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GENETIC VARIATION IN IMMUNITY TO *TRICHINELLA SPIRALIS* IN THE MOUSE
A review

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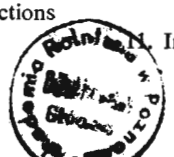
Introduction

Study of the immunogenetics of the host-parasite relationship is a recent development in parasitological research, despite the fact that the existence of variation in ability to resist infection has been recognised for many years (Wakelin, 1978). The results of such studies have clear relevance to human and veterinary medicine and indeed, research is already in progress in fields such as HLA - typing in patients infected with schistosomes (Salam, Ishaac & Mahmoud, 1979) and in selective breeding for resistance to parasitic infection in sheep and cattle (Dargie, 1982). Work of this kind, in man and in large animals, presents formidable biological and logistic problems and thus laboratory studies, using experimental model systems, have an important role in providing fundamental data. A very suitable model for such work is provided by *Trichinella spiralis* in the mouse. The latter is, in immunological and genetic terms, the best defined of all laboratory hosts and has already

TABLE 1

Parameters of infection with *Trichinella spiralis* in mice in which genetic variation has been described

1. Site occupied by worm
2. Survival of adults in primary infections
3. Duration of larval output by females
4. Number of muscle larvae maturing
5. Survival of adults in secondary infections
6. Occurrence of rapid expulsion after challenge
7. IgE/IgG$_1$ responses
8. Antibody responses to surface antigens
9. Mast cell responses
10. Lymphoblast localization after I/V injection
11. Immunodepression



been extensively used in comparable studies with other infectious organisms (Krco and David, 1981). *T. spiralis* is one of the most completely investigated nematode parasites. It has both enteral and parenteral phases in its life cycle, infection provokes a wide spectrum of quantifiable responses, many of which show marked variation when different strains of mouse host are used (Table 1), and it elicits high levels of immunity in the host (Wakelin & Denham, 1983). An additional, but as yet unexploited advantage of this parasite is the existence of clearly defined races/subspecies already known to be associated with distinct patterns of host response (Nelson, Blackie & Mukundi, 1966).

Earlier work on genetically determined variation in this system has already been reviewed (Wakelin, 1978) and it is intended here to concentrate on work published since that time.

Immunity to Primary and Secondary Infections

Immunity to *T. spiralis* can be measured by several parameters, but the simplest and most commonly used are the duration of survival of intestinal stages and the number of muscle larvae established. For many years it was widely assumed that the pattern of responses shown by the inbred Swiss albino mice studied by Larsh and co-workers (Larsh, 1963) was representative of the mouse host. Subsequent studies have revealed wide, host-strain dependent variation and it is now accepted that strains of mice show characteristic response patterns reflected in a) the patterns of adult worm survival in primary and secondary infections (Fig. 1a, b), the numbers of muscle larvae established from primary infections (Fig. 2) and c) the ability to express the rapid expulsion response on challenge (Table 2). Recent analysis has suggested that variation may occur independently in each of the phase-specific immune responses generated by infections (Bell, McGregor & Adams, 1982).

Genetic control of such variation appears to operate in a simple manner, in that greater resistance, whether assessed by earlier adult expulsion, fewer muscle larvae or stronger rapid expulsion, is inherited as a dominant characteristic and is expressed in all the F_1 progeny of a cross between resistant and susceptible (or rapid x slow responder) parents. Preliminary data from F_2 and backcross generations suggest that relatively few genes are involved in this control (Wakelin, unpublished). The genes controlling resistance appear primarily to lie outside the H-2 (major histocompatibility) complex, since in all three aspects of resistance, variation can be shown between strains of mice showing the same H-2 haplotype (Fig. 2, Table 3). However, as Wassom,

