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Immunity to *Dipetalonema viteae* (Filarioidea) infections in resistant and susceptible mice

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Summary

The course of infection with *Dipetalonema viteae* in mice shows marked genetically-determined strain variation. Subcutaneous implantation of 5 female *D. viteae* into C57BL/10 (B10) mice results in a short term, low level microfilaraemia compared with that seen in similar infections in BALB/c mice. Adult worm survival is similar, thus the different patterns of infections reflect responses directed against the microfilariae larvae (mf). A number of immunological parameters have been monitored during infection in an attempt to identify strain differences which may be correlated with levels of resistance. Blast cell activity in the spleen and lymph nodes showed little strain difference, peaking on day 10 and declining as mf disappeared from the circulation. Total serum IgG levels doubled in both strains during infection, the response being more rapid in B10 mice. Serum IgM levels increased threefold in BALB/c mice but fourteen-fold in B10. Radiosorbent assays identified comparable anti-adult antibody and anti-mf homogenate IgM antibody responses in both strains. Immunofluorescent assay showed that the appearance of IgM antibodies directed against the mf surface correlated with the clearance of mf from the blood of B10 mice, whereas similar antibodies were not detected in BALB/c mice. It is proposed that the efficient clearance of mf in B10 is mediated through an IgM-dependent mechanism and that the chronic microfilariaemia seen in BALB/c mice is facilitated by the absence of a specific IgM response to mf surface antigens.

Key words: filariasis; inbred mouse, immunity; *Dipetalonema viteae:* IgM.

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Introduction

The existence of individual host variability in the course of filarial infections of man and laboratory animals is a well documented but poorly understood phenomenon. In human lymphatic filariasis, for example, there is often a wide spectrum of disease manifestations amongst individuals in the same endemic region, where one might suppose exposure to infection to be relatively uniform. This variability implies that host factors play an important role in the determination of disease susceptibility and resistance and indeed, such factors have been implicated in the susceptibility of individuals to Bancroftian filariasis (Ottesen et al., 1981). Similarly, the host genotype has been found to influence filarial infections in inbred laboratory rodents (review, Philipp et al., 1984), although the mechanisms underlying such differences have received relatively little attention.

The paucity of laboratory models of filariasis involving immunologically and genetically well defined hosts has led to the use of "proxy" models whereby mice, which are generally refractory to filarial infections, are used as the hosts for transplanted parasites. Thus patent microfilaraemias have been established in mice by the transfusion of *Brugia malayi* and *Onchocerca* spp. microfilariae (Grove et al., 1979; Aoki et al., 1980; Townson and Bianco, 1982) or by the implantation of gravid *Dipetalonema viteae* females (Haque et al., 1980a; Storey et al., 1985). We have previously reported that when mice are infected by the subcutaneous (s.c.) implantation of adult, female *D. viteae* there is a host strain-associated variation in the pattern of the resulting microfilaraemia (Storey et al., 1985). Thus, BALB/c mice support a high level, long lasting microfilaraemia whereas in C57BL/10 (B10) mice the microfilaraemia remains at a relatively low level and is of short duration. The refractory phenotype of B10 mice is inherited as a dominant characteristic, is not obviously influenced by H-2 linked genes and is mediated via bone marrow-derived cells, suggesting the involvement of immunologically mediated resistance mechanism. Comparative studies of infections in B10 and BALB/c mice therefore provide a useful way of analysing the components of this immunity.

