

Chasing the genes that control resistance to gastrointestinal nematodes

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Abstract

The host-protective immune response to infection with gastrointestinal (GI) nematodes involves a range of interacting processes that begin with recognition of the parasite's antigens and culminate in an inflammatory reaction in the intestinal mucosa. Precisely which immune effectors are responsible for the loss of specific worms is still not known although many candidate effectors have been proposed. However, it is now clear that many different genes regulate the response and that differences between hosts (fast or strong versus slow or weak responses) can be explained by allelic variation in crucial genes associated with the gene cascade that accompanies the immune response and/or genes encoding constitutively expressed receptor/signalling molecules. Major histocompatibility complex (MHC) genes have been recognized for some time as decisive in controlling immunity, and evidence that non-MHC genes are equally, if not more important in this respect has also been available for two decades. Nevertheless, whilst the former have been mapped in mice, only two candidate loci have been proposed for non-MHC genes and relatively little is known about their roles. Now, with the availability of microsatellite markers, it is possible to exploit linkage mapping techniques to identify quantitative trait loci (QTL) responsible for resistance to GI nematodes. Four QTL for resistance to *Heligmosomoides polygyrus*, and additional QTL affecting faecal egg production by the worms and the accompanying immune responses, have been identified. Fine mapping and eventually the identification of the genes (and their alleles) underlying QTL for resistance/susceptibility will permit informed searches for homologues in domestic animals, and human beings, through comparative genomic maps. This information in turn will facilitate targeted breeding to improve resistance in domestic animals and, in human beings, focused application of treatment and control strategies for GI nematodes.

Introduction

The intestinal immune response to infection with nematodes comprises a plethora of interacting processes that are activated once the presence of the parasite and its antigens in the gut have been recognized. The host

protective response is driven by the Th2 lymphocyte subset and culminates in the activation of a range of potential effector mechanisms against the invading parasites (Else & Finkelman, 1999; Behnke *et al.*, 2000; Artis & Grencis, 2001). Figure 1 summarizes some of the key processes involved in this type of response.

As new tools for dissecting the component processes are developed, we learn in yet more intricate detail about the molecular interactions that are involved. Different cell

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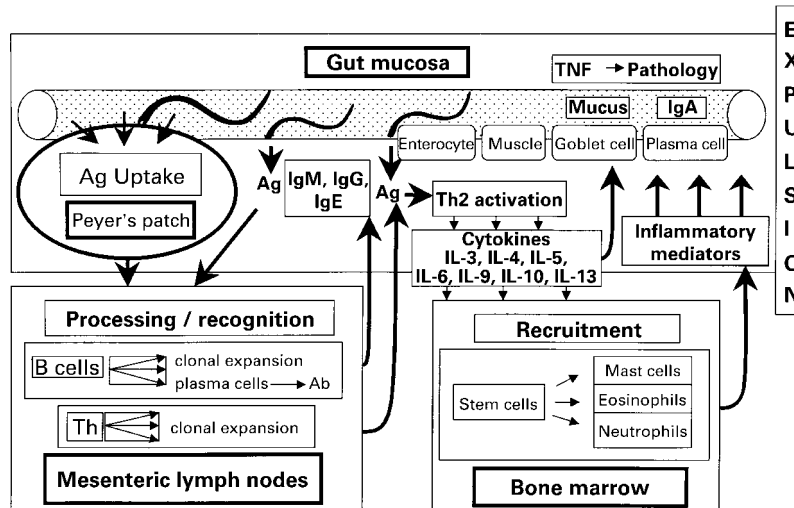


Fig. 1. Schematic diagrams summarizing some of the key processes that occur during the intestinal response to GI nematode infections. Three main compartments are illustrated (gut mucosa, mesenteric lymph nodes and bone marrow) and the arrows link boxes within these to illustrate the steps that follow after invasion of the intestinal lumen and mucosa (represented by the horizontal tube) by worms. These include initial recognition of foreign antigens, antigen processing, activation of B and Th2 lymphocytes, secretion of antibody and cytokines, recruitment of effector cells from the bone marrow and direct effects on the mucosa.

