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ACANTHOCHEILONEMA VITEAE (*DIPETALONEMA VITEAE*) IN MICE: ATTEMPTS TO CORRECT THE LOW RESPONDER PHENOTYPE OF THE BALB/c HOST

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Abstract—STOREY N., BEHNKE J. M. and WAKELIN D. 1989. *Acanthocheilonema viteae* (*Dipetalonema viteae*) in mice: attempts to correct the low responder phenotype of the BALB/c host. *International Journal for Parasitology* **19**: 723–727. Attempts were made to correct the low responder phenotype of microfilaraemic *Acanthocheilonema viteae* (*Dipetalonema viteae*) infected BALB/c mice through the transfer of immune spleen cells and immune serum from amicrofilaraemic B10 background mice. The transfer of immune cells and serum prior to infection failed to influence development of microfilaraemia in BALB/c recipients. Attempts to alter the course of an established microfilaraemia in BALB/c mice through the transfer of 3×10^7 immune spleen cells were unsuccessful but transfer of 3×10^8 cells reduced microfilaraemia temporarily. Treating microfilaraemic BALB/c mice with immune serum brought about a rapid reduction in microfilaraemia. This effect was only temporary and numbers of circulating microfilariae returned to control levels within a short time. Repeated serum transfers reduced the microfilaraemia only during the period of treatment. Similar results were obtained when immune serum was given to microfilaraemic, immunodeficient CBA/N mice.

INDEX KEY WORDS: *Acanthocheilonema viteae*; *Dipetalonema viteae*; mice; BALB/c; C57Bl/10; CBA/N; microfilaraemia; IgM; antibody; resistance.

INTRODUCTION

THE paucity of laboratory models of filariasis involving hosts which are genetically and immunologically well defined has led to mice being used as proxy hosts for one or more life cycle stages (Philipp, Worms, Maizels & Ogilvie, 1984). The model whereby adult female *Acanthocheilonema viteae* (*Dipetalonema viteae*) are implanted into mice has given rise to interesting observations regarding both the influence of host genotype on the resulting microfilaraemia (Haque, Worms, Ogilvie & Capron, 1980; Storey, Wakelin & Behnke, 1985) and the immunological mechanisms controlling patent infections. For example, following such infections the microfilaraemia in C57Bl/10 (B10) mice is of a low level and short duration in marked contrast to that seen in BALB/c mice (Storey *et al.*, 1985). In a previous paper, we had suggested that host-strain variations in the course of infection may be primarily determined by the level of IgM response mounted against antigens on the surface of microfilariae, this response being present in B10 mice but apparently absent in BALB/c mice (Storey, Behnke & Wakelin, 1987). However, Almond, Worms, Harnett & Parkhouse (1987), using several extremely sensitive assays, demonstrated IgM antibodies against microfilarial surface antigens in BALB/c mice harbouring a chronic microfilaraemia. Thus the role of IgM antibodies in protection is still not fully resolved. CBA/N mice, which have a defective ability to respond to certain T-independent antigens, and have a preferential IgM deficiency, also sustain a chronic *A. viteae* microfilaraemia which resembles that in BALB/c mice in terms of duration (Thomson, Crandall, Crandall & Neilson, 1979; Philipp *et al.*, 1984).

The demonstration that BALB/c mice respond to *A. viteae* with anti-microfilarial surface IgM antibodies implies that the susceptibility of this strain cannot be explained simply by a quantitative deficiency in this isotype but it does not rule out the possibility that the chronicity of microfilaraemia in BALB/c and CBA/N mice reflects a common lesion in the immune response preventing the generation of effective, host-protective responses. BALB/c mice can be made to resist *A. viteae* after treatment involving lethal whole body irradiation and reconstruction with bone marrow stem cells from B10 D2/n mice (a H-2 compatible congenic strain; Storey *et al.*, 1985). Therefore, resistance is

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determined by the properties of bone marrow-derived precursors of lymphoid and/or myeloid cell lines. In this paper we report the results of passive and adoptive transfer experiments which were carried out to determine whether immune effector components likely to be functional in responding resistant strains (C57Bl/10 and B10 D2/n) could transfer the capacity to reduce the numbers of circulating microfilariae in chronically infected susceptible strains (BALB/c and CBA/N).

