The genetic control of host responses to *Dipetalonema viteae* (Filarioidea) infections in mice

N.STOREY, D.WAKELIN & J.M.BEHNKE
MRC Experimental Parasitology Research Group, Department of Zoology, University of Nottingham, Nottingham, UK

Summary Dipetalonema viteae (Filarioidea) infections were established in inbred strains of mice by the s.c. implantation of adult female worms and the resulting microfilaraemia and adult worm survival monitored. BALB/c mice were the most susceptible strain examined, showing a high level microfilaraemia of approximately 6 month's duration. C57Bl/10, CBA/Ca and C3H/He mice were all equally resistant to infection, showing a low level of microfilaraemia of approximately 1 month's duration. The response of NIH mice was intermediate. Relatively little strain difference was seen in adult worm survival although worms lived slightly longer in C57Bl/10 mice than in BALB/c mice. The adult females became depleted of microfilariae over a period of approximately 1 month before becoming encapsulated in host tissue. Challenge infections given to mice previously implanted with worms resulted in lower level, shorter lasting microfilaraemias than those seen in the initial primary infections. All strains showed immunity when challenged. High responsiveness (resistance) was inherited as a dominant trait in F_1 hybrids produced by crossing high and low responder strains. Genes linked with the major histocompatability complex (H-2) were found to have no effect on the response phenotype as demonstrated by the similar responses of H-2 congenic mice on the BALB/c or C57Bl/10 backgrounds. The response phenotype of radiation chimaeras was determined by the phenotype of the donor from which bone marrow (BM) cells were taken for reconstitution. Susceptible BALB/c mice reconstituted with resistant B10D2/n BM behaved identically to the donor strain, indicating that the genetic variation which exists between mouse strains in their responses to *D. viteae* is expressed through a population of BM derived cells and is not simply a consequence of host structure or physiology.

Keywords: *Dipetalonema viteae*, mice, genetics, inheritance, major histocompatibility complex, radiation chimeras, immunity

Introduction

The influence of host genetic factors on susceptibility or resistance to parasitic infection has now been demonstrated in a wide variety of host–parasite systems (Wakelin 1978). In

human filariasis, there is a great deal of observational (Low 1908, Ogilvie & MacKenzie 1981) and some experimental data (Piessens *et al.* 1980) to show that susceptibility to infection and liability to pathological sequaleae vary markedly between individuals in the same endemic region. If it can be assumed that, in these regions, each individual is equally exposed to infection, then it can be concluded that such variations must reflect the effect of host genetic factors upon the outcome of infection. This conclusion is supported by the observation of familial clusterings of filarial disease (Ottesen *et al.* 1981) where this clustering is most compatible with genetic transmission of disease susceptibility. Variations in host responses to filarial infections have also been noted in laboratory animals, for example with *Dipetalonema viteae* in hamsters (Neilson 1978) and mice (Haque *et al.* 1980), *Brugia pahangi* in rats (Gusmao, Stanley & Ottesen 1981; Fox & Schacher 1976; Cruickshank *et al.* 1983) and *Brugia malayi* in mice (Fanning & Kazura 1983). However, the reasons underlying variation in host responses have received little attention and are inadequately explained. In part, this is because the analysis of host–parasite immunogenetics requires the use of hosts which are well defined both immunologically and genetically and there are few experimental systems where filarial worms will develop in mice and rats (Philipp *et al.* 1984). Consequently, there is a valid requirement to use mice as proxy hosts, i.e. to infect them by transfer of adult or microfilarial stages of the parasites. One such proxy system is the *Dipetalonema viteae*/mouse model (Haque *et al.* 1980) in which adult worms are implanted s.c. into mice and a patent microfilaraemia established. These workers noted host strain variations especially in terms of microfilaraemia but also adult worm survival. The present investigation has used this system to examine variations between inbred, hybrid and congenic mice in order to analyse the genetic basis of differential response to infection. An initial analysis of the mechanism through which this genetic variation is expressed has been made using radiation chimaeras.

