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Inheritance of immunity in mice to challenge infection with *Nematospiroides dubius*

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Two lines of mice (*Mus musculus*) were selectively reared over 10 generations for high (H) and low (L) levels of immune response to *Nematospiroides dubius*, an enteric nematode parasite. Filial and backcross families were derived from the two parent lines. The mode of inheritance of the trait, immune response to challenge infection with *N. dubius*, was analysed by comparing the levels of infection in the parental, filial and backcross (BC) families of mice.

The immunity of the F_1 mice was found to be dissimilar to both parents, but was closer to the H value than to the level of immunity in the L mice. The backcross to H progeny showed levels of immunity approaching that of the H mice, whereas only two of four backcross to L families were low immune responders. Analysis of these results indicated that the inheritance of immunity in these mice to challenge infection with *N. dubius* was quantitative, partially dominant for high immune response, and additive, in nature.

INTRODUCTION

Several recent reviews have outlined current knowledge of the genetic factors influencing the interaction of animal parasites with the mammalian immune system (Wakelin, 1978; Mitchell, 1979; Skamene, Kongshavn and Landy, 1980; Vadas, 1980; Sher and Scott, 1982). The genetic analysis of, and the selection of economically valuable species for, resistance to disease have long been recognised as beneficial in agriculture (see Day, 1974; Falconer, 1981). Parasites, particularly helminths, compound the analysis of host immunity to infection because they evade or suppress host immune responses and because they show considerable genetical variety themselves.

Mammalian reactions to various antigens involve control by the immune response (IR) genes and the major histocompatibility locus (MHC) of genes (Benacerraf and McDevitt, 1972; Biozzi, Stiffel, Mouton, Bouthillier and Decreuesefond, 1971; Wassom, David and Gleich, 1979; Klein, Juretic, Baxevanis and Nagz, 1981) which may explain intraspecific variation in susceptibility to parasites (Vadas, 1980; Wakelin, 1984). Sher and Scott (1982) consider that these genes might influence resistance to infection by regulating biochemical events involved in the physiological adaptation of parasites to the host environment.

In this paper we describe levels of immunity in families of mice derived from crosses and backcrosses between two lines which were selectively reared over 10 generations for high (H) and low (L) levels of immunity to *Nematospiroides dubius* (=*Heligmosomoides polygyrus*), (Sitepu, Brindley and Dobson, 1986). *N. dubius* is a trichostrongyloid nematode which parasitises various rodents. The third-stage larva, which is infective for mice, is ingested. It then moults and penetrates the intestinal mucosa. The dioecious adults emerge from the gut wall one week after infection, mature, copulate and shed eggs within the faeces of the host (see Bryant, 1973).

MATERIALS AND METHODS

Mice

High (H) and low (L) immune responder lines of mice, in terms of immune response to a challenge infection with *Nematospiroides dubius*, were selectively reared from a foundation, outbreeding

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population of *Mus musculus*. We have described this selection process in detail elsewhere (Sitepu and Dobson, 1982; Sitepu, *et al.*, 1986). Briefly, F_1 progeny from crosses between outbred Quackenbush (Q) strain mice and wild caught mice were infected with *N. dubius* and the mice with the highest and lowest levels of infection were selected in a two-way experiment. At the third generation of selection, and onwards, the H and L lines of mice showed a difference in their immunity to the nematode infection and this difference expanded with subsequent selection.

First filial, F_2 and backcross (BC) mice were derived from cross-matings between H and L mice from the eighth or ninth selection generations. Specifically, a series of hybrid combinations were bred, as listed below, in order to investigate the mechanism of inheritance of immunity in mice to infection with *N. dubius*, including the influence of the sex of the parents on the immune status of their progeny. The parent lines, H and L, were "inbred" at the same time as the filial and backcross mice were reared, and the progeny so derived—9th and 10th generation H and L mice—were also examined in this experiment. It is the mean number of eggs per gm (epg) for these H and L progenies, not those for the parents of these, and the filial and the backcross progenies, which are given in the tables and figures in this paper, alongside values for the filial and backcross mice. The symbolism for the filial and backcross progenies, with the female parent given first, is as follows:

$$H \times L = F_1H$$
$$L \times H = F_1L$$
$$F_1H \times H = BC1$$
$$H \times F_1H = BC2$$
$$F_1L \times H = BC3$$
$$H \times F_1L = BC4$$
$$L \times F_1H = BC5$$
$$F_1H \times L = BC6$$
$$L \times F_1L = BC7$$
$$F_1L \times L = BC8$$
$$F_1H \times F_1L = F_2.$$

Parasite

N. dubius was maintained in our laboratory in outbred Quackenbush strain mice. Third-stage larvae were harvested by irrigating cultures of infected faeces comminuted with tap water, streaked onto moist filter paper and incubated for 1 week at 26° (Dobson and Owen, 1977). Larvae were stored in a shallow layer of tap water at 4° for 4 weeks prior to innoculation into experimental mice.