MATERIALS AND METHODS

A. viteae was maintained cyclically in the Mongolian jird *Meriones unguiculatus* and the soft tick *Ornithodorous moubata* as described by Worms, Terry & Terry (1961). Male Syrian hamsters (DSN strain; Intersimian, U.K.) were injected subcutaneously with approximately 300 infective larvae and the adult worms recovered 90–100 days post-infection. Female BALB/c, C57Bl/10 (B10), B10 D2/n, CBA/Ca and CBA/HN/-Xid (CBA/N) mice were obtained from Olac 1976 Ltd (Bicester, U.K.); experimental groups consisted of at least six mice. Six- to 10-week old mice were infected with *A. viteae* by the subcutaneous implantation of five adult, female worms as described by Storey *et al.* (1985). Microfilaraemia was monitored by examining stained smears of 10 μl of retro-orbital blood, obtained under light ether anaesthesia in accordance with the procedure outlined in the UFAW handbook (Poole, 1987). Microfilaraemias are expressed as group geometric means following a log $x + 1$ transformation. Immune lymphocytes were taken from B10 D2/n donors [H2 compatible with BALB/c] and immune sera from B10 donors. Both B10 strains rapidly develop resistance to infection. Cells and sera were taken 27 days post-infection, a time chosen to coincide with the peak antibody response to the microfilarial surface (Storey *et al.*, 1987) and with rapidly declining microfilaraemia in mice with a B10 background. Cell suspensions were prepared and transferred intravenously using standard techniques. Immune serum was also injected intravenously. In order to determine whether a significant reduction in an established microfilaraemia occurred following cell and/or serum transfers, the test for outliers in statistical data (Barnett and Lewis, 1978) was used, and the 5% level taken as significant.

RESULTS

Attempts to alter the course of microfilaraemia in BALB/c mice by cell and serum transfer

Immune spleen cells (SC). Three experiments were carried out in which SC were transferred into BALB/c mice 24 h before (experiment 1) or 34 days after (experiments 2, 3) implantation of five *A. viteae* female worms. Transfer of 3×10^7 SC had no effect on the course of subsequent microfilaraemia in either experiment 1 or 2. When the number of SC transferred on day 34 was increased to 3×10^8 (experiment 3) the mean microfilaraemia of the recipients was significantly reduced (from 280 mf 10 μl^{-1} to 194 mf 10 μl^{-1}) by 5 days after transfer, but had returned to control values by day 44. Transfer of SC from uninfected mice had no significant effect upon microfilaraemia.

Immune sera (IS). In experiments 1 and 2 groups of mice were also injected with both 3×10^7 SC and 0.5 ml IS. Whereas no effect was recorded on microfilaraemia when transfers were made before infection (experiment 1), transfers made on days 34–35 (SC—day 34, IS—day 35) resulted in a rapid and significant fall in microfilaraemia, which was wholly due to the serum transfer. Mean microfilaraemia in mice given both SC and IS fell by 53% (from 300 to 142 mf 10 μl^{-1}) within 4 h; in mice given IS only, the fall was 58% (from 243 to 102 mf 10 μl^{-1}). In both cases pre-transfer levels of microfilaraemia were regained within 48 h.

Serum transfers into BALB/c and CBA/N mice

Time course of IS mediated effect upon established microfilaraemia. In order to determine the rapidity with which the level of microfilaraemia fell following treatment with immune serum, the numbers of microfilariae were monitored in infected BALB/c mice for 7 h directly following serum transfer. A similarly infected control group, given normal serum, was monitored in the same way. Mean microfilaraemia in the control group was 181 mf 10 μl^{-1} prior to treatment and did not alter significantly thereafter. In the group which received immune serum the mean microfilaraemia prior to treatment was 272 mf 10 μl^{-1}, this fell significantly to 115 mf 10 μl^{-1} 30 min post-treatment and returned to the original level within 48 h.

Effect of repeated IS transfers into microfilaraemic BALB/c mice. The above experiments showed that a single treatment with immune serum brought about only a temporary reduction in microfilaraemia. To determine whether maintaining the concentration of immune serum over a longer period of time might lead to a permanent reduction in the microfilaraemia the experiment was repeated with immune serum being administered on 4 consecutive days. As shown previously there was significant reduction in microfilaraemia from 367 to 131 mf 10 μl^{-1} (Fig. 1) within a

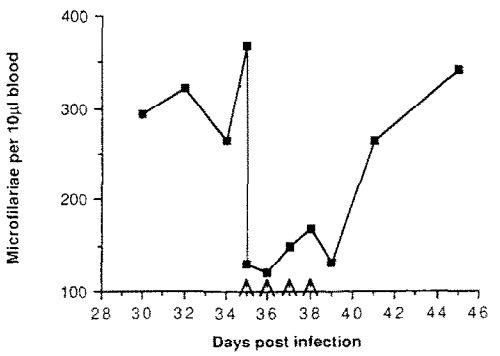

FIG. 1. The effect of repeated serum transfers on the microfilaraemia of BALB/c mice with an established *Acanthocheilonema viteae* microfilaraemia. Arrows indicate the i.v. injection of 0.25 ml day 27 (immune) B10 serum.

few hours of transfer. The microfilaraemia stayed depressed throughout the period of treatment, but returned to pre-treatment levels within 48 h of the final serum injection.