Previous studies of the mechanisms of immunity to *D. viteae* microfilariae in mice (Thompson et al., 1979; Philipp et al., 1984) have been based on a comparison of infections in CBA/H mice and the immunologically deficient CBA/N strain. The latter are unable to respond to certain T-independent antigens (Amsbaugh et al., 1972; Scher et al., 1975) and have a selective IgM deficiency (Perlmutter et al., 1979). It has been suggested that CBA/N mice are unable to control *D. viteae* microfilaraemia because they fail to produce an IgM response to the antigens present on the surface of the microfilariae whereas the normal, resistant CBA/H mice are capable of doing so (Philipp et al., 1984). However, it cannot be assumed that the factors underlying the susceptible phenotype of mutant CBA/N mice are necessarily the same as those determining

the susceptibility of BALB/c and other strains. Indeed, BALB/c mice are able to respond strongly to T-independent antigens such as pneumococcal polysaccharide (Amsbaugh et al., 1972) whereas the CBA/N strain has a negligible response. As both B10 and BALB/c mice are capable of responding to a wide variety of antigens (Festing, 1979) an initial analysis of the reasons underlying their contrasting response to *D. viteae* must involve the assessment of a broad spectrum of immunological parameters.

The experiments described in this paper were designed to compare the immune responses of B10 and BALB/c mice following the implantation of adult, female *D. viteae.* In order to assess the overall responsiveness of the two strains, parameters such as blast cell activity in lymphoid organs, splenomegaly and immunoglobulin levels were determined in relation to uninfected mice. Responses specific to the parasite were assessed by assaying antibody responses to semi-defined antigen preparations and by determining the presence and isotype of antibodies to the microfilarial surface.

Materials and Methods

Experimental design

The microfilaraemia resulting from the implantation of five adult, female *Dipetalonema viteae* into female B10 and BALB/c mice has been described previously (Storey et al., 1985) and is consistently reproducible in this laboratory. Based on this data, five timepoints during the course of infection in B10 and BALB/c mice were chosen to cover the duration of microfilaraemia. These were days 5, 10, 20, 27 and 32 post-infection for B10 and days 10, 20, 48, 120 and 170 for the BALB/c strains. At each point, the following parameters were measured in relation to uninfected mice: blast cell activity in the spleen and in the peripheral lymph nodes, spleen index, total serum IgM and IgG and specific antibody to adult worms and microfilariae. Sera, spleens and lymph nodes were pooled from the mice tested at each time-point. Additional serum samples were taken from BALB/c mice 88 days post-infection and from B10 mice 37 days post-infection.

Animals

Six to ten week old, female BALB/c/01a and C57B1/10/ScSn/01a (B10) mice were obtained from Olac 1976 Ltd (Bicester, England) and maintained under conventional animal house conditions.

Infection of mice with D. viteae

Twenty mice of each strain were implanted s.c. with 5 female *D. viteae* recovered from 96-day infected male DSN hamsters as previously described by Storey et al. (1985). Ten uninfected age matched mice of each strain served as controls.

Microfilariae counts

The course of microfilaraemia in the two strains was determined by examining stained smear preparations of 10 μl retro-orbital blood samples obtained from all surviving animals at various points post-infection. Microfilaraemia was calculated as a group geometric mean following a log x + 1 transformation.

Isolations of microfilariae

Microfilariae were isolated from the blood of infected jirds by a method based on that used to recover trypanosomes by Lanham and Godfrey (1970) (Worms M. J., personal communication). Jirds were bled by cardiac puncture into an approximately equal volume of phosphate buffered saline

(ionic strength 0.073, pH 7.98) containing glucose (4%) and heparin (500 units). The blood suspension was applied to a column of DEAE-cellulose (DE52, Whatman) having a bed volume four times greater than the blood volume, and pre-equilibrated with PBS/glucose prior to use. The column was connected to a reservoir of PBS/glucose (without heparin). Microfilariae passed through the column but blood cells were retained. The purified microfilariae were concentrated by centrifugation.

Blast cell assays

Blast cell activity was measured in the spleen and in the peripheral lymph nodes (pooled axillary, brachial and inguinal nodes). Cell suspensions were made in RPMI 1640 with L-glutamine (Gibco) supplemented with foetal calf serum (5%), HEPES (20 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) and $NaHCO_3$ (0.06%); their viability was determined by trypan blue exclusion. Aliquots of 5×10^5 viable cells in 250 μl of medium containing 1 μCi radiolabelled thymidine (^{3}H-thymidine, Amersham International) were incubated in flat-bottomed microtitre plates for two hours at 37° C in 5% CO_2. Cells were harvested using a Titertek Cell Harvester (Flow Laboratories) and beta activity determined by counting for one minute in a Packard Tricarb Liquid scintillation spectrometer. Results are expressed as counts per minute (cpm) for 5×10^5 cells.