Materials and methods

Experimental design

A panel of inbred mouse strains was used to determine whether the course of infection following implantation with female *D. viteae* showed genetically determined variations. Microfilaraemia and adult worm survival during a primary infection and the response to a challenge infection were determined. The most susceptible and resistant strains of mice were selected and crossed to give F_1 hybrids, which were then used to study the mode of inheritance of response phenotype. Congenic strains of mice on the genetic backgrounds of the resistant and susceptible strains were then used to assess the influence of the H-2 (MHC) haplotype. Radiation chimaeras between susceptible and resistant H-2 compatible strains were used to assess the expression of genetic control through bone marrow (BM) derived cells.

Animals

Inbred female mice obtained from Olac 1976 Ltd were used and are listed in Table 1. F_1 hybrids (NIH × B10, NIH × B10.G and BALB/c × B10) were bred in the Department of Zoology, Nottingham University, as were jirds and inbred DSN hamsters.

Table 1. List of mouse strains with their H-2 haplotype and the abbreviations used in this paper

Strain	H-2 haplotype	Abbreviation used
BALB/c/01a	d	BALB/c
BALB/b/01a	b	BALB/b
BALB/k/01a	k	BALB/k
CBA/Ca/01a	k	CBA
C3H/He/01a	k	C3H
C57BL/10/ScSn/01a	b	B10
B10.D2/n/01a	d	B10.D2/n
B10.BR/01a	k	B10.BR
B10.G/01a	q	B10.G
NIH/01a	q	NIH

Parasites

Dipetalonema viteae was cyclically maintained in jirds (*Meriones unguiculatus*) and the tick *Ornithodorus moubata* (Worms, Terry & Terry 1961).

Ticks were infected by feeding them on the abdomen of anaesthetised jirds showing a microfilaraemia of 20–40 microfilariae (Mf)/10 μl. Four to six weeks later the ticks were dissected in RPMI 1640 medium containing penicillin and streptomycin (100 u/ml) in order to collect infective (L_3) larvae. Approximately 300 L_3 were injected s.c. into the groin of male, inbred DSN hamsters. Ninety-six days later (at peak microfilaraemia) the hamsters were killed and skinned. Adult worms were removed from the pelt and carcass using fine forceps and transferred into RPMI 1640 (containing antibiotics) at 37°C. They were sexed and inspected for damage prior to transfer.

Implantation of adult D. viteae

Six to ten week old mice were anaesthetised with pentobarbitone sodium and the right flank shaved and surface sterilized with 70% alcohol. A 5–10 mm incision was made and a s.c. pocket created by inserting the tips of a pair of blunt forceps. Using blunt forceps, the worms were gently inserted into the pocket and the wound closed with silk sutures. The area was then dressed with antibiotic spray (polymixin/bacitracin/neomycin) and sealed with plastic skin (Nobecutane). Mice were allowed to recover, given food and water (containing the antibiotic oxytetracycline hydrochloride 165 mg/l—Terramycin, Pfizer Ltd) *ad libitum*, and housed under conventional animal house conditions in groups of six.

Microfilaraemia

Duration and level of microfilaraemia was monitored for each recipient mouse. A 10 μl blood sample was obtained from the retro-orbital venous plexus of ether anaesthetised

animals. The blood was expelled onto the surface of a clean, grease free microscope slide and spread into an area 10×20 mm. After air drying overnight, the smear was dehaemoglobinized in 1% acetic acid, fixed in methcol, stained with Mayer's haemalum and blued in tap water. Microfilariae were counted microscopically and the number of microfilariae per 10 µl blood expressed as a geometric mean using a $\log x + 1$ transformation. Microfilaraemia was considered extinct when 2/3 of the mice had been microfilariae negative for two successive bleeds. Challenge infections of five female worms were given to mice when the primary infection had been cleared. Age matched naive control mice were given similar infections at the same time.