Effect of IS transfer into microfilaraemic CBA/N mice. Seven CBA/Ca and 10 CBA/N mice were each implanted with five female *A. viteae*. The microfilaraemia in the CBA/Ca mice reached a mean peak of 42 mf 10 μl^{-1} and thereafter declined. In contrast, the microfilaraemia in CBA/N mice stabilized at approximately 500 mf 10 μl^{-1} 30 days post-infection. On day 34, five CBA/N mice were treated with immune serum and five with normal serum. Four hours after treatment the microfilaraemia had fallen significantly from 590 to 234 mf 10 μl^{-1} in the former group (Fig. 2) whereas there was no significant

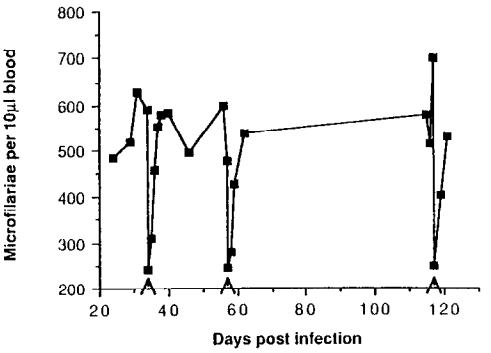

FIG. 2. The effect of injecting day 27 (immune) B10 serum into CBA/N mice with an established *Acanthocheilonema viteae* microfilaraemia. Arrows indicate the i.v. injection of 0.25 ml of serum.

reduction in the control group (data not shown). The microfilaraemia in the experimental group returned to pre-treatment levels after about 72 h. The serum transfers were repeated on days 57 and 117. In neither case was there a significant reduction in the control group. However, significant reductions occurred in the group treated with immune serum and on each occasion the pre-treatment levels were restored within 3–4 days.

DISCUSSION

The experiments described in this paper were concerned with the passive or adoptive transfer of immunity to *Acanthocheilonema viteae* microfilariae from high responder B10 background mice into low responder BALB/c mice and hence with correcting the poor responsiveness of the latter strain.

Immune cells and serum given prior to infection failed to influence the development of a subsequent microfilaraemia in recipients. That cells alone were ineffective may have been due simply to a quantitative inadequacy in terms of the number of cells transferred. However, the failure of serum transfer was more surprising. Previous studies (Storey *et al.*, 1987) indicated that immune serum contained microfilarial surface-specific IgM which has been strongly implicated in the control of *A. viteae* microfilaraemia (Weiss, 1978; Neilson, Crandall & Crandall, 1981; Philipp *et al.*, 1984). In addition, Haque, Lefebvre, Ogilvie & Capron (1978) have reported that sera from infected and latent hamsters were able to suppress the release of microfilariae *in vivo* and *in vitro*, the most suppressive antiserum being from latent hamsters. The inability of serum transfer to bring about a permanent reduction in the level of infection in the present experiments may indicate that the antibody half-life was too short for an adequate concentration to persist long enough to have had an effect; alternatively the specific antibody may have been absorbed by excess antigen.

Experiments involving the transfer of 3×10^7 spleen cells into mice with an established microfilaraemia failed to reduce the level of infection significantly. However, when spleen cells were transferred with serum, or when serum was transferred alone, there was a temporary reduction in microfilaraemia. Since it can be reasonably assumed that the transplanted female worms were no longer releasing microfilariae at the time of treatment (Storey *et al.*, 1985) then this reduction has two possible explanations. Either a proportion of the microfilariae was killed and permanently removed from the circulation, but the microfilaraemia re-stabilized at the previous level through the release of parasites harboured in the visceral tissues (e.g. lungs), or a proportion of the microfilariae was sequestered away from the circulation for a brief period. The first suggestion would have to imply a mechanism for stabilizing the microfilaraemia at a pre-set value.

