Helminth Immunogenetics: Why Bother?

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The importance of host genotype as a determinant of protective responses against helminth infection is well established. In contrast, there have been relatively few investigations of the role of helminth genotype, despite the importance accorded to the genetics of other disease-causing organisms. Here, Andrew Read and Mark Viney discuss the reasons for this oversight. They argue that it is not for any compelling empirical reason: there is at least as much evidence that worm genetics affects host protective responsiveness as there is that it does not.

The term 'immunogenetics' normally describes studies of the role of host genetics in immune function, and it is that bias with which we are concerned. Helminth immunologists have largely ignored parasite genetics, as several have noted[1–3]. Yet much of their effort is aimed at developing effective vaccines against geographically widespread parasites and, in the long term, the efficacy of vaccines and selection programmes to improve host resistance crucially depends on whether helminth populations can respond to immune-imposed selection. Parasite genetic heterogeneity can also have profound epidemiological effects[2,4,5] and is essential for host–parasite co-evolution. Given these implications, the negligible attention paid to helminth immunogenetics is rather striking. It is certainly not because parasitic helminths are peculiarly homogenous: genetic variability in parasitic helminth species is comparable to that of free-living organisms[6].

Here we assess whether there are good empirical reasons to ignore worm genetics in helminth immunology. We concentrate on host responses that are functionally protective. Reports of antigenic polymorphism in helminths (eg. Refs 7–12) or differences between worm lines in their ability to elicit or modulate particular immune parameters (eg. Refs 13, 14) are considered only when there is additional evidence that these phenomena are functionally important. Among helminths, there are reports of antigenic variation unrelated to variation in immune susceptibility[12] and, conversely, of clonal variation in immune susceptibility despite apparent antigenic monomorphism[15]. Likewise, interspecific differences will not be considered, except to note that the quite different infection profiles of different helminth species in the same host emphasize that parasite factors are important determinants of host immunoresponsiveness. Protection elicited by one parasite species against another could mean that intraspecific genetic variation is irrelevant, but only if cross-species immunity is sterilizing.

How might worm genetics affect or alter a host's immune response? Worms may vary in the extent to which they elicit host responses (immunogenicity) directed both against themselves and against subsequent conspecific infections. Similarly, they may vary in their ability to suppress the immune response (immunomodulation) or to mitigate or evade the immune-effector mechanisms. In fact, there is some evidence for a role of worm genetics in all of these.

Gastrointestinal nematodes.

By far the most comprehensive examination of the role of helminth genetics in host responsiveness has used different isolates of *Trichinella spiralis* in mice. Within the same mouse strain, different parasite isolates vary in the kinetics of primary infection[16–19]. This may be due, in part, to intrinsic differences in isolate fitness. However, these differences depend on the responsiveness of the host strain and, significantly, immunosuppression can remove differences between parasite isolates (Fig. 1).

There is direct evidence that host responses against *T. spiralis* are, in part, isolate-specific. Worm survival and reproduction in challenges with heterologous isolates was frequently different from that in challenges with homologous isolates, despite often considerable crossprotection*. This was so following immunization either with live worms[9] or with antigen preparations[20,21]. The outcome for any particular combination of isolates was repeatable, with worm survival and fecundity generally higher following heterologous challenges. Occasionally, homologous challenges did better, suggesting isolate differences in immunomodulatory ability. Less-direct evidence for a role for *T. spiralis* genetics comes from measurement of various immune parameters. Using immunization either with live worms or with antigen preparations, different parasite isolates have been shown to elicit qualitatively and quantitatively different cytokine, inflammatory, antibody and lymphocyte responses[9,13,14,17–21]. This together with the parasitological data, makes it clear that '*Trichinella* isolate' is an important determinant of functional protection.