Radiation chimaeras

Recipient mice were irradiated with approximately 900 rad of gamma irradiation using the Nottingham University ^{60}Co source (output ~ 2700 rad/h). BM cell suspensions were prepared from the long bones of donor mice and adjusted to approximately 5×10^7 cells/ml in Hanks' balanced salt solution with heparin (5 iu/ml). Between 1 and 2×10^7 cells were injected i.v. into the lateral tail veins of recipients within 3 h of irradiation. Recipient mice were allowed to rest for 90 days to ensure the donor BM graft had replaced all BM derived cells. Irradiated mice were given Terramycin in their drinking water.

Results

Microfilaraemia in inbred mouse strains

Five adult female *D. viteae* were implanted s.c. into each of six female mice per strain and the mice were then bled between one and three times a week. The results, in terms of peak microfilaraemia and duration are shown in Table 2. Mf were first seen in the peripheral blood at 2 days post-infection in all strains. Peak microfilaraemia occurred between days 4 and 10 in B10, CBA and C3H mice with mean values ranging from 10 to 14 Mf/10 µl. Thereafter, the microfilaraemia declined and was extinct by day 30 in B10 and day 58 in CBA and C3H. In contrast, microfilaraemia reached a much higher level in BALB/c mice peaking at a mean of 323 Mf/10 µl. This peak occurred between days 19 and 56 for individual animals and microfilaraemia was not extinct until about day 180 post-infection. NIH mice showed an intermediate pattern, reaching a peak of 89 Mf/10 µl at day 23 post-infection which declined to extinction at around 63 days post-infection.

Microfilaraemia in challenge infections

Upon extinction of microfilaraemia, B10, BALB/c and NIH mice were challenged with a further five female worms (Table 3). The observed microfilaraemias were of lower level and of shorter duration than in the primary infection for each strain. The microfilaraemia in age-matched challenge control mice was higher and longer in duration than in the challenged mice but did not normally reach the levels seen in the initial primary infection. This age-linked difference was particularly marked in the B10.G, B10.BR and BALB/k strains.

Table 2. Microfilaraemia in various mouse strains, congenic strains and F_1 hybrids following the s.c. implantation of five female *D. viteae*

Strain	H-2 haplotype	Microfilaraemia (Mf/10 l blood)		Duration of microfilaraemia (days)	
		Peak*	Range†	Duration‡	Range§
BALB/c	d	323	293–438	180	140–**200**
B10.D2/n	d	15	12–46	56	25–63
BALB/b	b	252	192–694	202	156–218
B10	b	14	9–31	30	20–44
BALB/k	k	266	289–389	238	127–265
B10.BR	k	19	24–82	63	29–77
CBA	k	10	3–39	58	12–**60**
C3H	k	11	10–59	58	24–**60**
NIH	q	89	61–300	63	44–**65**
B10.G	q	48	27–201	49	26–64
(NIH × B10.G) F_1	qq	34	14–98	49	21–64
(NIH × B10) F_1	qb	17	3–35	28	9–29
(BALB/c × B10) F_1	db	42	33–50	22	14–22

* Peak refers to the peak geometric mean.
† Range refers to the range of peak microfilaraemias recorded for individual mice.
‡ Duration refers to the period from infection to the point where 2/3 of the mice were amicrofilaraemic.
§ Range refers to the shortest and longest microfilaraemias recorded for individual mice. Bold writing indicates a positive microfilaraemia at the termination of the experiment.

Adult worm survival

BALB/c and B10 mice were implanted with five female worms and, on days 17, 22, 27, 32, 37 and 50 post-infection, groups of two to four mice were killed and dissected to recover adult worms.

At post-mortem, worms were generally to be found either beneath the dorsal skin or in the axillae. On single occasions a worm was found in the peritoneum and the thoracic cavity.