Spleen index

The weight of the spleen in relation to the weight of the body was calculated by the formula:

$$\text{Spleen index} = \frac{\text{weight of infected spleen/weight of infected mouse}}{\text{weight of control spleen/weight of control mouse}}$$

Serum immunoglobulin measurement

Serum IgM and IgG levels were measured by single radial immunodiffusion using monospecific sheep anti-mouse Ig antisera (Serotec), incorporated into 1% agarose in 0.2 M Tris buffer, pH 8.3, containing 3% polyethylene glycol 6000. Calibration curves were constructed using standardised mouse reference sera.

Antigen preparations

Adult antigen. – The crude antigen was prepared from whole male and female worms, and hence contained microfilarial as well as adult material. Adult male and female worms were recovered from hamsters 90 days post-infection. Equal wet weights of worms were pooled, washed in borate-buffered saline (BBS) at pH 8.4, and then homogenised in a minimal volume of BBS at 4° C. The homogenate was ultracentrifuged at 100,000 *g* for 60 min and the protein content of the decanted supernatant determined by the Bio-Rad assay (Bio-Rad).

Microfilarial antigen. – 1.5×10^6 microfilariae isolated from jird blood were homogenised in carbonate/bicarbonate buffer pH 9.6 at 4° C. The homogenate was then sonicated for 5 min (three times) at 4° C, ultracentrifuged and the protein content of the decanted supernatant determined.

Assays for antibodies to adult worms

Antibodies to adult worms were measured by a solid phase radio-sorbent assay. Adult worm antigen was diluted from 1 mg ml^{-1} to 60 μg ml^{-1} in carbonate/bicarbonate buffer pH 9.6 and aliquots of 50 μl (3 μg) of antigen were then placed in wells of flexible flat bottomed microtitre plates (Falcon, Becton & Dickinson) and incubated at 4° C overnight. The wells were then washed three times with phosphate-buffered saline (PBS) containing Tween 80 (0.05%) pH 7.4 A range of test serum dilutions was prepared, 50 μl added per well and the plates incubated at room temperature for 3 h. The wells were then washed a further four times with PBS-Tween, after which 50 μl of ^{125}I-labelled rabbit anti-mouse immunoglobulin (Birmingham University, Department of Immunology) was added to each well (approx. 10,000 counts per well). The plates were incubated at 4° C overnight, following which the wells were washed five times with PBS-Tween. The wells were then individually detached from the plates and gamma-activity determined in a Packard 800 Gamma Counter. Results are expressed as counts per minute following the subtraction of background counts obtained using normal serum as a control.

Assays for antibodies to microfilarial homogenate

A radiosorbent assay similar to that described for adult worms was used except that 1.5 μg of antigen were placed in each well and a ^{125}I-labelled rabbit-antimouse IgM (Birmingham University, Department of Immunology) was used. Bound labelled antibody was counted as above.

Assay for antibodies to the microfilarial surface

10^4 pelleted microfilariae were suspended in 100 μl of test serum (at a range of dilutions) for 1 h at 37° C. The microfilariae were then washed three times with PBS and resuspended in 100 μl of a $^1/_{10}$ dilution of sheep anti-mouse IgM or IgG (Serotec, Bichester, England) and incubated for 30 min at 4° C. The microfilariae were again washed three times in PBS and resuspended in 100 μl of a $^1/_{20}$ dilution of FITC conjugated donkey anti-sheep serum (Serotec) and incubated for 30 min at 4° C. Following two washes in PBS the microfilariae were resuspended in Hanks' Balanced Salt Solution/ Glycerol (50:50) and examined for fluorescence using an Olympus BHS microscope fitted with an Olympus BH-RFL-W reflected light fluorescent attachment. The degree of fluorescence was scored from 0 to +++.