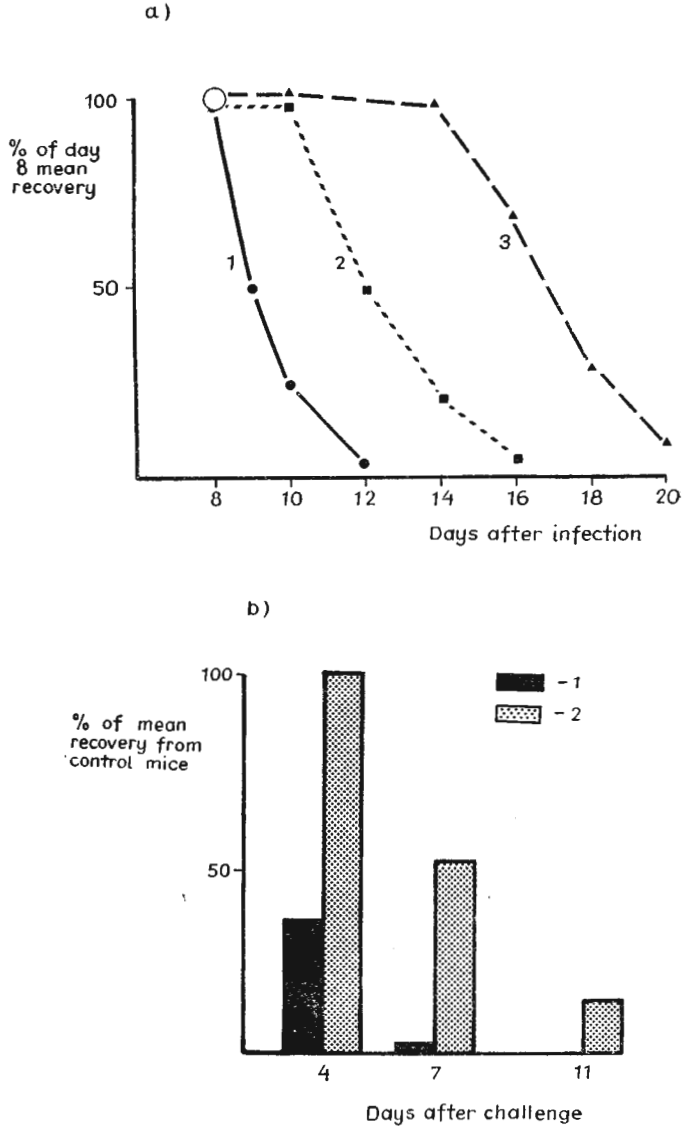


Fig. 1.a. Course of primary infection with *T. spiralis* in NIH *(1)*, CBA *(2)* and C57BL/10 *(3)* strain mice. Values are expressed as % of the day 8 mean worm recovery for each strain b. Course of secondary infection with *T. spiralis* in NIH *(1)* and C57BL/10 *(2)* mice immunized 3 to 4 weeks previously with 300 larvae. Values are expressed as % of the mean worm recovery from controls killed at each time point

David & Gleich (1979, 1980) have pointed out, H-2-linked genetic control can be masked by strong background genetic influences, and indeed, when congenic strains of mice are compared, H-2-linked

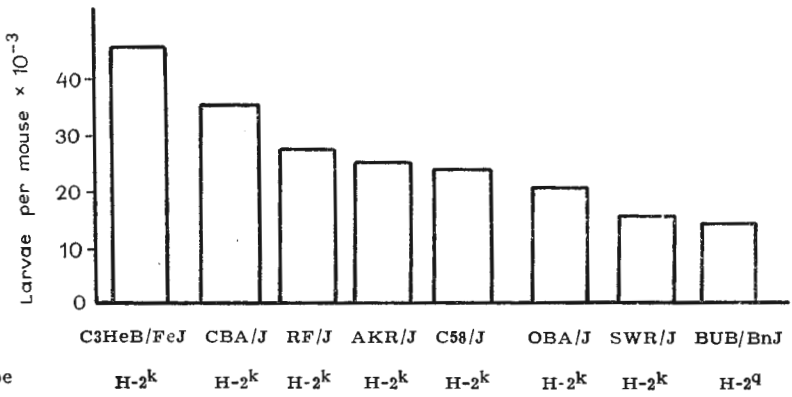


Fig. 2. Numbers of muscle larvae recovered from mice of different genetic backgrounds after primary infection with *T. spiralis*. (Data from Wassom et al., 1979)