However, parasite genetics may be unusually important in the *T. spiralis*–mouse interaction. First, *T. spiralis* is one of the most widely distributed and least host-specific of all nematodes. The isolates used in these studies come from different hosts (foxes, bears, pigs, cats, rats) and various geographical locations (Hong Kong, New Zealand, Spain, UK, USA) and may actually represent highly divergent gene pools. The taxonomy of *T. spiralis* has proved both difficult and controversial. In addition, responses of pigs are known to be qualitatively different to those of the mouse and it is not clear how relevant studies of *T. spiralis* in mice are to natural infections[13]. Finally, under some circumstances, host debilitation and death enhance predation risk and hence transmission, so that

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* Throughout this article, the term 'heterologous' refers to conspecific parasite lines of different origin.

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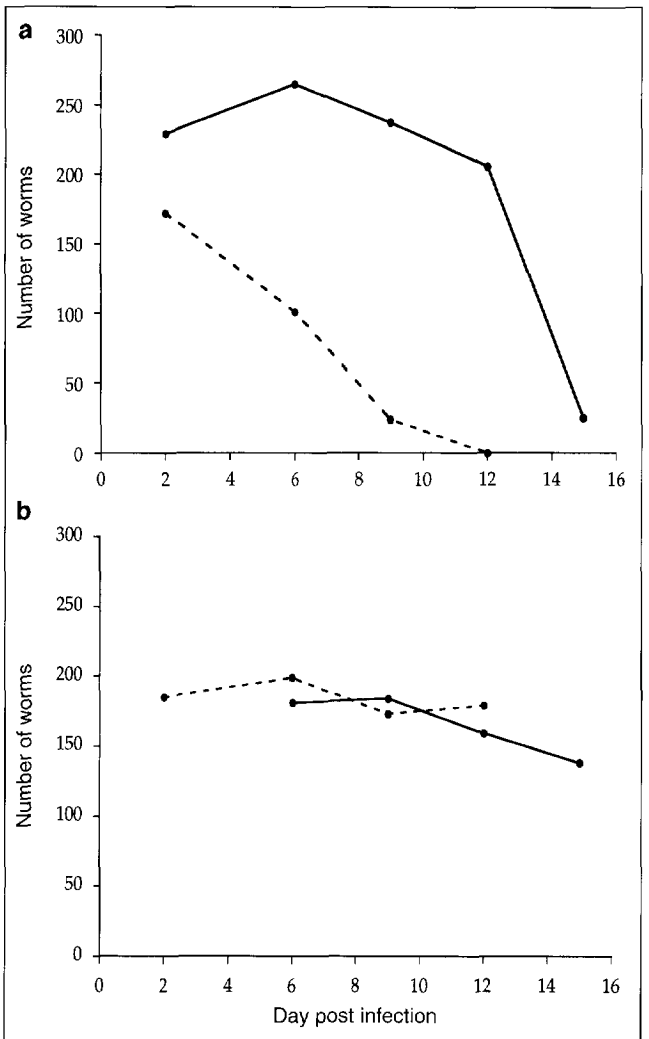


Fig. 1. Kinetics of primary infections with 300 larvae of *Trichinella spiralis* isolates S (solid line) and W (dashed line) in normal NIH mice (a), and immunosuppressed NIH mice (b). The difference between isolates is removed in immunosuppressed animals. (Data from Ref. 17.)

T. spiralis may retain its immunogenicity in order to promote immunopathology[22]. The importance of these caveats is unclear.

Some of the pioneering experiments examining differences in host responses to different parasite isolates remain unpublished. These utilized a natural host–parasite system. Using two geographical isolates of *Strongyloides ratti* in rats, Katherine C. Carter (PhD Thesis, University of Edinburgh, UK, 1986) showed that a primary infection with one isolate afforded greater protection against homologous challenge than against heterologous challenge; primary infection with the other isolate afforded protection that was the same against both homologous and heterologous challenge. This effect could be reproduced using adoptive transfer of mesenteric lymph node cells. Using that technique, she also showed that the two isolates differed quantitatively in the immune reaction that they stimulated, with the isolate that protected equally against homologous and heterologous challenge being the more immunogenic.