Results

The course of microfilaraemia as seen in the B10 and BALB/c mice used in this study is depicted in Fig. 1, and was essentially similar to that reported previously (Storey et al., 1985) and which is consistently reproducible.

Blast cell activity

Figs. 2 and 3 compare blast activity in the spleen and peripheral lymph nodes, of infected and control animals at increasing times post infection. Overall, the patterns for the two strains were similar. Peak activity in both the spleens and nodes was seen on day 10 post-infection and declined thereafter. At the final time points examined – day 32 for B10 mice and day 170 for BALB/c mice – blast activity was little more than that seen in control mice. The spleen index increased to approximately 2 during the course of infection. The peripheral lymph nodes were also seen to be considerably enlarged throughout the infection.

Concentration of serum immunoglobulin isotypes

IgM and IgG levels in control animals of both strains remained relatively constant throughout the period of study. After 32 days of infection in B10 mice the serum IgG concentration was 13.8 mg ml^{-1}, representing an approximate doubling over that of the controls. By comparison, a similar increase in BALB/c mice was reached after about 88 days, the IgG concentration being 15.2 mg ml^{-1} on day 120 (Table 1). In infected BALB/c mice IgM levels were increased threefold by day 88 and thereafter increased only slightly. In contrast, IgM levels in the resistant B10 strain increased throughout the period of observation and on day 32 reached 1.6 mg ml^{-1}, representing an approximate fourteen-fold increase over the control mean.

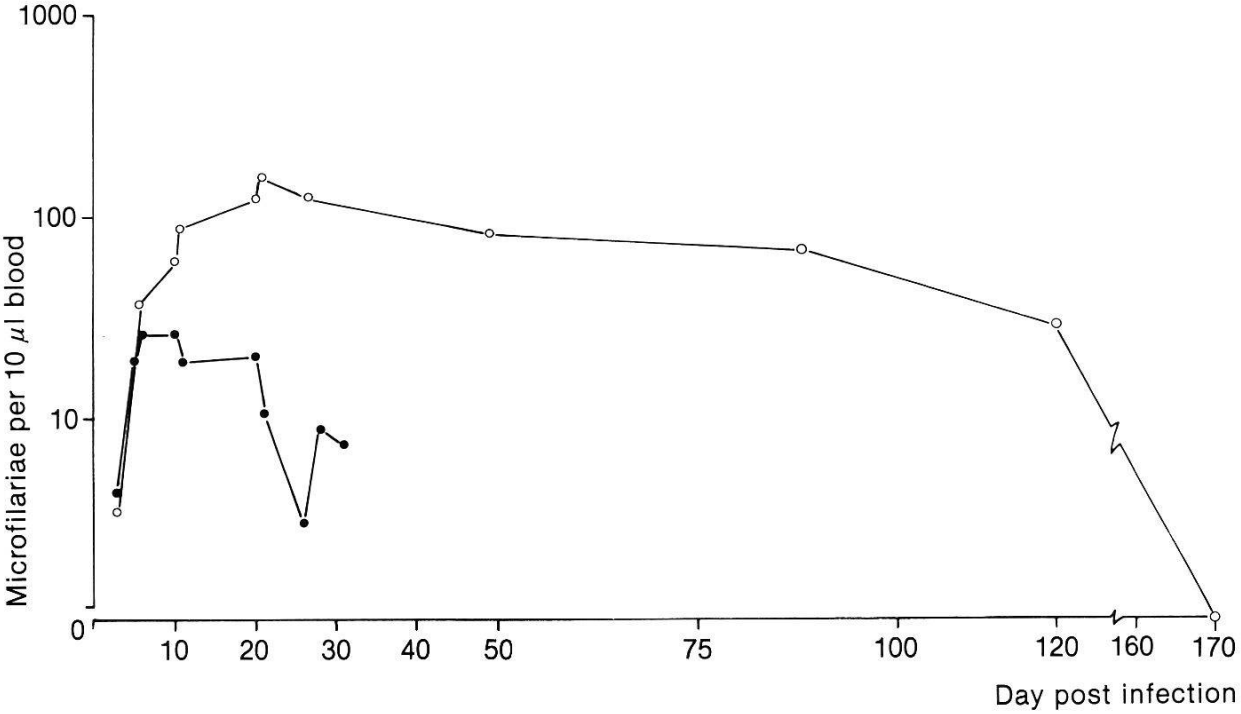

Fig. 1. The course of microfilaraemia in B10 and BALB/c mice following s.c. implantation of five female *Dipetalonema viteae*: B10 ●——●, BALB/c ○——○.