Assessing the phenotype

Experimental mice were infected when 7 to 8 weeks old with 100 infective larvae by gastric gavage. Three weeks later they were drenched free of *N. dubius* infection with an anthelmintic (20 mg kg^{-1} levamisole phosphate, "Nilverm", ICI, Australia), also by gastric gavage. Four days later the mice were reinfected with 100 larvae. On days 20 and 21 after reinfection the numbers of *N. dubius* eggs per gm (epg) of mouse faeces from individual mice were determined using the McMaster technique (Roberts and O'Sullivan, 1950). Faeces for epg counts were collected between 0900 and 1100 hours only.

In total, 975 mice from the parental, filial and backcross families were examined in this experiment. At each infection of batches of these mice, 10 female Quackenbush (Q) mice were also infected with 100 larvae as a control for the infectivity of the parasite stock, which can be influenced by environmental parameters (Kerboeuf, 1978). These Q mice were killed 21 days after infection and the numbers of adult *N. dubius* that they harboured were counted. The means of these numbers recovered from each control group of Q mice were used to standardise the faecal epg counts from the experimental mice to overcome any variations in the infectivity of the parasite larvae, using Sitepu and Dobson's (1982) formula.

RESULTS

The results for the filial and backcross progeny are presented in two ways. Firstly, mean values for faecal *N. dubius* epg counts for the F_1, F_2, backcross to high (BCH) and backcross to low (BCL) immune responder mice were compared with the parental lines to examine a role for additive effects and dominance in this trait. Secondly, mean values for F_1 (F_1H and F_1L) and backcross (BC1, BC2 ... BC8) progeny, with reference to the sex of each parent, were compared with values for the parental H and L mice to examine the influence of the sex of parental genotypes in this trait.

Fig. 1 summarises all the epg data. Fig. 2 and table 2 are based on the same mice as in fig. 1 and

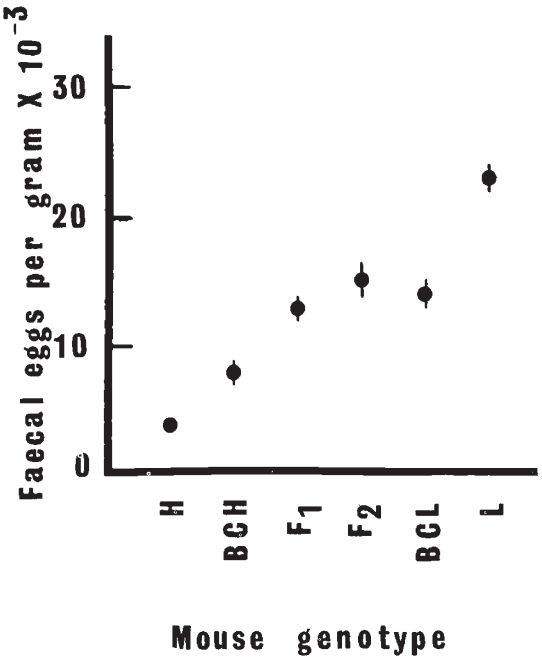

Figure 1 Mean ±S.E. faecal *Nematospiroides dubius* epg counts from mice selectively reared for high (H) and low (L) immunity to infection and from filial and backcross families derived from them.

table 1, but the results have been broken down into finer categories.

H, L, F_1, F_2, BCH and BCL progeny

Mean values for these families are given in fig. 1. The mean value for the F_1 mice was distant from both the H and L values, but was closer to the H than to the L mean. The F_2 mean was equidistant between the H and L progeny means. The F_1 and F_2 mean epg values were not statistically different from each other, but the variance of the F_2 epg values was greater than that of the F_1's—307 and 180 respectively. The mean for the BCH progeny was midway between the H and F_1 means, whereas the mean BCL was not significantly different from the F_1 mean.