There has been little direct experimentation with isolate differences in other gastrointestinal nematodes. Recent work on two geographical isolates of *Trichuris muris* revealed substantial differences in the expulsion of the primary infections from the same mouse strain[23]. These differences were not just due to intrinsic viability differences: reproductive performance in immunosuppressed mice was similar, and the quantitative differences in worm burden between isolates in normal mice were different in high- and low-responder mouse strains. Interestingly, these authors also found repeatable differences between two lines derived from one isolate but maintained as separate laboratory populations for less than 100 generations. Whether this divergence is a result of founder effects or of mutation accumulation and drift is unclear; the former would suggest intra-isolate genetic variation.

Other isolate comparisons provide more equivocal results. Two geographical isolates of *Trichostrongylus colubriformis*, one drug sensitive, the other drug resistant, differed in their fecundity in rabbits (not the normal host). This may have been due to observed differences in the severity of the intestinal mucosa response (greatest response against the drug-resistant isolate), or may represent a fecundity cost to drug resistance[24]. H11 is a *Haemonchus contortus* gut membrane-derived antigen that is being developed as a candidate vaccine for sheep. H11 derived from an Australian and a UK isolate were used in homologous- and heterologous-challenge experiments[25]. Vaccination by the subcutaneous route resulted in less-effective protection against heterologous challenges compared to homologous challenges; worm burdens were three times higher in the heterologous challenge infections. Whether this was simply due to slight differences in the quality of the extracted H11, or genuine isolate-specific protection cannot be determined from the experimental design used. When the experiment was repeated with vaccination by the intra-muscular route, which provoked a more-rapid antibody response, there was no evidence of isolate-specific protection. Intramuscular vaccination with H11 from two Australian isolates also revealed no differences between homologous- and heterologous-challenge infections[25].

Gastrointestinal nematodes can be used in selection experiments and several attempts have been made to see whether worm survival in previously exposed animals differs between nematode lines maintained in high-responder or immunized animals and those maintained in low-responder or naive animals. The results have been mixed. In a heroic but minimally reported effort, Dobson and Tang[26] passaged lines of *Heligmosomoides polygyrus* (presumably from the same ancestral stock) through groups of (1) naive, (2) once previously exposed, and (3) multiply exposed mice. This selection regime produced lines that differed in fitness when tested in semi-immune animals, with the lines selected in the most-resistant hosts surviving best. The response to selection was particularly marked during the first ten generations. The underlying mechanism is unclear. Subsequent analysis of the lines revealed minor molecular differences and some differences in antigens recognized on western blots[12]. These differences may be related to the different survival of the lines in immune hosts or may just be due to genetic drift between separate lines. In an even briefer report, Windon[27] describes a selection experiment using *T. colubriformis* in sheep, where it was found

that worms passaged just once through vaccinated sheep survived better in high-responder sheep than did those passaged through naive sheep.

Two selection experiments on different host–parasite systems have thus revealed evidence for heritable variation of worm fitness in previously exposed hosts. The necessary corollary is that there is natural variation present for this trait, even in laboratory parasite lines that are likely to be highly inbred. In contrast, no response to selection was observed in *H. contortus* lines passaged repeatedly through the same (increasingly resistant) individual sheep[28,29] or passaged in genetically resistant or susceptible sheep for 14 generations[30]. This absence of response is particularly notable, given the rapidity of the response in the *H. polygyrus* and *T. colubriformis* experiments. These contrasting results may arise because of qualitative differences in immune evasion or regulation by these taxa, or differences in the selection regimes employed.

Filarial nematodes

We are not aware of any studies that have examined filarial worm variation in eliciting functional responses, but there is a recent suggestion that such variation might exist. Ravindran *et al.*[31] investigated the reactivity of sera from asymptomatic microfilaraemic individuals to microfilariae (Mf) from five patients infected with *Wuchereria bancrofti*. The sera reacted with none or only one of the five sources of Mf; one serum sample reacted with two. One possible explanation for this is that the immune response elicited by Mf is specific and that Mf vary. These are the basic elements of strain-specific immune responses. The chronicity of filarial infections seems to be due, at least in part, to the immunomodulatory efforts of the worms so that immune responses may have a large strain-independent component. Even so, a comparison of the ecologically and genetically distinct[32] forest and savannah 'forms' of *Onchocerca volvulus* might be worthwhile. They show some clinical and antigenic differences[7,32] and it would be interesting if, despite extensive crossreactivity between filarial species, protection was, in part, 'form'-specific. Apparently, that possibility has not been examined.