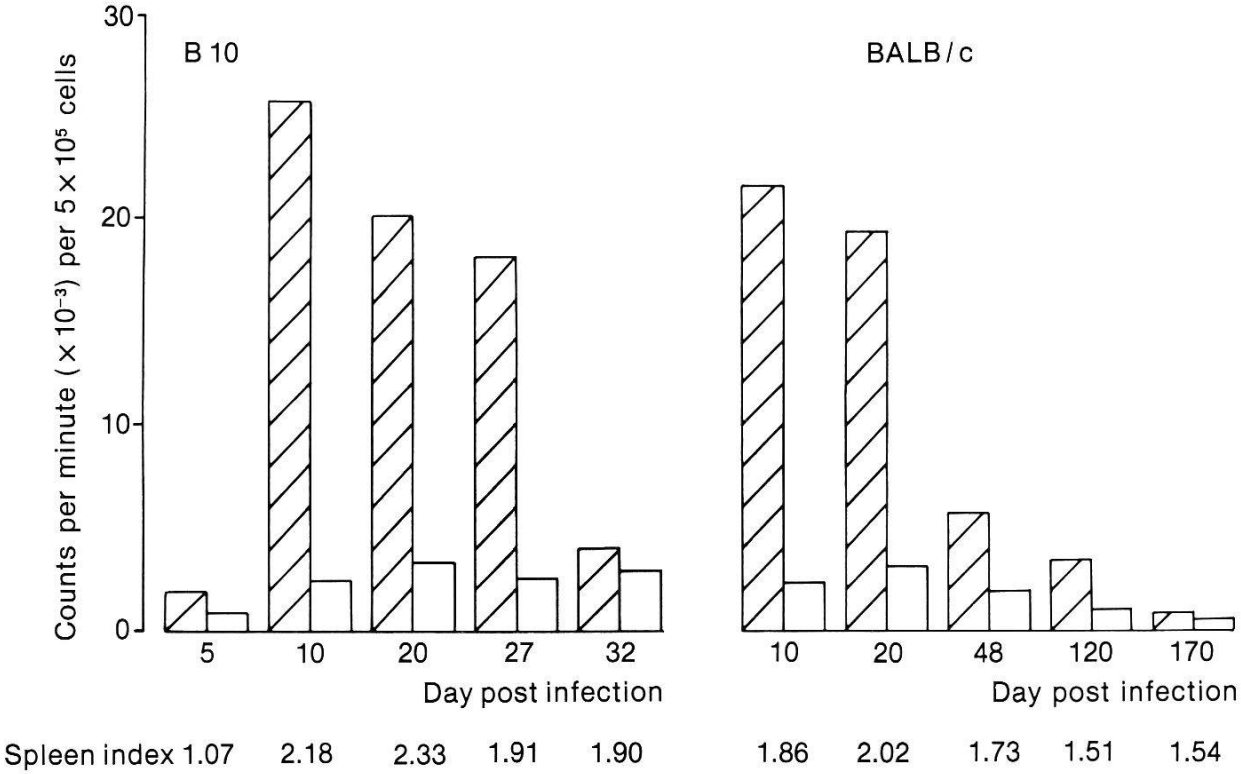

Fig. 2. Blast cell activity in the spleen cells of B10 and BALB/c mice following s.c. implantation of five, female *Dipetalonema viteae*, as determined by the in vitro incorporation of tritiated thymidine: infected ▨, control □.