All the H mice used to breed the mice described in this experiment had expressed the phenotype "0 epg" after challenge *N. dubius* infection. Of 200 H progeny, 71 (or 36 per cent) expressed the "0 epg" phenotype of their parents. Of 128 L progeny, 39 (29 per cent) expressed faecal epg values ">40,000 epg", which was the phenotype of the L parents used for breeding. Of the 58 F_2 progeny, one showed the "0 epg" phenotype and 3 showed the ">40,000 epg" phenotype (table 1).

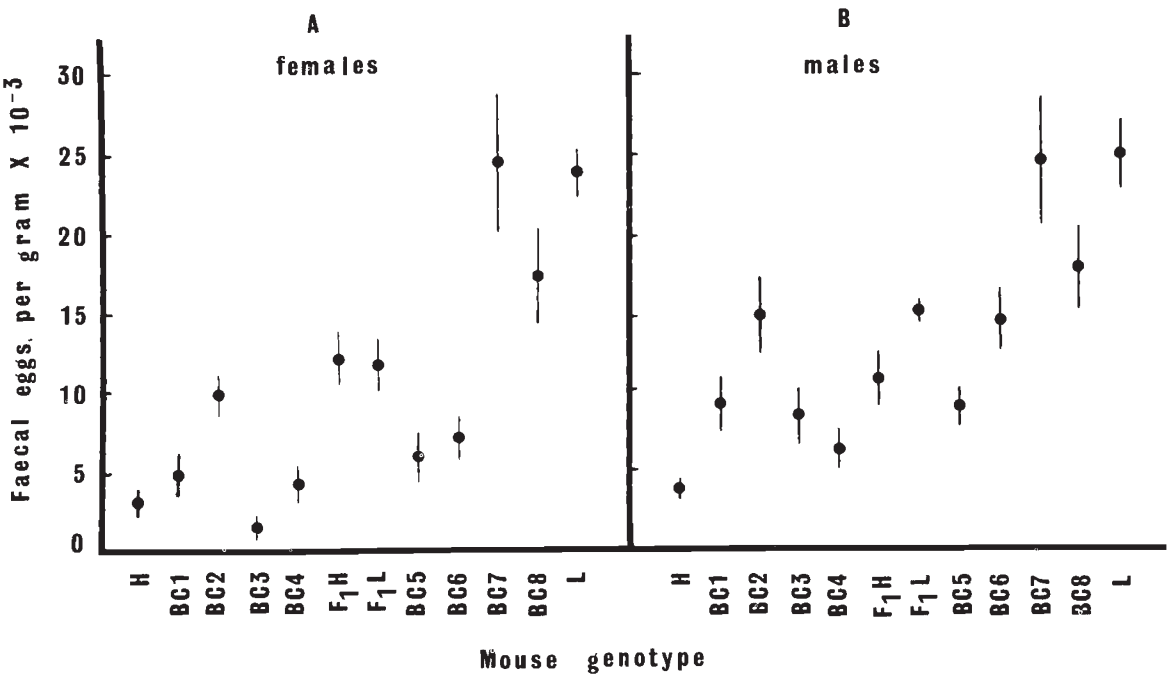

Figure 2 Mean ±S.E. faecal *Nematospiroides dubius* epg counts from mice selectively reared for high (H) and low (L) immunity to infection, and from F_1, F_2 and backcross families derived from them.

Table 1 Percentages of families of mice displaying the parental H ("0 epg") and L (">40,000 epg") phenotypes

Family	Percentage of progeny exhibiting parental phenotype "0 epg" phenotype	">40,000 epg" phenotype	Number of mice
H	36	0	200
BCH	20	1	215
F_1	3	4	179
F_2	2	5	58
BCL	4	8	195
L	0	29	128

Table 2 Percentages of families of mice displaying the parental H ("0 epg") and L "(>40,000 epg)" phenotypes

Family	Percentage of progeny exhibiting parental phenotype "0 epg" phenotype	"40,000 epg" phenotype	Number of mice*
H	36	0	200
BC1	12	0	51
BC2	4	1	72
BC3	53	0	36
BC4	27	2	56
F_1H	1	4	92
F_1L	5	2	87
BC5	12	0	49
BC6	3	4	68
BC7	0	18	40
BC8	0	13	38
L	0	29	128

* Similar numbers of females and males within each family.