types respond to different combinations of molecular signals during the induction phase and depend on different signals for their eventual homing to the sites where they express their effector functions (Urban *et al.*, 2000; Else & deSchoolmeester, 2003). Thus, overall orchestration of the protective intestinal response is a complex process that we are still unravelling at the molecular level (McDermott *et al.*, 2001; Else & deSchoolmeester, 2003). The availability of knock-out mice, in which the genes for specific components of the response have been inactivated, has clarified the essential requirement of cell adhesion molecules, cytokines such as interleukin(IL)-4, IL-9, IL-10 and IL-13 and the STAT6 signalling system in the expulsion of *Trichinella spiralis*, *Trichuris muris*, *Nippostrongylus brasiliensis* and resistance to *Heligmosomoides polygyrus* (Urban *et al.*, 1991, 1998, 2000; Bancroft *et al.*, 1998; Greenwald *et al.*, 1999; Schopf *et al.*, 2002) and has identified interesting and informative differences in the precise requirements for host-protective immunity for different species (e.g. *N. brasiliensis* where IL-4 is not essential for worm loss, at least in mice; Lawrence *et al.*, 1996).

Such studies, as well as those employing transgenic strains in which specific cytokine secretion is up-regulated and the recent use of chimaeric mice in combination with knockout genetic status, have demonstrated that there is considerable redundancy in the overall response (Finkelman *et al.*, 1991; Meeusen & Balic, 2000). While in each case, the afferent arm of the response, in which antigen is recognized and Th2 cells are activated, is very similar and drives events, eventually a range of effector mechanisms is activated, only some of which may be essential for loss of the initiating species. One of the most dramatic consequences of infection with gastrointestinal (GI) nematodes is the enormous influx of mast cells that occurs in the intestinal mucosa, first noted during *N. brasiliensis* infections in rats (Wells, 1962; Miller,

1971; Nawa & Miller 1979). However, the mast cell response in this host-parasite combination is now considered to be redundant and not essential for worm loss (Abe *et al.*, 1992). Rather, it is the goblet cell response involving the secretion of mucus that is crucial for expulsion of *N. brasiliensis* from rats and mice (Ishikawa *et al.*, 1993; Nawa *et al.*, 1994). Similarly, mast cells play little role in protective immunity to *T. muris* (Betts & Else, 1999), even though marked mastocytosis occurs in the caecum during primary infection (Lee & Wakelin, 1982). In contrast, the mast cell response is essential for expulsion of both adult *T. spiralis* and *Strongyloides ratti*. This range of effectors presumably evolved over time as hosts had to contend with a variety of different intestinal nematodes with varying effects on host fitness, and employing different strategies for evading specific components of host protective resistance (Behnke *et al.*, 2000).

The essential nature of key genes

The complexity of the overall response, and the requirement for it to be precisely orchestrated, implies that many genes must be involved, that hierarchical relations exist between them and that they become activated in a particular sequence (Artis *et al.*, 1999; Schopf *et al.*, 2002). The deletion of a key gene for an essential central process in the sequence may abrogate the response altogether. In fact the discovery of the nude mouse is one such example. Nude, or athymic mice, are unable to expel GI nematodes because they lack T cells (Jacobson & Reed, 1974), and this is attributed to just a single gene defect in a transcription factor that controls the generation of the thymic anlage and concurrently the development of hair papillae (Nehls *et al.*, 1994). Deletions of additional genes downstream can also block worm expulsion (Schopf *et al.*, 2002), but nevertheless may

enable some of the other component processes to proceed relatively normally and vice versa. For example IL-13 knockout mice, which do not expel *T. muris*, eventually generate strong Th2 responses including mastocytosis and exaggerated parasite-specific IgG1 antibody responses (Bancroft *et al.*, 1998). In contrast, IL-5 knockout mice do not show eosinophilia but expulsion of GI nematodes is not slowed (Finkelman *et al.*, 1991; Meeusen & Balic, 2000).

Natural variation in genes – alleles for resistance and susceptibility

Natural mutations that severely affect immunocompetence exist in nature (e.g. immunodeficiency diseases such as severe combined immunodeficiency or agammaglobulinaemia, DiGeorge syndrome, Janeway *et al.*, 1997). However, in nature, animals do not survive such dramatic deficiencies in key components of the immune response, and genetically-determined variation is usually quantitative, allowing survival but resulting in differences between individuals. Under conditions of natural exposure to infection a range of other intrinsic (e.g. age, sex, reproductive status) and extrinsic (season, location, climate, etc.) factors may also influence susceptibility and resistance to infection, so the genetic component is not always easy to identify. When the conditions of exposure are similar, differences in immunocompetence may be attributable to a large degree to differences in alleles of some of the key genes in the gene cascade that drives the response. One set of such alleles (haplotype) may enable a host to respond quickly, facilitating rapid clearance of worms, whilst a different haplotype can at best mediate a slow response or at worst no effective host protection.

