</sub> cells can be  
209 further classified as Th1, Th2 or Th17 cells depending on the cytokines they produce. CD8  
210 and CD4 T cells bind MHC class I and MHC class II molecules respectively. Regulatory T  
211 cells (T<sub>reg</sub>) suppress the activity of other lymphocytes, and are critical for the maintenance of  
212 immunological tolerance.

### 213 *The T cell response*

214 The Th1 response has been traditionally associated with the immune response to intracellular  
215 bacteria, protozoa and viruses. The Th1 cascade is triggered by the production of IL-12 by  
216 dendritic cells, macrophages and B cells (65), which stimulates the production of the pro-  
217 inflammatory cytokine IFN- $\gamma$  by natural killer (NK) cells (66). IFN- $\gamma$  is important for  
218 differentiation of naive CD4<sup>+</sup> T cells into IFN- $\gamma$ -producing Th1 cells (67). The T-box  
219 transcription factor T-bet plays a critical role in this process, accounting for Th1 cell  
220 development and the Th1 cell-specific IFN- $\gamma$  production (68, 69). Both IL-12 and IFN- $\gamma$  also  
221 inhibit the production of the Th2 cytokine IL-4 in mice infected with intestinal nematodes  
222 (70). The effector molecules of the Th1 response are specialised to stimulate proliferation of  
223 CD8<sup>+</sup> Tc cells and activate macrophages and increased expression of these effectors has been  
224 associated with GIN susceptibility in sheep in a number of studies (71-73).

225 An antibody-stimulating protective Th2-type response is commonly elicited by  
226 helminth parasites. Common features include expression of Th2-type cytokines (IL-4, IL-5  
227 and IL-13), infiltration of eosinophils, basophils and mast cells (all of which can produce  
228 several types of Th2-type cytokines), and IgE production (74). The presence of IL-4 early in  
229 *Trichuris muris* infection has been shown to be critical for the activation of the protective Th2  
230 response in mice (75). IL-4, through activation of STAT6, up-regulates GATA3 expression,  
231 inducing differentiation of naïve Th cells to Th2 cells while suppressing differentiation into  
232 Th1 cells (76). Upon activation, Th2 cells produce additional IL-4 in a positive feedback loop,  
233 along with other Th2 cytokines including IL-5, IL-9, IL-13 and IL-25. IL-4 induces class  
234 switching in activated B cells, leading to production of IgE (77). The antibody IgE primes the  
235 IgE-mediated type 1 hypersensitivity response by binding to Fc (Fc $\epsilon$ RI and II) receptors on  
236 the surface of mast cells and basophils (78). When helminth antigen binds to cell bound IgE it  
237 leads to mast cell degranulation, and the release of soluble mediators (74). The sensitivity of  
238 target cells to mast cell and basophil-derived mediators is increased by IL-4 and IL-1 3

239 signalling. In mice it has been shown that together the two cytokines promote increased  
240 contractility of smooth muscle cells (79), increased permeability of epithelial cells (80), and  
241 elevated goblet-cell hyperplasia during nematode infection (81). The presence of IL-4 in  
242 extravascular tissue induces alternative activation of resident tissue macrophages, which  
243 function in wound healing and tissue repair. IL-5, aside from triggering eosinophilia,  
244 enhances secretion of IgA by B cells (82). The Th2 cytokine IL-13 induces epithelial cell  
245 repair and mucus production, and together with IL-9 recruits and activates mucosal mast cells.  
246 In sheep, the timely induction of a Th2 response to GIN infection, characterised by mast cell  
247 hyperplasia, eosinophilia, recruitment of IgA/IgE producing cells and the expression of Th2  
248 cytokines, is considered to promote the development of resistance (83, 84).

249         The roles of the more recently discovered Th17 and Treg cells in the ovine response to  
250 GIN remains to be elucidated. Th17 cells promote inflammation through the recruitment of  
251 neutrophils and macrophages to the site of infection. Early in infection IL-6, produced by  
252 dendritic cells, acts with TGF- $\beta$  (also required for the differentiation of regulatory T cells) to  
253 produce the Th17 response. This results in the production of IL-17 family members and IL-  
254 21, a subset of cytokines particularly important in clearing pathogens during host defence  
255 responses and in inducing tissue inflammation in autoimmune disease (85). Later, dendritic  
256 cells along with other antigen-presenting cells produce cytokines to promote either Th1 or  
257 Th2 development, and suppress Th17 development. Increased expression of Th17-associated  
258 genes has been associated with both susceptibility (86) and resistance (87) to GIN in sheep  
259 depending on the experimental model. Treg cells are a subpopulation of T cells that are  
260 involved in the maintenance of immunological self-tolerance and homeostasis through  
261 immune suppression (88). Expression of the forkhead transcription factor FOXP3 is critical  
262 for the development and function of Treg cells (89). Treg (CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>) cells are an  
263 important “self-check” in the immune system, and have been shown to be activated and  
264 expanded during helminth infection in mice (90-92). A faster switch from a Th1 to a

265 Th2/Treg response was found in resistant Suffolk lambs compared to susceptible lambs (93).

266 The human T cell response may be more functionally diverse than previously thought.

267 Pathogen stimulation of naïve T cells may give rise to multiple T cell subtypes, suggesting

268 that Th cell polarisation could be the results of preferential expansion of particular clones

269 rather than preferential priming (94). The implication of this for sheep Th cell polarisation

270 remains to be determined.