TABLE 2

Mouse strain variation in ability to express the rapid expulsion response when challenged with *T. spiralis**

Strain	No. of worms recovered 24h after challenge as % of control mean
BALB/c	115
C3H/HeJ	97
C57BL/KsJ	82
CBA	68
NFR/N	30
CFW	7

* Mice infected with 400 larvae for 7/8 or 12/14 (CFW) days, treated from day 3 with thiabendazole to suppress production of larvae, and challenged on day 10 or 21 (CFW) (Data from Bell et al., 1982)

control of variation can be detected (Figs. 3, 4). It is striking that identical associations between H-2 haplotype and response phenotype have emerged independently from work using quite different parameters of resistance. For example, the haplotypes H-2^s and H-2^q are linked with greater resistance whether total muscle larval recovery (Wassom et al., 1980), anti-adult or anti-fecundity responses (Bell et al., 1982) or adult worm expulsion (Wakelin & Donachie, 1982a) is taken as the criterion.

Detailed genetic analysis of the H-2-linked alleles influencing resistance have been made by Wassom et al. (1980) and more recently by Wakelin & Donachie (1982a). By using a number of congenic and H-2 recombinant inbred strains Wassom et al. were able to show

TABLE 3

Mouse strains showing rapid (< 12 days) or slow (> 14 days) expulsion of primary infections with *T. spiralis*

RAPID		SLOW	
Strain	Haplotype	Strain	Haplotype
NIH	H-2^q	B10.G	H-2^q
DBA_1	H-2^q	B10.D2	H-2^d
SWR	H-2^q	B10.BR	H-2^k
SJL	H-2^s	B10.S	H-2^s

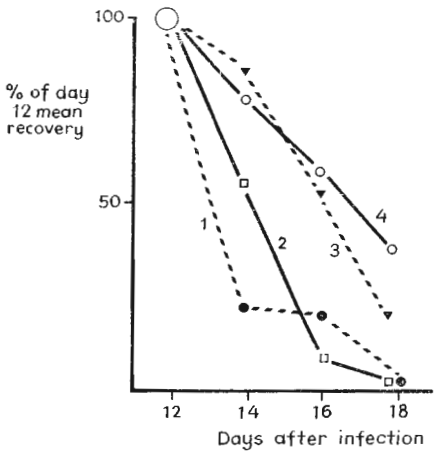


Fig. 3. Course of a primary infection with T. spiralis in B10 background congenic mice, differing only at the H2 loci. B10.AKM (*1*); B10.G. (*2*); B10.BR (*3*); B10 (*4*). (Data from Wakelin & Donachie, 1982)

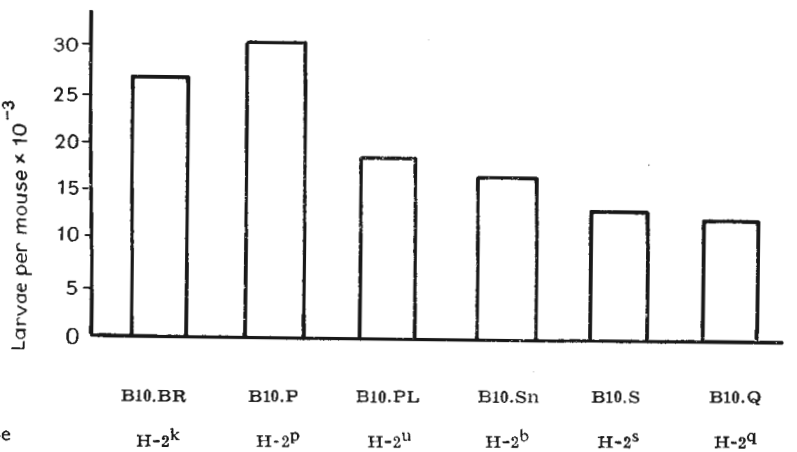


Fig. 4. Numbers of muscle larvae recovered from mice of similar genetic background but different H-2 haplotype after primary infection with *T. spiralis*. (Data from Wassom et al., 1979)