At day 17, the worms recovered from both strains were of normal appearance with motile microfilariae *in utero*. On day 22 the worms were being enclosed by host tissue although there was little cell adherence and healthy worms could be removed with little difficulty. By day 27, in both B10 and BALB/c mice, some of the worms were dead and these were enclosed in soft nodules; some live worms were also in soft nodules. There was obvious depletion of *in utero* microfilariae and some worms had no Mf. By day 32 all the worms in the BALB/c mice were dead and showed evidence of internal disintegration. No Mf were visible *in utero* and the worms were all enclosed in nodules. Some worms in B10 mice were still alive at this time but had no Mf *in utero*. By day 50 all worms were dead in both strains.

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Table 3. The microfilaraemia in mice following a secondary challenge infection of five female worms

Strain	Peak microfilaraemia (Mf/10 µl blood)			Duration of microfilaraemia (days)		
	Primary*	Secondary†	Control‡	Primary	Secondary	Control
BALB/c	323	23	169	180	120	180
BALB/b	252	39	nd	202	63	nd
BALB/k	266	39	81	238	106	99
B10	14	4	22	30	22	22
B10.BR	19	3	6	63	32	30
B10.D2/n	15	3	22	63	23	50
B10.G	48	3	7	49	22	29
NIH	89	3	48	63	27	43

* Primary infection of five female worms in 6–10 week old mice.
† Homologous challenge infection given following extinction of microfilaraemia.
‡ Age matched challenge controls.
nd = not done.

Microfilaraemia in H-2 congenic mice and F_1 hybrids

On the basis of the above results, B10 and BALB/c mice were selected as representative high responder (resistant) and low responder (susceptible) strains respectively. A selection of H-2 congenic mice on the B10 and BALB/c backgrounds were then examined to assess the influence of H-2 haplotype on response phenotype. The strains used and their H-2 haplotypes are listed in Table 1.

F_1 hybrids were made between resistant and susceptible strains, as listed in Table 2.

In each case six female mice were implanted with five female worms and the results are given in Table 2.

In the congenic strains the microfilaraemia followed the pattern seen in the corresponding background strain, i.e. the response of BALB/b and BALB/k was essentially the same as BALB/c, and that of B10.D2/n, B10.BR and B10.G was essentially the same as B10 (Figures 1 & 2). Thus, no significant influence of the H-2 haplotype on the course of microfilaraemia could be detected.

The pattern of microfilaraemia in F_1 hybrids followed that of the most responsive parent, i.e. (NIH × B10.G) F_1 were essentially the same as B10.G and (NIH × B10) F_1 and (BALB/c × B10) F_1 were as B10.