It has been known for some time that breeds of domestic livestock differ in their capacities to control or resist GI nematode infections (Ackert *et al.*, 1933; Stewart *et al.*, 1937). Native breeds that have developed under conditions where parasite challenge is high, often have enhanced resistance to the locally most important GI nematode species, (e.g. *Haemonchus contortus*; Gray *et al.*, 1995). Such breeds as the Red Massai in East Africa, Louisiana Native in the USA, St. Croix in the Caribbean and the Javanese Thin Tail sheep in Indonesia, have been naturally selected for resistance (Preston & Allonby, 1979; Courtney *et al.*, 1985; Baker *et al.*, 1992, 1999, 2003; Baker, 1998) and therefore, carry alleles for resistance on some of the crucial genes involved.

This variation in capacity to respond to GI nematodes is also replicated in laboratory model systems that exploit combinations of syngeneic (inbred) mouse strains and murine nematodes (Wakelin, 1978, 1985, 1988, 2000; Bell, 1998).

Heritability of resistance to GI nematodes

The genetic basis of variation in resistance to nematode infections in mammals was initially suggested in the 1950s in work with *H. contortus* (Whitlock, 1955), but the first laboratory demonstration that indisputably confirmed the heritability of resistance to GI nematodes was based on work with *T. muris* in Schofield mice. Wakelin

(1975) showed that Schofield mice, approximately 70% of which normally clear *T. muris* infection, could be selectively bred to enhance resistance from 70% to 100%. In contrast, whilst initially approximately 30% of the parental stock failed to clear infection and therefore sustained chronic infection, the percentage could be increased to 70–80% by selective breeding for susceptibility (fig. 2). The experiments were continued until the sixth generation, at which time the resistant line was solidly resistant to infection whereas the susceptible line still included about 20–30% of individuals that managed to resist infection. This experiment suggested that more than a single gene is involved in determining whether Schofield mice resist or expel worms, and that the alleles for resistance are dominant. In the 1980s many other studies, based both on laboratory model systems (Brindley & Dobson, 1982; Bell, 1998) and on parasites of livestock, predominantly sheep parasites, expanded on this earlier work (Windon & Dineen, 1984; Albers & Gray, 1987; Gray, 1987) and established that in livestock heritability of resistance is generally of the order of about 0.2–0.4 (Kloosterman *et al.*, 1992; Gasbarre & Miller, 2000).

MHC based genes

Mouse strains differ profoundly in their responses to *T. muris* (fig. 3), *T. spiralis* (Wakelin, 1988; Bell, 1998) and *H. polygyrus* (Behnke & Wakelin, 1977; Behnke & Robinson, 1985; Enriquez *et al.*, 1988a). However, it was soon realized that, in certain systems, strains that showed the same MHC haplotype behaved similarly although not identically to one another (Wassom *et al.*, 1979). Thus, whilst mouse strains with the H-2^d haplotype (DBA/1, SWR, BUB/Bn) showed varying levels of resistance, all these strains were better at resisting *T. spiralis* than strains with the H-2^k haplotype (e.g. C3H, CBA, AKR). As in other parasitic, bacterial and viral infections, H-2 based genes may therefore account for some of the variation between strains and, by extrapolation, for a significant proportion of naturally occurring variation in disease resistance (Wakelin & Blackwell, 1988; Cooke & Hill, 2000).

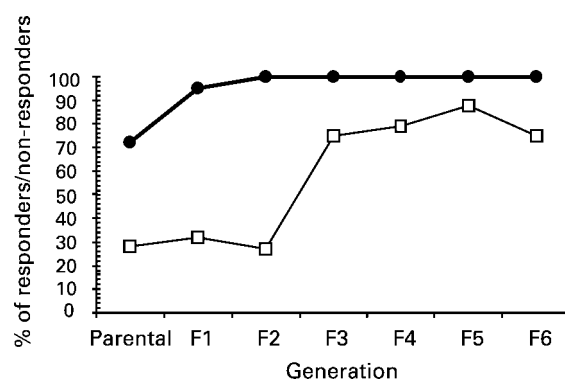


Fig. 2. Selection for resistance and susceptibility to infection with *Trichuris muris* in Schofield mice (Wakelin, 1975). Non-responder line (□) and responder line (●) mice.