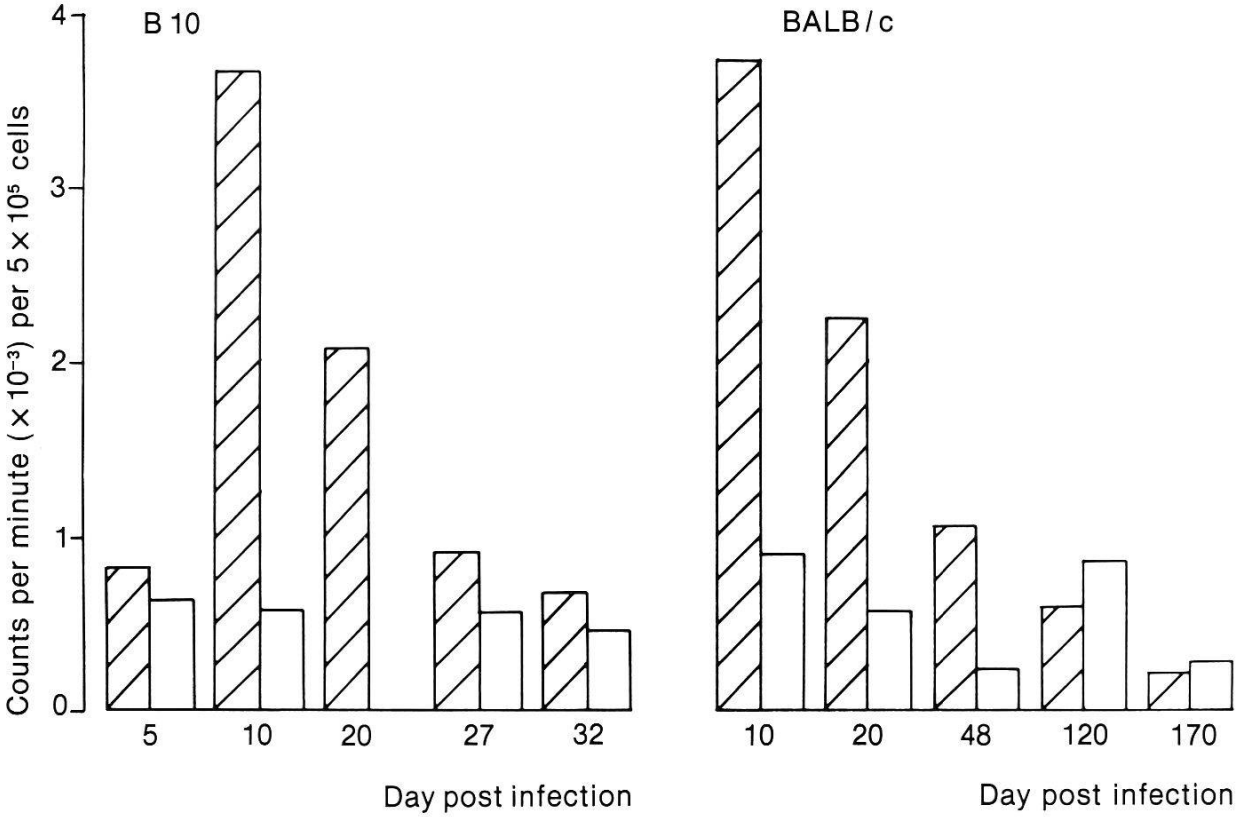

Fig. 3. Blast cell activity in the peripheral lymph node cells of B10 and BALB/c mice following s.c. implantation of five, female *Dipetalonema viteae*, as determined by in vitro incorporation of tritiated thymidine. Infected ▨, control

Table 1. Serum IgM and IgG levels in B10 and BALB/c mice following the s.c. implantation of five, female *Dipetalonema viteae*