Microfilaraemia in BM radiation chimeras

Twelve 6–10 week old BALB/c mice were irradiated and six were reconstituted with B10.D2/n BM. Six 6–10 week old B10.D2/n were similarly irradiated and reconstituted
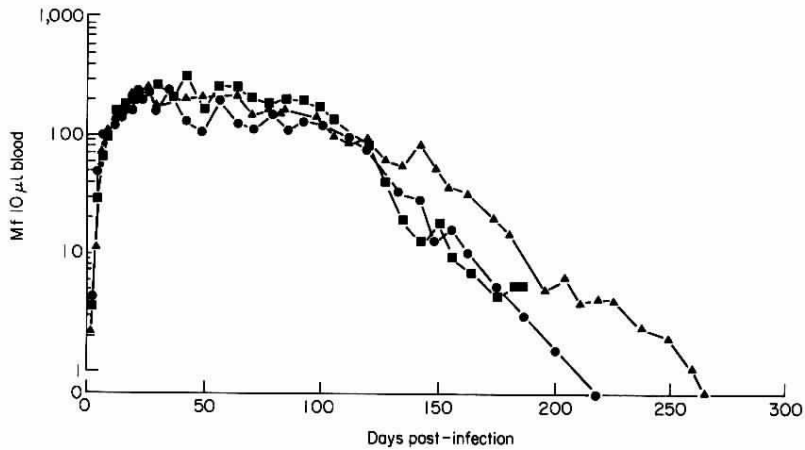
Figure 1. Microfilaraemia in H-2 congenic mice on the BALB/c background following the s.c. implantation of five female *D. viteae*: BALB/b ●——●, BALB/c ■——■, BALB/k ▲——▲.
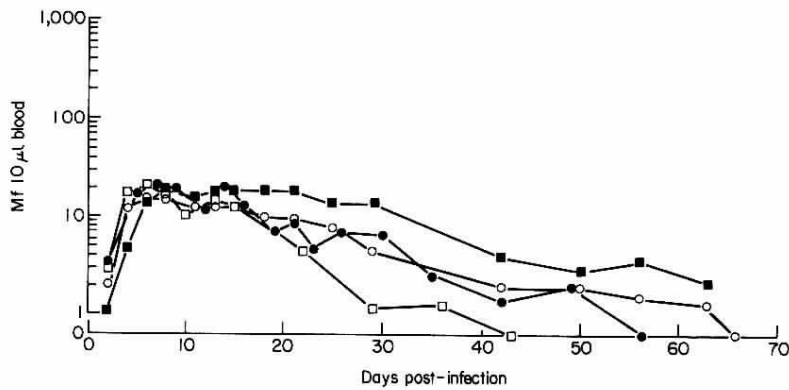

Figure 2. Microfilaraemia in H-2 congenic mice on the B10 background following s.c. implantation of five female *D. viteae*: B10 ●——●, B10.D2/n ○——○, B10.BR ■——■, B10.G □——□.

with B10.D2/n BM to provide controls for the effects of irradiation. Unirradiated, age matched BALB/c and B10.D2/n were included as additional controls. The chimeras were allowed to recover and then all the mice were implanted with five female worms. Results are shown in Figure 3.

The microfilaraemias in B10.D2/n mice irradiated and reconstituted with syngeneic BM did not differ significantly from those seen in the controls. However, whereas normal BALB/c mice showed the characteristic high and prolonged microfilaraemia, in BALB/c mice reconstituted with B10.D2/n BM, microfilaraemia followed the B10.D2/n type response and was not significantly different from that recorded in syngeneically reconstituted or normal B10.D2/n mice.
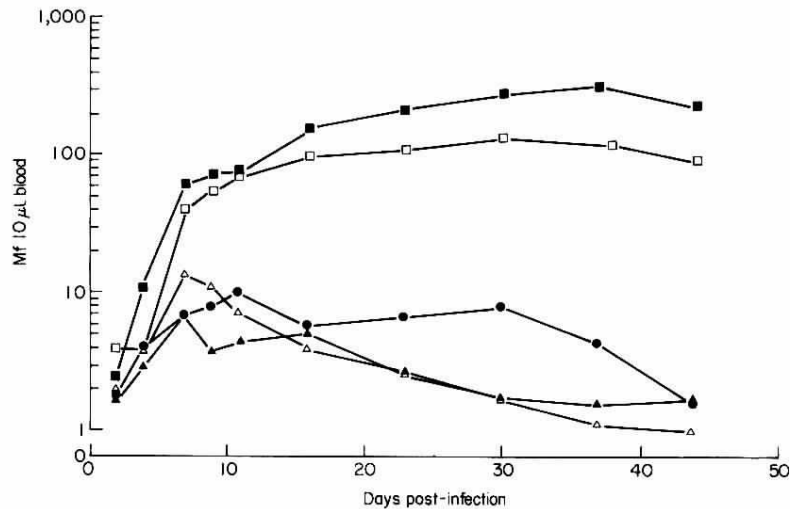
Figure 3. Microfilaraemia in radiation chimaera mice following subcutaneous implantation of five female *Dipetalonema viteae*: irradiated BALB/c reconstituted with B10.D2/n BM ●——●, irradiated BALB/c reconstituted with BALB/c BM ■——■, normal BALB/c □——□, irradiated B10.D2/n reconstituted with B10.D2/n BM ▲——▲, normal B10.D2/n △——△.