### 271 *Antibody response*

272 The principal function of B cells is to make antibodies (immunoglobulins) against

273 antigens. The binding of an antigen to a naïve B cell, coupled with the accessory signals from

274 Th cells, stimulates the lymphocyte to proliferate and differentiate into plasma cells, which

275 secrete large amounts of antibodies. A number of antibodies isotypes have been shown to be

276 correlated with GIN resistance in sheep, including IgA, IgG1 and IgE. IgA, which has both

277 circulating and secretory isoforms, is the isotype most closely associated with intestinal

278 mucosal immune responses. Increased levels of IgA have been positively associated with

279 resistance to *T. circumcincta*, regulating both worm length and fecundity (95-98). This

280 resistance is regulated through suppressed parasite growth, development and fecundity, and is

281 mediated by IgA activity against 4th-stage larvae. In Scottish Blackface lambs the presence of

282 arrested L4 larvae has been shown to be positively associated with both worm burden and the

283 size of the local IgA immune response (12). Elevated levels of both IgA and IgG1 were

284 observed in *T. colubriformis*-challenged sheep (99).

285 Increased levels of IgG1 and IgE have also been negatively correlated with FEC in

286 Romney selection line sheep in New Zealand (100-102), although IgE was positively

287 correlated with breech soiling (102). IgE mediates mast cell, eosinophil and basophil

288 degranulation in response to GIN and elevation of total and/or parasite-specific IgE serum  
289 antibodies have been reported during infection with *H. contortus* (103), *T. colubriformis* (104)  
290 and *T. circumcincta* (105, 106). In addition, an association between a polymorphism at the 5'  
291 end of the sheep IgE gene and resistance to *T. colubriformis* has been reported, although  
292 attempts to confirm this finding in other flocks failed (107).

293           A significant number of activated antigen-specific B cells and T cells persist after an  
294 antigen has been eliminated, and these are known as memory cells. These cells form the basis  
295 of immunological memory and can be reactivated much more quickly than naïve  
296 lymphocytes, and usually provide lasting protective immunity.

297

## 298 DEVELOPMENT OF RESISTANCE TO GIN IN SHEEP

299           Studies comparing naïve and previously infected animals have shown that  
300 development of immunity to GIN is associated with a predominantly Th2 response,  
301 characterised by an increase in Th2 cytokines, recruitment of eosinophils, mast cells and  
302 globule leucocytes, and increased production of parasite-specific IgA, IgG1 and IgE (108-  
303 110). However, there is conflicting evidence on whether a Th2 response can be used to select  
304 resistant or susceptible animals. While an increase in inflammatory cells and parasite-specific  
305 IgA were generally inversely associated with *H. contortus* worm burden and FEC in three  
306 breeds of sheep, mean values were not found to differ between the resistant (Santa Ines) and  
307 susceptible (Suffolk and Ile de France) breeds (111). This is in contrast to a study comparing  
308 genetically resistant sheep with random-bred Merino lambs, which found resistant lambs had  
309 increased *IL-5* expression, increased IgG1 and IgE antibody production, and higher densities  
310 of mucosal mast cells and eosinophils in response to *H. contortus* infection (71). During  
311 repeated experimental infections with *T. colubriformis*, genetically resistant sheep were also  
312 able to respond earlier than susceptible animals with nematode-specific IgA and IgG2 (112).

313 Resistant Barbados Black Belly lambs have also been shown to develop a more rapid Th2-  
314 type response than the susceptible INRA 401 lambs after a primary infection with *H.*  
315 *contortus* (113). A differential interplay between Th1/Th2 and Treg genes has also been  
316 proposed to modulate the immune response to GIN rather than a straightforward Th1 or Th2  
317 pathway (93) and failure to observe consistent gene expression profiles between resistant and  
318 susceptible animals could be due to variation in response time between studies. Additionally,  
319 multiple studies have suggested that the mechanisms of resistance may vary between animals  
320 with different genetic backgrounds, and may be parasite-specific (111, 114).

321

## 322 CONCLUSION

323 The host-parasite interaction is a complex relationship which determines the outcome of  
324 infection. Sheep GIN display a variety of surface and secretory/excretory antigens which can  
325 be stage-specific. Such molecules trigger the host's immune response generally resulting in  
326 the development of a protective immune response, although the level of immunity is  
327 dependent on age, nutritional status and genotype. Increased mucus and bioactive molecule  
328 production, activation of mast cells, eosinophilia, polarisation of the immune response to a  
329 Th2 response and the production of anti-nematode antibodies are all associated with the  
330 development of immunity. A protective immune response can be considered an expression of  
331 resistance and a detailed understanding of the genes and biological mechanisms involved in  
332 protective immunity will aid the development of non-chemical effective and sustainable  
333 nematode control methods. Understanding the genetic and molecular basis of disease  
334 resistance also has many advantages and applications such as the development of novel  
335 genetic markers for inclusion in genetic improvement programmes.

336

337

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343

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