H, L, F_1H, F_1L and BC1 through BC8 progeny

The mean values for each sex in each of these families are compared in fig. 2. Several trends appeared. A comparison of fig. 2(a) with fig. 2(b) showed that the level of immunity was generally higher *i.e.*, lower faecal *N. dubius* epg, in females than males. The mean faecal epg value was significantly lower in females than in males in the BC2 and BC3 families (t-values 2·81 and 2·03, $P<0·01$ and $P<0·05$, respectively) and was lower, though not significantly so, in 8 of the other 10 families. In the other two families, F_1H and BC7, the faecal egg count was not significantly different between males and females within each family, although the epg values were lower for males than females in both these families.

The BC2 family was notable because the BC2 mean epg was higher than that of each of the three other backcross to H families (male mice, t-values 1·69, 1·74 and 2·69, P N.S.—not significant, N.S. and <0·05; female mice, t values 2·08, 2·33 and 2·69, $P<0·05$ all cases). The female F_1H and F_1L showed similar mean epg values whereas the F_1L male progeny showed a mean epg value that was 40 per cent higher than, though not significantly different from, the mean for male F_1H mice. In the backcross to L families, the BC5 and BC6 mice were more immune than the BC7 and BC8 mice. (All t-comparisons significantly different at $P<0·05$ to <0·01 except male BC6 versus male BC8 where P N.S.) Indeed, the BC5 and BC6 more closely resembled the backcross to H families than they resembled BC7, BC8 or L mice. Mice in BC7 and BC8 showed poor immunity to reinfection with *N. dubius*, as did the L progeny.

Table 2 lists the percentage of each of the F_1 and backcross families exhibiting the parental phenotypes "0 epg" or ">40,000 epg". These data emphasise the same trends in immunity seen in fig. 2. In particular, the BC7 and BC8 mice appear most like the L progeny, much more so than do the BC5 and BC6 progeny.

Analysis for sex-linkage

We designed these series of matings to produce F_1H, F_1L and BC1 through BC8 families in order to determine whether sex-linked genes were involved in the inheritance of immunity in mice to challenge infection with *N. dubius*. According to Mather and Jinks (1971), the diagnostic properties of sex-linkage are (a) a difference between reciprocal crosses in the F_1 generation which is confined to males, and (b) a difference between reciprocal crosses in the two backcrosses, in both sexes, but a difference between the two reciprocal crosses using the inbred line as the female parent which is confined to the female progeny.

When we made these comparisons, we found no significant difference between the epg means for male F_1H and male F_1L. Concerning the reciprocal crosses in the two backcrosses, only two of the eight comparisons showed significant differences. These were BC1 vs. BC2, females, and BC5 vs. BC6, males (t-values, 2·15 and 2·78, $P<0·05$ and <0·01), respectively. Furthermore, when we compared the two reciprocal crosses using the inbred line (H or L) as the female parent, we indeed found significant differences between families in female progeny means (BC2 vs. BC4, females, $t=2·08$, $P<0·05$; BC5 vs. BC7, females, $t=3·86$, $P<0·01$), but there were also significant differences between corresponding male families (BC2 vs. BC4, males, $t=2·66$, $P<0·01$; BC5 vs. BC7, males, $t=5·74$, $P<0·01$).

In summary, there were numerous inconsistencies with the results described here in relation to a positive diagnosis of sex-linkage in this trait.

DISCUSSION

Selectively reared outbred mice rather than syngeneic laboratory strains were used in this experiment because the H and L mice were bred for high and low immunity to a natural infection—syngeneic strains do differ in their responses to reinfection with *N. dubius* (Prowse, Mitchell, Ey and Jenkin, 1979) but these differences to this and other diseases in such strains occur by chance. The extremes in infection shown by the H and L lines resulted from changes in frequencies of genes responsible specifically, and perhaps only, for resistance to challenge infection with *N. dubius*.