Vast numbers of Mf are produced by sexual reproduction and persist in the host; it is difficult to believe that this mode of reproduction is without consequence for the survival of the larvae. Moreover, a variable clinical outcome is one of the striking features of filarial infections, but the possibility that genetic variation among parasites may play a role in this has not been examined.

Schistosomes

There have been several analyses of the importance of schistosome variation in eliciting protective responses. However, egg-associated liver pathology arising from primary infections can provoke non-specific loss of challenge larvae[33], making studies using live larvae for immunization difficult to interpret. Experiments immunizing with UV-attenuated or -irradiated cercariae avoid this problem. Many of these have found evidence of cross-species protection, and frequently report extensive crossprotection, at least among closely related species[33]. However, protection against homologous challenge is generally stronger,

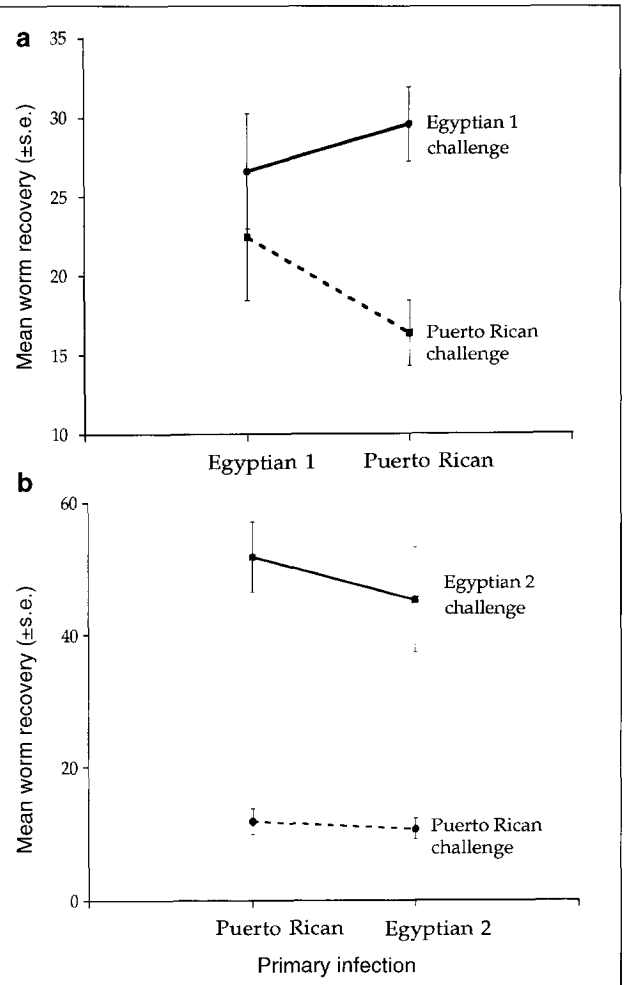


Fig. 2. Homologous and heterologous challenges with lines derived from isolates of *Schistosoma mansoni* following immunization with irradiated cercariae. Isolate-specific protection following immunization with ~250 cercariae is shown (a) (data from Ref. 8). Isolate differences in adult worm burden in immune animals can be seen following immunization with ~500 cercariae (b) (data from Ref. 38). Puerto Rican parasites are probably from the same isolate in both studies; the Egyptian isolates probably differ. Each point represents mean for seven and ten mice in the two studies, respectively. s.e., standard error.

leaving open the possibility that intraspecific genetic variation could also play a role. Attempts to investigate this focus on comparisons of lines derived from isolates from different geographical regions, or of clones derived from inbred laboratory lines. The picture that emerges is somewhat mixed.