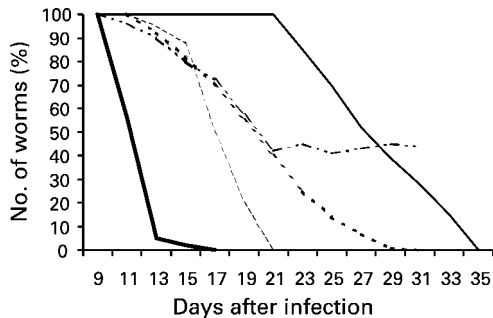


Fig. 3. Variation in the course of infection with *Trichuris muris* in five strains of inbred mice (—, NIH; ---, CBA; ···, C3H; - · - ·, DBA2; —, A) (Data from Wakelin 1978).

A complementary laboratory approach exploited H-2 congenic strains, in which the background genes are identical and the H-2 genes have been switched by selective breeding and backcrossing. The availability of a large range of strains on the B10 (C57BL/10) background and a limited range on the BALB background enabled the role of MHC genes to be confirmed. Again, in respect of *T. spiralis*, the H-2^k haplotype was shown to be associated with susceptibility (B10-BR), whereas H-2^q (B10.Q) and H-2^s (B10.S) were both resistant haplotypes. Much the same picture emerged for *H. polygyrus* (Enriquez *et al.*, 1988b) although for *T. muris*, H-2^k carrying mouse strains varied, some showing capacity to expel the parasite (CBA and BALB/k) while others failed to do so (B10.BR; Else & Wakelin, 1988).

Collectively these studies established an important role of variation among the MHC alleles in predisposing resistance/susceptibility to infection with GI nematodes in rodents. They were followed by studies demonstrating that MHC genes also play a role in regulating GI nematode infections in sheep (Outteridge *et al.*, 1985, 1988; Schwaiger *et al.*, 1995; Stear *et al.*, 1996; Paterson *et al.*, 1998) and other domestic animals (Stear *et al.*, 1988; Stear & Wakelin, 1998). The MHC locus encodes primarily genes that are involved in recognition of foreignness (non-self), including the class 1 and class 2 antigen presenting molecules on cells such as macrophages and dendritic cells. These findings were therefore in accord with the proposed mechanisms of resistance as illustrated in fig. 1. However, they emphasized that genes outside the MHC, background genes, also play a role, in fact if anything, a more important role in this respect (Bell, 1998). Figure 4 shows that loss of *H. polygyrus* from B10.S (H-2^s) mice is more rapid than from B10 (H-2^b) mice, which differ only in alleles at the MHC, but it is considerably faster in SJL mice, that share H-2 with B10.S but differ in other parts of their genome (Behnke & Wahid, 1991).

Whilst there was no possibility at this stage to investigate further the background genes, the availability of MHC recombinant mice in Chella David's laboratory enabled the MHC genes involved in resistance to *T. spiralis* to be localized more precisely (Wassom *et al.*, 1979). This led to the hypothesis that at least two genes, mapping within the MHC were

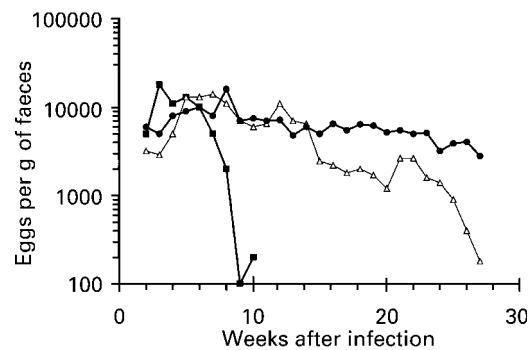


Fig. 4. Comparison of the rate of loss of *Heligmosomoide polygyrus*, as reflected through faecal egg counts, from three strains of mice. B10 (●) and B10.S (Δ) mice differ in MHC haplotype but have the same alleles for other genes. B10.S and SJL (■) mice share MHC haplotype but the alleles of their non-MHC background genes differ. (Data from Behnke & Wahid, 1991).

involved. The first of these, the Ts-1 gene was shown to map to the I-A locus, and in this case the *s* haplotype facilitated resistance and the *k* haplotype susceptibility. The second was shown to map to a region between the S and D loci of the H-2. Here the *b* and *s* haplotypes were linked to resistance, and the *d* haplotype with susceptibility (Wassom *et al.*, 1983). Comparable studies on *H. polygyrus* established that two MHC genes were also involved and that the Ts-1 locus was probably also crucial for resistance to this species (Enriquez *et al.*, 1988b).