	Day post-infection	Immunoglobulin level (mg/ml)	
		IgM (increase)[a]	IgG (increase)
B10	Control	0.12 s.d. 0.03[b]	6.32 s.d. 0.58
	5	0.15 (×1.3)	4.21 (×0.7)
	10	0.84 (×7.3)	10.51 (×1.7)
	20	0.78 (×6.8)	9.83 (×1.6)
	27	1.31 (×11.4)	13.00 (×2.1)
	32	1.60 (×13.9)	13.77 (×2.2)
BALB/c	Control	0.30 s.d. 0.04	5.54 s.d. 1.28
	10	0.31 (×1.3)	4.95 (×0.9)
	20	0.65 (×2.1)	6.44 (×1.2)
	48	0.92 (×3.0)	8.67 (×1.6)
	88	0.93 (×3.0)	13.24 (×2.4)
	120	0.94 (×3.1)	15.24 (×2.8)

[a] increase = relative increase in infected mice compared with control
[b] s.d. = standard deviation of mean of control sera

Parasite specific antibodies

Antibodies specific for the crude adult worm extract were detected in both strains of mice, and there was no obvious difference between the strains in the pattern and intensity of their response. Similarly, both strains of mice had IgM antibodies which recognized the antigen extract prepared from homogenised microfilariae, and the intensity and pattern of the responses were similar.

Microfilaria surface specific antibody

Using microfilariae harvested from the blood of infected jirds, no surface specific IgG could be detected in sera from either strain at any of the time points examined. Specific IgM was detected in B10 mice on day 20, although staining was weak and titred out at 1:20. By day 27 the staining was intense (titre 1:160). Sera collected on days 32 and 37 showed a progressive decline in intensity of fluorescence; both titres were 1:40. In contrast no surface specific IgM could be detected in sera from BALB/c mice at any time.

Sera were later re-tested for IgM antibodies to the microfilarial surface using microfilariae harvested directly from the uteri of adult female worms, and similar results were obtained (data not shown).

Discussion

The present study was concerned with the relationship between susceptibility and resistance of mice to infection with *Dipetalonema viteae* and their immune responses to the parasite. The survival of subcutaneously implanted adult worms is similar in both B10 and BALB/c mice (Storey et al., 1985) but the BALB/c strain is highly susceptible in terms of microfilaraemia when compared with B10 mice. Previous evidence from radiation chimaera studies has indicated that the resistance of B10 mice is mediated via a population of bone marrow-derived cells (Storey et al., 1985) and may therefore have an immunological basis.

In the present study, both strains were found to respond similarly in terms of blast cell activity in the spleen and peripheral lymph nodes and thus are capable of responding immunologically to the infection. Although this assay was not designed to indicate specificity for particular parasite antigens, it demonstrated that BALB/c strain is not anergic in terms of immunological activity following infection. The degree of splenomegaly was similar in both strains. Splenomegaly has also been observed during the microfilaraemic phase of *Brugia* spp. infections of rodents (Vincent and Ash, 1978; Grove et al., 1979) and probably reflects an intense antigenic stimulus. However, the persistent splenomegaly seen throughout the chronic microfilaraemia in BALB/c mice suggests that this organ is unlikely to play a major role in removing microfilariae from the circulation. Indeed, intact and splenectomised B10 mice are equally capable of controlling *D. viteae* microfilaraemia (Storey, unpublished).

It was observed that in both B10 and BALB/c mice there were marked increases in both IgM and IgG levels following infection. Both strains showed similar two-fold increases in total serum IgG over the course of infection. In contrast, the increase in IgM levels was considerably greater in B10 than BALB/c mice. B10 mice are known to produce high levels of IgM antibody in response to antigens such as sheep erythrocytes and it is believed that they do not readily switch to IgG (Haber and Winn, 1981). Both strains produced antibody reacting with the crude adult worm extract, although caution must be exercised in the interpretation of this result since the antigen preparation also contained material of microfilarial origin and thus the response may have been directed against adult and/or microfilariae stages, or even other antigen sources such as uterine fluids. The assay for IgM antibodies reactive with antigen prepared from homogenised microfilariae appears to provide evidence that both strains are capable of producing a response to antigens of microfilarial origin. However, the fact that antibodies are produced in response to microfilarial antigens does not necessarily mean that these antibodies have a protective capacity. For example, Weiss (1978) detected antibodies to somatic antigens of papain digested microfilariae as early as two weeks following an L3 infection of *D. viteae* in hamsters – long before the onset of microfilaraemia. Thus it may be concluded that different stages of *D. viteae* share common or related antigens, a hypothesis supported by protein composition analysis (Prusse et al., 1982) and by Western Blotting experiments using sera from infected jirds (Lucius et al., 1983).