Discussion

This study has shown that inbred mouse strains differ in their response to infection with s.c. implanted female *D. viteae*. The microfilaraemia in B10, CBA and C3H mice was of low level and short duration in comparison with the microfilaraemia in BALB/c mice; NIH mice demonstrated an intermediate response. The ability to respond rapidly in terms of Mf clearance was inherited as a dominant trait, as demonstrated by the use of F_1 hybrids, and was not significantly influenced by MHC linked genes. In all strains the adult female worms became depleted of Mf after about 30 days and were then killed. Hence the low microfilaraemia in B10 mice does not appear to be due to a suppression of Mf production. In B10 mice microfilaraemia is controlled before the adult worms are killed, whereas the opposite is true for BALB/c mice in which Mf persist long after adult worm death. Thus, independent mechanisms appear to act against adults and microfilariae. When amicrofilaraemic mice were challenged with a homologous infection the resultant microfilaraemia was lower than in age matched controls, and the latter generally exhibited lower microfilaraemias than in the initial primary infections. Hence, all strains appear to develop both age immunity and acquired immunity to infection with *D. viteae*.

Using methods similar to those described here, Haque *et al.* (1980) found BALB/c mice to be more susceptible (in terms of microfilaraemia) than C57Bl/6 (B6) mice, with C3H/He mice intermediate. Our study found that the response of C3H/He was essentially the same as B10 mice which are genetically closely related to B6; this difference in strain designation may be due to substrain variations between the mice used in different laboratories. However, our results also contradict those of Haque *et al.* (1980) in terms of adult worm survival. Whereas these workers found worms to survive longer in BALB/c mice than in B6 mice, we found worms to survive longer in B10 than in BALB/c.

Inheritance of resistance to *D. viteae* was dominant in F₁ hybrids produced by crossing resistant and susceptible strains and this has also been reported for resistance to *Brugia malayi* Mf in mice (Fanning *et al.* 1983). In Bancroftian filariasis, Ottesen *et al.* (1981) found that the familial clustering of disease in an endemic area was best explained by postulating the existence of a recessive gene for filariasis susceptibility, with frequency 0.82 ± 0.15 and a penetrance 0.62 ± 0.14, such an explanation being twice as likely as the influence of environmental factors.

Our study showed no significant influence of the H-2 haplotype on response phenotype. Resistance to several parasites has been shown to be influenced by genes linked to the MHC (Vadas 1980) but this has not so far been demonstrated for filarial infections. Ottesen *et al.* (1981) found no significant linkage of susceptibility to Bancroftian filariasis with either HLA-A or HLA-B locus specificities. In rodents, Fanning *et al.* (1983) found that susceptibility to *B. malayi* microfilaraemia was independent of H-2 and Cruickshank *et al.* (1983) found no influence of the RTI (rat MHC) on resistance to infection with *B. pahangi*.

Although a detailed study of the mechanisms through which genetically determined variation in response is expressed was not included within this particular study, the results of the experiments using radiation chimaeras imply that expression is associated with a cell population of BM origin. It cannot be excluded that the early death of transplanted adult worms results from a physiological incompatibility with the murine host, but this is clearly not the cause of the variation in microfilaraemias, which must therefore arise from variation in the ability to mount an effective immune response. The cell populations involved could therefore be lymphoid or myeloid or both, as both antibody-mediated and granulocyte- or macrophage-mediated protective responses are known to operate against *D. viteae* in rodents (reviewed by Philipp *et al.* 1984). Analysis of immunological responses during the course of infections in high and low responder mice will form the subject of a subsequent paper.

Acknowledgements

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