that alleles located within the IA and IB regions of the H2 complex exerted a major influence upon resistance, but that the effects of these genes in determining resistance could be modulated towards greater susceptibility by alleles present at H2D. The *d* allele at this locus was particularly effective and its influence overrode that of resistance alleles elsewhere in the H-2. This modulating influence of H-2D^d was confirmed by Wakelin & Donachie (1983a), who showed that expulsion of adult worms from the intestine was less rapid in B10.T(6R) mice, which carry the *d* allele at H-2D and the *q* allele elsewhere in the H-2 complex, than in the B10.G (H-2^q) mice with which they are related.

Analysis of Expression of Non-H2 Control in Worm Expulsion

More is understood of the mechanisms underlying expulsion of adult *T. spiralis* from the intestine than any other aspect of resistance. There exists, therefore, a sound basis for determining the means through which genetic variation in this parameter is expressed. Expulsion is thought primarily to be the outcome of T cell-dependent inflammatory changes in the intestine (Wakelin & Denham, 1983) and thus variation may be expressed through one or both components involved, i.e. through the T cells which initiate the inflammation or the myeloid cells which respond to T cell factors and infiltrate the mucosa.

One approach to analysis has been the use of adoptive transfer of immunity within and between strains of contrasting response phenotype (i.e. rapid- and slow-responders). Adoptive transfer can be achieved easily by transfer of immune mesenteric lymph node cells (IMLNC) taken from infected donors and it is now established that the cells responsible are T lymphoblasts (Grencis & Wakelin, 1982; Wakelin, Grencis & Donachie, 1982). Transfer of immunity results in an accelerated expulsion of adult worms and is therefore easily monitored. Much of the work has made use of two histocompatible, inbred strains of mice, namely albino NIH and black B10.G (a C57BL/10-background congenic line).

The results of adoptive transfers in these strains can be summarized as follows:

Homologous transfer

1. NIH mice given IMLNC from infected NIH donors show accelerated worm expulsion, loss of worms beginning about 2 days earlier than in controls, i.e. after day 6.

2. B10.G mice given IMLNC from infected B10.G donors show accelerated worm expulsion, loss of worms beginning 3 - 4 days earlier than in controls, i.e. after day 10.

3. IMLNC capable of transferring immunity appear at the same time, day 4 after infection onwards, in both strains.

Reciprocal transfers

1. The response of NIH mice to transfer of B10.G IMLNC is the same as when NIH cells are used, i.e. loss occurs after day 6.
2. The response of B10.G mice to transfer of NIH IMLNC is the same as when B10.G cells are used, i.e. loss occurs after day 10.
(data from Wakelin & Donachie, 1980).

Thus, exchange of the lymphocyte component of immunity does not influence the strain characteristic response to adoptive transfer. This fact, coupled with the similarity in time of appearance of IMLNC capable of transferring immunity, appears to imply that genetic variation is expressed through the inflammatory (myeloid) component. More direct evidence for this conclusion has been obtained from the use of bone marrow chimaeras, prepared by reconstituting lethally irradiated (B10.G $\times$ NIH) F_1 mice with parental bone marrow (Wakelin & Donachie, 1981). In primary infections and after transfer of NIH IMLNC, NIH$\rightarrow F_1$ chimaeras behaved as NIH mice and B10.G$\rightarrow F_1$ chimaeras behaved as B10.G mice. Thus response phenotype is determined by the BM donor employed, not by the source of IMLNC, and this may reflect the ability of BM-derived non-lymphoid cells to respond to factors released from sensitized T cells.