It is particularly interesting that antibodies to the surface of microfilariae were detected only in the resistant B10 strain. The correlation between the appearance of microfilariae-surface specific IgM and the clearance (i.e. maximal reduction) of microfilariae in B10 mice suggests that the long term survival of microfilariae in BALB/c mice might arise from the absence of a comparable antibody response. Further evidence for a role of surface specific IgM in the control of microfilaraemia comes from studies with implanted *D. viteae* in mutant CBA/N mice. Normal CBA/H mice clear microfilaraemia after around 70 days and this is associated with the appearance of IgM antibody which reacts with the surface of microfilariae (Thompson et al., 1979). In contrast, the immunologically defective CBA/N strain fails to produce a comparable response and sustains a chronic microfilaraemia. Immuno-precipitation and PAGE analysis studies by Philipp et al. (1984) showed that both normal (CBA/N × BALB/c) F1 and defective (BALB/c × CBA/N) F1 male mice produced antibodies to the surface of microfilariae and both recognised the same pattern of surface antigens. However, only the normal mice produced surface specific IgM antibodies. Thus it appears that the clearance of microfilariae has strict antibody isotype requirements.

The results presented in this paper confirm the data obtained with mutant CBA/N but extend them significantly by the important observation that defective antibody responses, associated with increased susceptibility to a filarial

parasite, can occur in normal, non-mutant mouse strains. As such, this has obvious significance for analysis of the individual variations in response seen in human populations.

The observation of Haque et al. (1980a) that outbred nu/nu (athymic) mice control *D. viteae* microfilaraemia similarly to their nu/+ littermates further confirms the T-independent nature of the response as suggested by CBA/N. Hamsters also mount a T-independent IgM response to microfilariae (reviewed, Weiss and Tanner, 1981) and it is thought that neutrophils, eosinophils and macrophages act as the effector cells in antibody mediated microfilarial destruction (Weiss and Tanner, 1979; Rudin et al., 1980). In rats, however, it has been suggested that microfilarial control involves IgE and macrophages (Haque et al., 1980b), IgG, IgE, complement and neutrophils (Aime et al., 1984) and IgE and platelets (Haque et al., 1985).

Correlation between the clearance of microfilariae from the peripheral blood of the host and the appearance of antibodies directed against their surface has been demonstrated in several other filarial systems. In individuals infected with *Brugia malayi* immunofluorescent studies demonstrated antibodies to the microfilarial sheath almost exclusively in the sera of amicrofilaraemic patients (Piessens et al., 1980) and these antibodies were of IgM or IgG isotypes. Subrahmanyam et al. (1978) found antibodies to the surface of *Wuchereria bancrofti* microfilariae in persons in an endemic area only in amicrofilaraemic individuals. Furthermore, the relationship between amicrofilaraemia and the appearance of antibodies to the microfilarial surface is particularly clear in laboratory models (reviewed by Philipp et al., 1984).

Since microfilariae are necessary for the transmission of filariasis and play a major role in the pathology of certain filarial diseases, an understanding of the immunological mechanisms which determine the outcome of the host-parasite relationship at this stage, i.e., lead to microfilarial clearance, is important in the planning of immunological strategies aimed at prophylaxis.

The *D. viteae* mouse model presents an opportunity to study both control by the host and survival by the parasite. Whether the survival of microfilariae in BALB/c mice is achieved by parasite mediated modulation of the host responses or is the consequence of an inherent inability of the host to produce qualitatively or quantitatively appropriate responses remain to be elucidated.

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