Drawing on all of their individual and combined studies, in 1984 Wassom *et al.* put forward their hypothesis concluding that 'the anti-adult response, the anti-fecundity response and the rapid expulsion response are under independent genetic control and influenced by the interacting products of both H-2 and non H-2 genes'. In other words, genetic differences between animals at several loci, both in the MHC and outside the MHC, influence resistance to infection and different combinations of genes may be responsible for different manifestations of resistance, such as effects on faecal egg counts, growth of worms, and worm burdens (fig. 5).

Other studies supported the idea that non-MHC genes played a significant role in worm expulsion (Bell, 1988) and that MHC genes moderated or fine-tuned the influence of non-MHC genes (Bell, 1998), but the loci of these non-MHC genes have proved to be elusive. Currently with just two exceptions, mapping of the genes whose different alleles influence susceptibility/resistance to infection has been focused on the MHC (table 1). One exception is a gene on chromosome 4, linked to the FV-1 locus, that influences the accumulation of muscle larvae in *T. spiralis* (Wassom *et al.*, 1987). The second exception is the interferon-gamma (IFN-γ) gene on chromosome 3 in the ovine genome, which has been identified as playing an important role in predisposing resistance to *H. contortus* in farmed sheep in Australia (Crawford & McEwen, 1998; Paterson *et al.*, 2001) and in a wild population of Soay sheep living on the island of Hirta in the outer Hebrides (Coltman *et al.*, 2001).

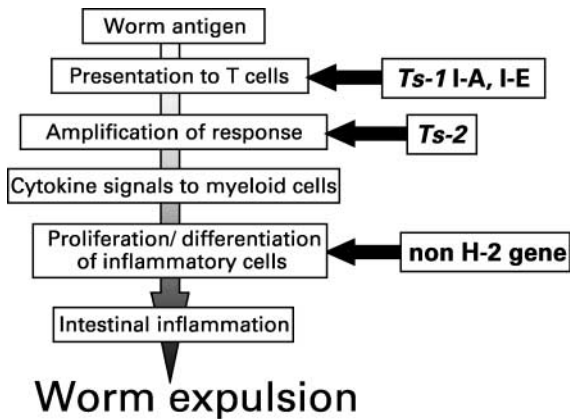


Fig. 5. Schematic representation of the stages at which Wassom *et al.* (1984) hypothesized that genes controlling the response to *Trichinella spiralis* exerted their influence on worm expulsion.

A different approach – linkage mapping exploiting microsatellites

The exploitation of syngeneic mouse strains, their H-2 congenic and recombinant strains enabled significant progress, and was facilitated largely by the perceived importance of the MHC not only in resistance to parasites but also many other diseases. However, the MHC genes constitute only a small fraction of the murine genome, spanning less than 10% of chromosome 17 (approximately 8 Mb, and 5 cM) and occupying less than 0.3% of the genetic distance across all the chromosomes combined (Allcock *et al.*, 2000). Loci for the other, non-MHC, genes were not known and could potentially reside anywhere among the remaining 19 murine chromosomes as well as on non-MHC encoding sections of chromosome 17. An alternative approach was required.

By the 1990s microsatellite technology had achieved major progress and by 1996 over 7000 microsatellite markers were available mapping right across the mouse genome (Dietrich *et al.*, 1996). Since then many

additional markers have been developed for the murine genome as well as those of humans and domestic animals, and these have been exploited successfully to map quantitative trait loci (QTL) for various traits including disease resistance (Crawford, 2001; Korstanje & Paigen, 2002). The first QTL to be mapped successfully for resistance to parasites were those for trypanosome infections in mice (Kemp *et al.*, 1997; Kemp & Teale, 1998; Iraqi *et al.*, 2000).

Considerable progress has been made since with mapping the QTL for resistance to GI nematode infections in sheep, where attention has been focused on a region of chromosome 3 that includes the IFN- γ gene (Crawford & McEwen, 1998; Paterson *et al.*, 2001). Polymorphism in this region (o(IFN)- γ_{126}) was also linked to reduced faecal egg counts and increased parasite specific IgA in a wild population of Soay sheep on the island of Hirta in the St Kilda archipelago in the Outer Hebrides (Coltman *et al.*, 2001).

In the human context, Williams-Blangero *et al.* (2002) have recently identified QTL for susceptibility to *Ascaris lumbricoides* in a community in eastern Nepal, mapping to chromosomes 1 and 13. The strongest effect was on chromosome 13, where the QTL spanned a 20-cM region located in the distal end of this chromosome, a region where relatively few genes have been identified. However, a gene from the tumour necrosis factor superfamily of cytokines (TNFSF13B) is located here and since TNFSF13B is considered to be a regulator of B lymphocyte function it was proposed as the candidate gene responsible for the QTL. No candidates were identified for the gene on chromosome 1, but another interesting locus on chromosome 8 that did not achieve overall significance in genome-wide analysis coincided with the loci for IL-7 and for activated B-cell factor (ABF1), which are involved in mucosal immunity and B cell function respectively.