Mice vaccinated with a Chinese isolate of *S. japonicum* were better protected against homologous challenge than against heterologous challenge with a Philippine isolate[34]. There is no evidence from DNA studies that these strains represent sister species[35]. However, different experiments with four geographically distinct Chinese isolates revealed no significant differences between homologous and heterologous challenges[36]. Work on *S. mansoni* cercariae has produced similarly contrary results. In one study, a Puerto Rican isolate of *S. mansoni* elicited protection twice as effective against homologous than against heterologous challenge (Fig. 2a). Tantalizingly, an antigen present in the Egyptian isolate, but not in the Puerto Rican isolate, was observed by immunoprecipitation[8]. In contrast, two other studies found that immunity

induced by isolates from Egypt, Kenya, Brazil, St Lucia and Puerto Rico was wholly crossprotective[37,38]. However, data from one of these studies[38] demonstrate differences between isolates in their survivorship in immunized hosts (Fig. 2b). This may be due, in part, to line differences in intrinsic viability independent of host response.

Oddly, there have apparently been no comparisons of the specificity of responses against different schistosome isolates from the same population. There have, however, been several attempts to analyse the protection elicited by different clones from single isolates. These have provided a consistent picture: no evidence of intra-isolate variation. No response to selection was observed after passage of a Puerto Rican line through pre-exposed hosts for five generations[39], and experiments involving nine clones of an Egyptian isolate[8] or seven clones of the Puerto Rican isolate[40] revealed no differences in protection against homologous or heterologous challenge. It is difficult to know what to make of these results. Laboratory lines are frequently maintained in genetically uniform (and naive) vertebrate and invertebrate hosts. Such selection has been shown to reduce genetic variability in *S. mansoni*[41,42]. In addition, parasite lines are often highly inbred and must have gone through frequent bottlenecks in their history: one widely used Puerto Rican line was derived from just a few patients half a century ago.

Conclusions

Available data support the idea that helminth genetics can be an important determinant of host responsiveness: there is at least as much evidence that worm genetics plays a role in host responses as there is that it does not. Evidence for isolate-specific responses has been reported in *Schistosoma* spp, *T. spiralis*, *H. contortus* and *Strongyloides ratti*. Furthermore, selection of *H. polygyrus* and *T. colubriformis* have demonstrated heritable variation in the ability to survive in previously exposed hosts, and line differences in the kinetics of infection in hosts of differing immune status have been reported in *Trichuris muris*, *Trichinella spiralis* and *S. mansoni*. Yet most studies of helminth immunology, even those analysing worm variation (!), involve one (or, at most, a few) inbred parasite lines.

Generalizations about the importance of helminth genetics in host responsiveness are not easy, not least because there have not been many relevant studies. The quest is compounded by problems with much of the data that we do have. One notable oversight has been analyses of variation within populations. For the most part, only differences between geographical isolates, or between sub-lines or clones derived from single inbred laboratory lines have been analysed. Within-population variation is the stuff of evolution. Furthermore, there is a common problem running though experimental analyses of isolate-specific protective responses. Typically, hosts are exposed either to live worms or to antigen preparations and the relative success of homologous and heterologous challenges compared (unfortunately, not always with the same sized dose within an experiment!). For the most part, such experiments were designed to look for evidence of crossprotection, which they usually show to be extensive. But experiments demonstrating significant crossprotection are not necessarily capable of determining whether there is an additional isolate-specific component to protection. Line differences in the ability to survive in immune hosts (irrespective of the immunizing strain) or in innate viability irrespective of host immune status (eg. Fig. 2b) can confound the picture. It is possible to untangle these effects, but only by including the reverse treatments in the experimental design. Formally, if there is isolate-specific protection, an interaction between immunizing genotype and challenge genotype will be a statistically significant determinant of the outcome of a challenge (Box 1). Only one of the experiments summarized above was formally tested for such an interaction; most did not include sufficient treatment groups to look for it. Nevertheless, statistically significant results from incomplete designs are of interest in the current context because they demonstrate some sort of effect of 'parasite line'. Insignificant results are far less interesting: they can easily arise because isolate-specific protective responses are confounded with isolate differences in immunogenicity or immune susceptibility, producing evidence for neither when, in fact, both are occurring (Box 1).