These results naturally raise the question of the myeloid cell population concerned in the expulsion response and at present this cannot be answered with any precision. Although there is no direct evidence to implicate mucosal mast cells (MMC) in the expulsion of adult worms, a marked increase in number of these cells does occur during primary infections and can be used as a marker of the intestinal inflammatory changes that accompany infection. Rapid responder strains (e.g. NIH) show a large increase in MMC by day 8 after infection, whereas in slow responders (e.g. B10.G) this increase is not evident until day 12 (Alizadeh & Wakelin, 1982). Intestinal mastocytosis can be transferred adoptively with IMLNC, but response phenotype is not altered by reciprocal adoptive transfer. In BM chimaeras, as with worm expulsion, response phenotype is determined by the BM donor employed. These results reinforce the conclusions based on worm expulsion data and suggest that slow-responder mice may show a generalized slow-response of myeloid cell precursors to T-lymphocyte derived signals. Preliminary data, using peripheral blood eosinophil responses to parasite antigens, support this hypothesis. Rapid responder NIH mice show high eosinophil responses to complete or abbreviated infections with *T. spiralis*, to infections with *Nematospiroides dubius* and to parenteral injection of

worm antigens after cyclophosphamide pretreatment, whereas slow responder B10 mice do not (Wakelin & Donachie, 1983b).

An interesting corollary of the intestinal changes which take place after infection with *T. spiralis* is an effect upon the traffic to the intestine of lymphoblasts from the gut-associated lymphoid tissues (Rose, Parrott & Bruce, 1976). Within a few days, there is a marked and selective increase in localization of intravenously injected T lymphoblasts in the intestinal mucosa. This localization is not antigen specific, and although correlated in time with the onset of inflammatory changes, is not related to alterations in blood flow through the mucosa (Ottaway, Manson-Smith, Bruce & Parrott, 1981). Comparison between rapid-responder NIH and slow-responder BALB/c mice has shown a strain-specific pattern in lymphoblast homing (Manson-Smith, Bruce, Rose & Parrott, 1979). In the former localization peaks 4 days after infection, in the latter the peak localization time depends upon the time after infection when lymphoblasts are taken, being 4 days with day 4 cells and 14 days with day 12 cells. In addition, localization is confined to the anterior intestine in NIH mice but is largely posterior in BALB/c mice. Whereas in NIH mice, blast activity in the draining node is maximal 4 days after infection, in BALB/c mice there are two distinct peaks, at day 4 and at day 12. Clearly these characteristics must be related in some way with the response phenotype of each strain, but the relationship is at present unclear.

Analysis of Expression of H-2-Linked Genetic Control

In a recent paper, Krco, David & Wassom (1982) have developed an in vitro assay for lymphocyte proliferation in response to *T. spiralis* antigens. This approach has been tested using cells from strains of mice identified in previous work (Wassom et al., 1980) as showing greater or less resistance to the establishment of muscle larval burdens. Primed cells from B10.S mice (resistant) gave a high proliferative response when stimulated in vitro with *T. spiralis* antigen, cells from B10. BR mice (susceptible) gave a 50% lower response. The good response of B10.S mice was dependent upon the presence of cells with the phenotype Thy-1 +ve, Lyt-1 +ve and was reduced considerably by antisera directed against I-A and I-E gene products. If antigen-induced lymphocyte proliferation can be correlated with the ability to develop protective immunity then these data confirm the in vivo studies of Wassom et al. in associating particular H-2 loci with resistance to infection. However, the results of Wakelin & Donachie (1980) showed that there was no relation between reponse phenotype and lymphocyte capacity to transfer immunity adoptively in mice of different background but

similar H-2 haplotype, and similar results have now been obtained with congenic mice differing only at H2 loci (Wakelin & Donachie, 1983a). Thus B10.T(6R) mice, which possess the *d* allele at H2D and are relatively slow at expelling adult worms compared with B10.G ($H2D^q$), nevertheless, on infection, develop lymphocyte populations (IMLNC) capable of transferring immunity to B10.G recipients as effectively as homologous cells. These results again imply a recipient-determined control of the expression of intestinal immunity and suggest the involvement of cells other than IMLNC in this expression.