Mapping the QTL for *Heligmosomoides polygyrus*

In 1998 we initiated a project to begin mapping the QTL for resistance to *H. polygyrus* in mice. We chose

Table 1. Loci of genes known to be involved in resistance to gastrointestinal nematodes.

Species	Host	Designation	Locus	Alleles		Reference
				R*	S*	
<i>T. spiralis</i>	Mice	Ts1	H-2, I-A β	s, q, f, b	k	Wassom <i>et al.</i> , 1983
<i>T. spiralis</i>	Mice	Ts2	H-2, between S & D	s, b	d	Wassom <i>et al.</i> , 1983
<i>T. spiralis</i>	Mice	?	near FV-1 chrom 3			Wassom <i>et al.</i> , 1987
<i>H. polygyrus</i>	Mice	?	H-2 left of I-E α	q, f, s	k	Enriquez <i>et al.</i> , 1988b
<i>H. polygyrus</i>	Mice	?	H-2, D end			Enriquez <i>et al.</i> , 1988b
<i>T. muris</i>	Mice	?	H-2, I-A	q, b	k, d	Else <i>et al.</i> , 1990
<i>T. muris</i>	Mice	?	H-2, D end	q	b, d	Else <i>et al.</i> , 1990
<i>T. circumcineta</i>	Sheep	?	MHC-DRB1	G2	1	Schwaiger <i>et al.</i> , 1995
<i>T. circumcineta</i>	Sheep	?	MHC-DRB1	?	?	Buitkamp <i>et al.</i> , 1996
<i>T. circumcineta</i>	Sheep	?	MHC-DY	?	?	Buitkamp <i>et al.</i> , 1996
<i>T. circumcineta</i>	Sheep	?	o(IFN)- γ chrom. 3q	126	130	Coltman <i>et al.</i> , 2001
<i>T. circumcineta</i>	Sheep	?	MHC OLADRB	263	257, 267	Paterson <i>et al.</i> , 1998
<i>H. contortus</i>	Sheep	?	IFN- γ chrom.3q	A	B	Paterson <i>et al.</i> , 2001

*R, alleles associated with resistance; S, alleles associated with susceptibility.

H. polygyrus because this is a well studied species (Monroy & Enriquez, 1992) with which we have worked for many years in Nottingham, and one with which we were very familiar (Behnke, 1987). Moreover, in contrast to *T. spiralis* and *T. muris*, it is arguably more relevant as a model for understanding resistance in domestic livestock because it is more closely related to species such as *H. contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Durette-Desset, 1985). Another key difference between *H. polygyrus* and the other murine species is that it does not normally elicit a rapid protective response in mice, but rather single pulse laboratory infections result in worms surviving for more than 30 weeks and even longer (Robinson *et al.*, 1989). It is therefore a convenient and relevant model of chronic GI nematode infections, such as those experienced by domestic ruminants grazing on pasture (Behnke, 1987; Behnke *et al.*, 1992; Monroy & Enriquez, 1992). We also chose a trickle infection protocol to mimic the continuous acquisition of larvae that occurs as animals graze on pasture (Brailsford & Behnke, 1992).

The selection of mouse strains to be used as founders of the resource population for mapping was also critical. We chose CBA (H-2^k) mice because this strain is well documented as harbouring long chronic primary infections, responding slowly with antibody and mucosal mastocytosis, and not readily acquiring immunity to re-infection (Behnke & Robinson, 1985; Robinson *et al.*, 1989; Wahid *et al.*, 1994). Our second strain was the SWR (H-2^g), which expels primary infections within 6–8 weeks of exposure to third-stage larvae (L3s), responds rapidly with mast cells and antibodies, and becomes solidly resistant to re-infection after minimal experience of L3s (Wahid *et al.*, 1989). Moreover, these two strains showed the contrasting MHC haplotypes that had earlier been linked with susceptibility and resistance.