Furthermore, immunizing dose and experimental protocol may affect the likelihood of detecting line effects. Evidence of isolate-specific responses in *Haemonchus* was detected following H11 vaccination by a route which results in a less efficient host response; no such evidence was found using a method which elicits a stronger response[25]. Similarly, differences in the kinetics of infection between *Trichinella* isolates were often undetectable in high responder mouse strains[16], and it may not be entirely coincidental that the irradiated cercariae studies which revealed an effect of geographic isolate on protection used smaller immunizing doses (typically <400) than those that failed to find any such effect (typically $\geqslant 500$). Such considerations raise the interesting possibility that helminth genotype may be of greater relevance in low-exposure situations (as occurs in Nature, and following a successful intervention programme) or in hosts less able than warm, well-fed laboratory animals to mount a strong response. Finally, it is not obvious how much the pervasive use of non-natural hosts affects the overall picture. In general, host responses are stronger against parasites that are not adapted to exploit them.

The oversight

Why has helminth genetics been largely ignored by immunologists? Certainly, worms are less often controlled by direct interaction with immunologically specific effectors than are many other parasites, but this does not rule out isolate-specific immune recognition as a trigger of non-specific mechanisms. Similarly, there is a perception of antigenic monomorphism within helminth species compared to, for example, protozoa[43]. But it is a large step from a perception of antigenic monomorphism to demonstration of functional protection and an even bigger step to conclude that helminth genetics is unimportant. For one thing, as protozoan immunology has demonstrated, immunodominant antigens are not necessarily involved in protective responses.

Box 1. How to Demonstrate Protective Genotype-specific Responses

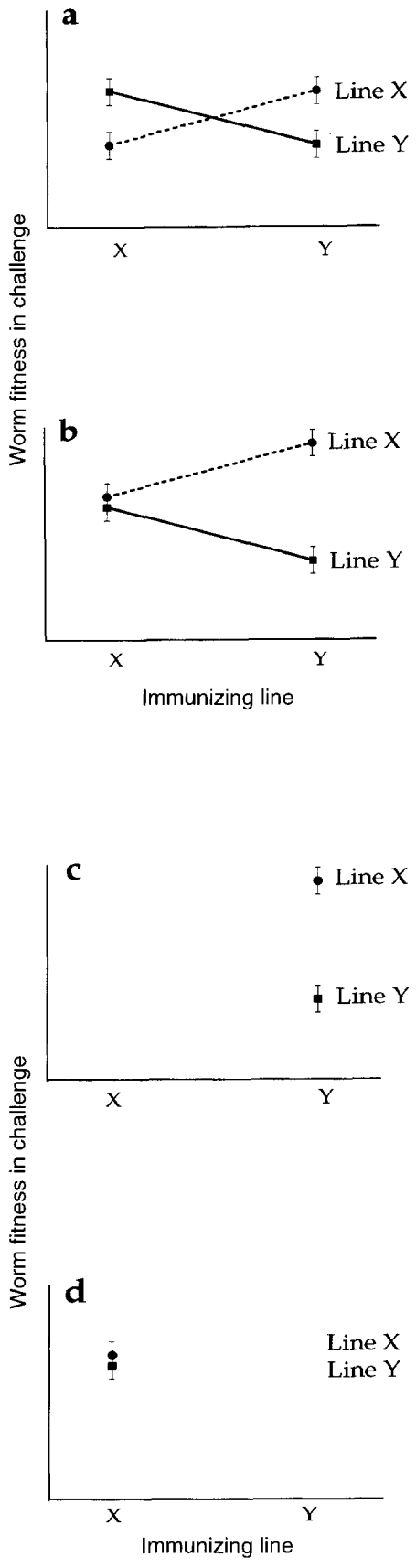
Do helminth genotypes differ in their ability to survive and reproduce in immune hosts? Do they elicit responses that are more protective against themselves than against other genotypes? These two questions are biologically quite different, but can be confounded by inappropriate experimental designs. At best, this leads to evidence for either genotype-specific protection or genetic variation in immune susceptibility. At worst, it results in evidence for neither when, in fact, both phenomena are occurring. We illustrate how this problem arises by first showing how to avoid it.