It was assumed that if this temporary reduction was solely an antibody-mediated effect then the transfer of a large number of immune spleen cells might produce a similar result because there should be enough antibody secreting cells to reproduce the effects of a serum transfer. Indeed, transferring 3×10^8 immune spleen cells temporarily reduced the microfilaraemia for around 4 days, although the depression only became evident 2–3 days after transfer. This time lag can be explained as the interval necessary for the generation of a sufficient quantity of specific antibody and its release into the circulation. The fact that such a large number of cells was necessary in order to effect a change in parasite numbers is itself indicative of a B cell-mediated effect rather than a T cell-mediated effect since the latter generally requires transfer of many fewer cells due to amplification mechanisms. The control of *A. viteae* microfilaraemia in both hamsters and mice has been suggested to be T-independent (Weiss, 1978; Thomson *et al.*, 1979; Haque *et al.*, 1980; Philipp *et al.*, 1984).

A time course study of the reduction of microfilaraemia following the transfer of immune serum demonstrated that the depression occurred as early as 30 min post-treatment. Treating similarly infected mice with normal serum had no effect and

hence the reduction in microfilaraemia was due to factors present only in immune serum and not due to stress or physiological disturbances induced by the injection. Repeated treatment with immune serum depressed the microfilaraemia only for as long as the injections were continued, and the restoration of intense microfilaraemia followed rapidly after the cessation of treatment.

It is particularly interesting that closely comparable results to those in BALB/c mice were obtained in CBA/N mice. Thus, either both CBA/N and BALB/c mice fail to effect killing of microfilariae in the presence of supposedly requisite antibody (e.g. through competent effector mechanisms) or the effect on the circulating microfilarial population is insignificant and transient, being countered by release from tissue reservoirs.

A radiolabelling experiment (unpublished data) aimed at tracing the movement of microfilariae away from the blood did not give any indication of the organs in which parasites might be sequestered or killed, since it was found that the circulating parasites represented only a small fraction (3% or less) of the total parasite population. This means however that although changes in peripheral microfilaraemia might be relatively dramatic they do not reflect a major impact on the parasite pool. This study compares with those of Grove, Davis & Warren (1979) and Pacheco (1974) which indicated that only 3% of transfused *Brugia malayi* microfilariae circulate in mice and 7% of *Dirofilaria immitis* microfilariae circulate in dogs.

The factors controlling microfilaraemia in an infected individual are probably very complex and are not well understood. Accordingly, the demonstrated effects of immune serum on *A. viteae* microfilariae can be interpreted in several ways. If only a proportion of the microfilariae was being killed through mechanisms mediated by the transferred antibody then either there was insufficient antibody to clear all the parasites or the microfilariae represent an antigenically heterogeneous population and some of the parasites are not 'targeted' by specific antibody. If it is the case that the specific antibody is having the effect of an opsonin and microfilariae are being sequestered away from the circulation only to return later, then this suggests that BALB/c and CBA/N mice lack the ability to kill microfilariae through competent effector mechanisms, that the adherent antibody is rapidly absorbed by excess free antigen or that it is shed from the surface of the parasites. Little is known about the dynamics of the microfilarial surface, although Hammerberg, Rikihisa & King (1984) found *D. immitis* microfilariae to shed bound antibody in less than 24 h. A final more intriguing hypothesis would be that microfilarial surface-specific IgM is ineffective in BALB/c mice because its action is blocked by the presence of 'blocking' antibodies of another isotype. Such antibodies are believed to be important in the IgG modulation of IgE mediated allergic symptomatology in *Wuchereria bancrofti* infections (Hussein & Ottesen, 1985) and in *B. malayi* infections (Sim, Kwa & Mak, 1984). Thus B10 mice are successful at controlling *A. viteae* microfilaraemia because they produce large quantities of IgM in response to infection (Storey *et al.*, 1987) and this host-protective activity is not hindered by blocking antibodies of another isotype [this strain of mice being known to switch less readily from IgM to IgG production (Haber & Winn, 1981)]. BALB/c mice, which produce less IgM in response to *A. viteae* infection (Almond *et al.*, 1987), may switch to IgG (blocking) antibody production to their detriment. To support this synthesis we have preliminary data indicating that BALB/c mice make an early IgG response to certain antigens of *D. viteae* to which B10 mice do not respond until much later in the infection, and that BALB/c serum may contain factors able to block the cell mediated killing of microfilariae by B10 peritoneal cells. This will constitute the subject of further reports.

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