The time course of infection in a pilot experiment is summarized in fig. 6a. This shows that worm burdens accumulated in CBA mice to achieve a plateau in week 4, by which time the worm burden was about 400–500 worms per mouse. In contrast, SWR mice resisted infection from the outset and by week 4, despite continued weekly re-infection, were solidly resistant to further infection. We monitored several accompanying immune responses throughout these pilot experiments and eventually chose four for inclusion in our search for QTL for resistance. As can be seen from fig. 6b, the mucosal mast cell 1 (MMCP1) response differed markedly between the two strains, peaking in weeks 2 and 3 in SWR mice just preceding the loss of established adult worms, whilst increasing comparatively slowly in CBA mice.

Week 6 was eventually chosen as the optimal time to conduct worm counts. We monitored egg counts in weeks 2, 4 and 6. Mucosal mast cell 1 levels were recorded in week 3, and the granulomatous response, IgE to fourth stage larvae and IgG1 to adult worm antigens in week 6, when the responses in the parental strains were most divergent. In total, we phenotyped 514 F2 mice in 13 separate experiments (40 F2/experiment), each of which was controlled by the inclusion of parental strain mice of both sexes. For genotyping we selected 272 mice

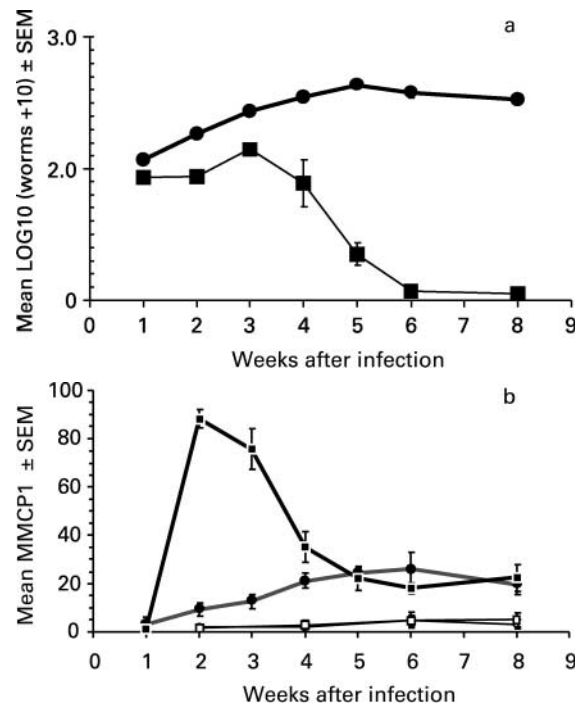


Fig. 6. (a) Changes in worm burden during the course of trickle infection with *Heligmosomoides polygyrus* in SWR (■) and CBA (●) mice. (b) The concentration of MMCP1 in the serum of SWR (■) and CBA (●) mice during the course of trickle infection with *H. polygyrus* and in naive uninfected SWR (□) and CBA (○) control mice.

representing 155 of the most resistant and 117 of the most susceptible animals. These were screened on chromosomes 1, 11, 12 and 17. Of the 272, 204 were screened on all the remaining autosomes and the X chromosome. To achieve nearly 100% genome coverage 175 informative microsatellites were used.

Figure 7 summarizes the details for the four QTL that were unequivocally significant, and as can be seen these are located on chromosomes 1, 2, 13 and 17 (Menge *et al.*, 2002; Iraqi *et al.*, 2003). Each QTL spans regions of 20–30 cM but in all four cases the alleles for resistance came from SWR mice, and in all cases the alleles for resistance were dominant, as we had predicted from our earlier observations. There is no certainty as to which genes are involved at this stage but some candidate genes can be suggested. Consistent with earlier studies in congenic mice, a significant QTL for worm counts lies on chromosome 17 in the region of MHC. However, the MHC region also encompasses other, non-MHC (e.g. TNF locus), genes and it is possible that fine mapping may eventually identify some of these as playing a significant role in resistance. The QTL on chromosome 13 encompasses the gene for IL-9 and this is also of interest since upregulation of this cytokine in IL-9 transgenic strains, which constitutively express high levels of IL-9, leads to rapid clearance of adult worm burdens (R.K. Grecnis *et al.*, personal communication). We have also recognized a significant QTL on chromosome 17 for the early fecundity response (faecal egg counts in week 2), that maps to the distal region of this chromosome and for which the allele