If there are line-specific protective responses, they will act more against challenge with the parasite line that elicited them than on another line. Thus, the fitness of parasites in semi-immune animals will depend both on their own genotype and on the line that elicited the protective response; heterologous challenges will do better than homologous challenges. In a statistical model explaining performance in previously exposed animals, there will be two factors: (1) immunizing line, and (2) challenge line. If there are line-specific responses, the interaction between the two factors will be significant (shown schematically in Fig. a; right), with heterologous challenges doing better than corresponding homologous challenges. But lines might also differ in their fitness in semi-immune hosts, irrespective of immunizing line (as is shown, for example, in Fig. 2). If so, the interaction will still be significant, but the average fitness of the lines will also differ [Fig. b (right); here line X (circles) has, on average, higher fitness than line Y (squares)]. Statistically, there would be a significant main effect of 'challenge line'. Thus, for two lines, four treatments are required to separate the effects of line-specific responses and line differences in immune susceptibility. Line differences in immunogenicity (or, conversely, immunomodulatory ability) in primary infections would also be revealed by a significant main effect of 'immunizing line'. In principle, then, reciprocal challenge experiments like this are potentially very informative (if the four treatments are performed in the same experiment at the same time!). Further treatment groups would be necessary to determine whether any effect of 'challenge line' was actually due to intrinsic viability differences between lines irrespective of host immune status (eg. Ref. 45). The meaning of significant interactions and main effects is somewhat complicated where there is immunomodulation by either the primary or challenge infections (particularly if this is line-specific), but careful examination of treatment means should reveal that.

Problems of interpretation arise when all the treatments are not performed, as was the case in the majority of studies reviewed here. Frequently, only one line was used to elicit immunity (resulting in just two treatment groups). If both line-specific immune responses and line differences in immune susceptibility exist, incomplete experimental designs lead to two outcomes. The least problematic is that shown in Fig. c (right): by chance, the treatment combination revealing a difference has been chosen, and there is clear evidence of some sort of 'line' effect. This could be either line-specific responses or line differences in immune susceptibility (or both), but with that experimental design it is impossible to distinguish them. More problematic is the other outcome (Fig. d, right): the combination of the two phenomena results in no difference between the groups. Clearly, this provides no evidence of anything.

An alternative experimental design, where different lines are used to immunize hosts and a single line used for the challenges, suffers the same problem. An analogous argument invoking line differences in immunogenicity (or immunomodulatory ability) in primary infections (eg. Ref. 46) can explain an absence of evidence for line-specific responses even though they exist. Note that it does not help to have three of the treatment groups: for the two genotype case, four are required in order to separate main effects and the interaction.

It is more than just a theoretical possibility that combinations of different types of line effects can occur and confound incomplete experiments. Many of the experiments that have used the full design have found non-reciprocal crossprotection (K.C. Carter, op. cit.; Refs 8, 34; and see, for example, Fig. 2a). This must be generated by a combination of line-specific responses and line differences in either immunogenicity in primary infections or immune susceptibility in challenge infections. Thus, the absence of a full design makes it even harder than normal to make much of a null result, but just such designs underpin many of the published conclusions that worm genotype is unimportant (eg. Refs 8, 37, 40, 46, 47). Unfortunately, because the same lines repeatedly appear in different experiments, the subsequent non-independence makes it difficult to estimate statistically how many of the null results emerging from the incomplete experiments might suffer from this problem by chance alone.



Sometimes an empirical justification is given for ignoring helminth genetics. For example, a recent review of some of the evidence discussed above led to the conclusion[44] that... 'results [of irradiated cercariae vaccines] obtained using one *S. mansoni* strain may be extrapolated to other strains'. This optimism seems somewhat premature: as described above, line effects on host responsiveness to schistosomes have been found, even though these experiments have for the most part involved inbred laboratory lines and incomplete experimental designs (Box 1). For helminths in general, the bulk of what little evidence there is argues for caution against such sentiments, and at the very least justifies a more thorough examination of the situation.