There appear to us to be two reasonable ways to analyse these progeny data. One is to compare the frequencies in the inbred, filial and backcross progenies of mice displaying the extreme phenotypes of the parent lines, *viz.*, "0 epg" or ">40,000 epg". The levels of infection in the H and L progeny showed that only 36 per cent and 29 per cent, respectively, displayed the extreme phenotypes of resistance and susceptibility of the parental line breeders, which suggests that neither of the parental lines was yet homozygous by generation 9. However, these proportions may have been influenced by environmental effects and are complicated by possible genetic heterogeneity in the parasite population. Responses from the F_1, F_2, and backcrosses suggested quantitative rather than qualitative inheritance—the F_1 progeny mean epg, for instance, was different from the mean for either parent line. The F_2 progeny were more varied in their response to infection than the F_1 mice, perhaps resulting from the segregation of several allelic pairs and the formation of many new genetic combinations. The presence of one in 58 F_2 progeny with "0 epg" and 3 F_2 progeny in 58 with ">40,000" suggested that at least 2 or 3 genes were segregating. Three allelic pairs produce a frequency of 1 in 64 for each parental phenotype among the F_2 progeny, whereas 2 allelic pairs produce a frequency of 1 in 16. The proportions of extreme phenotypes observed among the backcross progeny also support an additive mode of inheritance for the trait because 1 in 16 (17 per cent) or 1 in 4 (25 per cent) of the BCH and the BCL progeny would be expected to resemble the parental phenotype if 3 or 2 pairs controlled the trait; the BCH and BCL progeny were observed to approach these proportions (20 per cent and 8 per cent respectively).

We consider that another way to interpret these data involves analysis using the mean epg values for the inbred progeny of the parent H and L lines rather than using the epg score of the H and L mice used to breed them *i.e.*, 4000 and 24,200 epg, instead of "0 epg" and ">40,000 epg", H and L mice, respectively. Accordingly, it seems plausible to postulate that the L progeny expressed the minimal effect of immunity, observed as 24,200 epg (fig. 1), by an unknown number of genes. The expression of resistance by the H progeny, observed as 4000 epg, would then represent contributions from other genetic factors. The range of protective immunity expressed by the filial and backcross progeny was contained in a phenotypic value of 20,200 epg (*i.e.*, 24,200–4000). When this value is attributed to the additive effect of three pairs of genes, for instance, each allele would contribute 3500 epg. F_1 mice, which would inherit three alleles from each of H and L parents would display immunity midway between the H and L progeny values. This was close to the observed result, though the F_1 mean was somewhat closer to the H than the L mean, which in turn indicated partial dominance for high immune responsiveness. Similarly, the action of two rather than three genes would nearly satisfy the observed result for the F_1 mice.

No role for sex-linked genes was found in this trait, although female mice were more resistant to *N. dubius* reinfection than male mice in two families. That females are more resistant than males to infection with *N. dubius* has been noted previously by Dobson (1961) who showed that testosterone depresses the resistance of female mice to infection. Accordingly, we speculate that the action of genes involved in this trait in mice can be regulated by sex hormones.

Most examples of genetic control of immunity to helminth infections in mammals are quantitative (see Wakelin, 1978; Mitchell, 1979), though examples of qualitative control are known. In particular, Wassom, De Witt and Grundmann (1974) showed that immunity in deer mice to infection with *Hymenolepis citelli* was controlled by a single dominant allele, as is the immune expulsion of *Trichinella spiralis* from the laboratory mouse (Bell, Adams and Ogden, 1984). In contrast, Correa-Oliveira (1985) demonstrated that vaccine-induced resistance in mice to *Schistosoma mansoni* was inherited as a single recessive allele. Wakelin (1975) carried out a backcross trial using mice selected for their immune response to the enteric nematode *Trichuris muris*, and found ratios of progeny expressing the parent phenotypes which indicated the involvement of 3 genes but which expressed dominance rather than addition for protective immunity against that infection. The

analysis of Wakelin's (1975) results was simplified because immunity to *T. muris* is an all-or-nothing event *i.e.*, qualitative rather than quantitative genetic inheritance as is seen with *N. dubius* in the H and L mice (Sitepu, *et al.*, 1986).

In summary, the mechanism of inheritance of immunity in these mice to challenge infection with *N. dubius* is complex, but it exhibits partial dominance for high immune responsiveness, and additive effects. Complexity arises from the influence of host immunity on a variety of parasite biological functions including establishment, growth and reproduction (Brindley and Dobson, 1982, 1983; Sitepu *et al.*, 1986) and, furthermore, parasites also have genetic mechanisms which enable them to respond to changes in host resistance (Day, 1974).

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