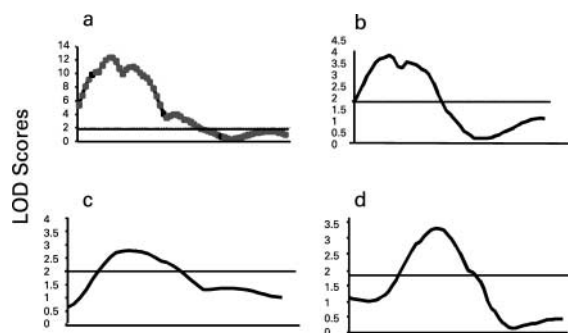


Fig. 7. Quantitative trait loci for worm burdens on four murine chromosomes. The figure shows the LOD score for worm burdens at week 6, across each of the chromosomes (proximal end is on the left and the distal on the right of each graph) where a significant QTL was found to segregate. Some candidate genes known to have loci within the range of each QTL are given in brackets. (a) chromosome 1 (IL-1r (type 1 and 2), Stat 4, and CD 28); (b) chromosome 19 (MHC, MCP 6 and 7 and trefoil factors 1-3); (c) chromosome 13, (T cell receptor γ chain and IL-9); (d) chromosome 2 (Integrin $\alpha 2$ and RAG1 and RAG2). A value greater than 2 is indicative of a QTL segregating at the locus but note that all data were fully analysed by appropriate statistical methods. (Data from Iraqi *et al.*, 2003.)

for resistance comes from CBA mice (Iraqi *et al.*, 2003). Several QTL for the accompanying immunological responses have been identified (Menge *et al.*, 2002) but it is still too early to present a clear picture of these. However, it is apparent that neither worm counts, nor faecal egg counts are prominently linked to any QTL for the immunological measures that we quantified other than those on chromosome 17. Here the QTL for all four measures superimpose on those for worm counts in the proximal region of this chromosome. If these QTL are eventually fine mapped to the MHC, one interpretation may be that once recognition has occurred, all the effectors are activated, even if they do not reflect a crucial component of the mechanism responsible for protective immunity. This concept of redundancy in the effector mechanisms elicited by type 2 responses to any specific nematode has been discussed earlier. It is consistent with the idea that the current complexity of intestinal responses evolved through selection pressure on hosts from a large range of mucosal pathogens with their own antigenic characteristics and intricate evasion strategies (Finkelman *et al.*, 1991; Behnke *et al.*, 2000).

The future

In order to fine map the QTL identified in the F2 resource population, we have already completed the phenotyping for worm counts and faecal egg counts of 1095 F6/F7 hybrids (advanced intercross line (AIL) population). The accompanying immune responses are still undergoing analysis and the genotyping is well under way. After completion of the fine mapping, we will apply multiple approaches for high resolution mapping (Iraqi, 2000) of the QTL to even more precise genomic regions and eventually confirm the genes underlying the

QTL by cloning. In turn, this information will facilitate and simplify the search for homologues in the genomes of humans and sheep (Zadissa *et al.*, 2001). The latter will be aided by the parallel programme at the International Livestock Research Institute mapping QTL for resistance to GI nematodes in sheep (Okomo *et al.*, 2000) and, once these are known, they will provide a valuable tool for selective breeding of sheep to improve resistance. Genetic markers will greatly accelerate the development of genetic resistance in productive breeds that are currently threatened throughout the pastoral regions of the globe by the rapid spread and growing threat of resistance to anthelmintic drugs among GI nematodes of domestic small ruminants (Waller, 1986, 1997; Coles, 1998; Jackson & Coop, 2000).

If economically feasible, genetic markers for susceptibility in humans could facilitate targeted anthelmintic use and thereby help to offset and hopefully avoid altogether the threat of anthelmintic resistance. Species such as hookworms still pose a continuing threat to communities in the tropics and poor responses to anthelmintic treatment, suggestive of drug resistance, have been reported recently (De Clercq *et al.*, 1997; Reynoldson *et al.*, 1997). Alongside development programmes aiming to improve water quality and sanitation facilities, another approach may be through education attempting to change sanitary behaviour and emphasizing the need for footwear in the case of hookworms, focusing in particular on the susceptible sectors of human communities, once these have been identified. Finally, awareness of the genetically determined resistance status of subjects may have implications for efficient vaccine delivery in the future, since it is precisely the susceptible individuals who will benefit most from vaccines.

Acknowledgements

The authors gratefully acknowledge grants 053419/Z/98/Z and 063810/Z/01/A from the Wellcome Trust. We thank Simon Clifford, Anna Lowe, Jill Brown and David Fox for contributions to this work in Nottingham and Bob King, John Mugambi, John Wambugu, Moses Ogugo, Daniel Mwangi and Nemwel Nyemweya at ILRI in Kenya.

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(Accepted 17 February 2003)
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