It may be, of course, that helminth genetics was somehow just forgotten by immunologists. That is certainly possible with recent emphasis on immunomodulatory mechanisms of parasite survival, where perhaps antigenic monomorphism is favoured by natural selection, rather than on immune evasion. However, an alternative (if uncomfortable) possibility is that it has been largely ignored because it is convenient to do so. Laboratory groups typically maintain a single, inbred strain; selection experiments and controlled crosses with dioecious endoparasites are not easy. Even with protozoa, where there is a greater emphasis on genetics, genetically pure lines are maintained, but occasionally muddled. Worse still, if there really are line-specific differences in eliciting and evading host responses, then the experimental challenge is enormous.

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A British Society for Parasitology Symposium entitled
Molecular Biochemistry and Physiology of Helminth Neuromuscular Systems
organized by D.W. Halton and R.J. Martin, will be held 18–19 September 1996 at the City University, London, UK.
The two articles that follow will whet the appetite of those interested in this topic.

Nematode Neuropeptides: Localization, Isolation and Functions

D.J.A. Brownlee, I. Fairweather, L. Holden-Dye and R.J. Walker

Historically, peptidergic substances (in the form of neurosecretions) were linked to moulting in nematodes. More recently, there has been a renewal of interest in nematode neurobiology, initially triggered by studies demonstrating the localization of peptide immunoreactivities to the nervous system. Here, David Brownlee, Ian Fairweather, Lindy Holden-Dye and Robert Walker will review progress on the isolation of nematode neuropeptides and efforts to unravel their physiological actions and inactivation mechanisms. Future avenues for research are suggested and the potential exploitation of peptidergic pathways in future therapeutic strategies highlighted.

The nervous system of parasitic nematodes is exceptionally well-defined in terms of the number, location and projections of the small number of neurones involved in regulatory behaviours vital to their survival[1–3]. This is largely because of the vast amount of information available on the free-living nematode *Caenorhabditis elegans*[4], and the neuroanatomical similarity between this species and parasitic species such as *Ascaris suum*. However, functional information has not paralleled these advances, and it is only recently that the chemical complexity of the nematode nervous system has become apparent[5]. This fresh insight has resulted from studies on the peptidergic component of the nervous system and the isolation of an increasing number of peptides from nematodes. This review will summarize data on the distribution of neuropeptides in the nematode nervous system, survey progress in the isolation of peptides 'native' or endogenous to nematodes and examine evidence supporting physiological roles for peptides in these organisms. Although immunoreactivities to a variety of peptides have been localized in the nervous system, all the endogenous peptides isolated to date are FMRFamide-related peptides (FaRPs). Consequently, the main focus of the review will be on this family of peptides.

The first indication that nematodes possess peptidergic nerve cells came in 1958 with the ascertainment of paraldehyde fuchsin-positive neurosecretory cells in *Ascaris*[6]. Neurosecretory cells have been identified in a number of nematodes and the results supported by ultrastructural evidence of typical dense-cored neurosecretory vesicles[7]. The main function attributed to the neurosecretions was in the ecdysis phase of moulting. Work on the cod worm, *Phocanema decipiens*, by Davey and colleagues (reviewed in Ref. 7) showed that a cycle of secretory activity in nerve cells belonging to ganglia associated with the anterior nerve ring was linked to the moulting of the third stage larva. The cells were envisaged to produce an 'ecdysial' hormone which acts on the excretory cell to activate and bring about the release of enzymes comprising the moulting or exsheathing fluid. The fluid is secreted via the excretory duct into the space between the old and new cuticles and serves to digest the old cuticle[7]. The identity of the presumptive 'ecdysial' hormone or, indeed, other neurosecretions is unknown.

Localization of peptide immunoreactivities

Neurosecretory products are now recognized as being typically peptidergic in nature, and the concept

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