Genetic Variation in Antibody Responses to Surface Antigens

A major development in the immunobiology of *Trichinella* infections has been the demonstration of stage-specific antigens present on the cuticular surface (Philipp, Parkhouse & Ogilvie, 1980). These antigens are of limited heterogeneity and occur in characteristic patterns in each developmental stage. The host responds to these antigens by the production of antibodies capable of combining with them directly and of mediating attachment of eosinophils to the cuticle. The nature of these antigens and of the responses which they elicit provide sensitive tools with which to probe genetically determined variations in host capacity to recognize and respond to *Trichinella* infections. Jungery & Ogilvie (1982) have shown in two strains of mice (NIH and C3H) that there are both qualitative and quantitative differences in their patterns of antibody response. For example, NIH mice produced antibodies to all four

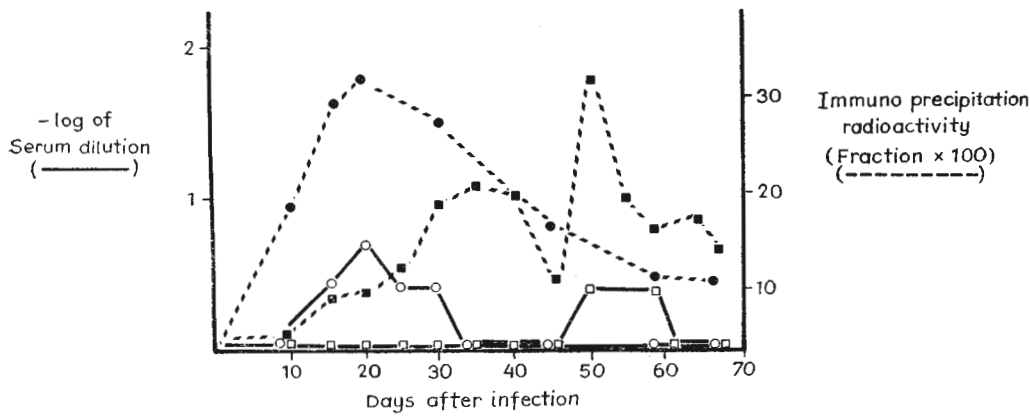


Fig. 5. Time course of anti-adult *T. spiralis* antibodies measured by eosinophil adherence (————) and immunoprecipitation (- - - -) of surface antigens in sera from NIH (● ○) and C3H (■ □) mice. Antigens from day 2 or day 7 (C3H eosinophil data) worms. (Data from Jungery & Ogilvie, 1982)

surface antigens of 2 day-old worms by the 10th day of infection, but C3H mice recognized only three at this time, not recognizing the fourth until day 35. The patterns of appearance of antibodies capable of precipitating surface antigens or of mediating eosinophil adherence also showed distinct differences (Fig. 5) with NIH mice responding earlier and to a greater degree. It is striking that NIH mice are the more strongly responsive of the two strains in terms of mounting protective responses against the intestinal stages of infection, and although, at present, the strain differences in antibody response to surface antigens cannot be related to this form of immunity, analytical approaches of this type will be invaluable in uncovering the precise responses underlying genetically-determined variations in resistance to infection.

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GENETYCZNE ASPEKTY ODPORNOŚCI MYSZY NA ZARAŻENIE *TRICHINELLA SPIRALIS*

D. WAKELIN

Badania immunogenetycznego układu żywiciel-pasożyt są najnowocześniejszym kierunkiem w immunoparazytologii. Autor na podstawie własnych badań oraz najświeższych publikacji z literatury dokonuje oceny danych dotyczących genetycznych uwarunkowań w powstawaniu odporności u myszy przeciw *T. spiralis*.

Ze względu jednak na podjęte na świecie próby wyhodowania owiec i bydła odpornego na inwazje pasożytnicze artykuł ten może mieć nie tylko teoretyczne znaczenie.