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Sensitization with anti-inflammatory BmAFI of *Brugia malayi* allows L3 development in the hostile peritoneal cavity of *Mastomys coucha*

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ABSTRACT

Filarial parasites survive by inducing tolerance in host but the antigens and mechanisms involved are not clear. Recently we found that BmAFI, a Sephadex G-200 eluted fraction of *Brugia malayi* adult worm extract, stimulates IL-10 release from THP-1 cells. In the present study, we determined the SDS-PAGE profile of BmAFI and infective 3rd stage larva (L3), investigated the effect of pre-sensitization of host with BmAFI on the survival and development of L3 in the non-permissive peritoneal cavity (p.c.) of the permissive host *Mastomys coucha* and in the p.c. of non-permissive Swiss mice, and studied immunological correlates for the observed effects. The parasite development and burden in p.c., was determined in sensitized infected *M. coucha* and Swiss mice and the release of TGF-β, IL-4, IL-10, IL-13, IFN-γ and NO, cellular proliferative response to Con A and BmAFI and levels of IgG subclasses and IgE were determined in sensitized infected *M. coucha*. Cellular proliferative response to Con A and BmAFI, mRNA expression of GATA-3, CTLA-4 and T-bet were determined in sensitized Swiss mice. In addition, the parasitological parameter was also studied in BmAFI-sensitized *M. coucha* exposed to the infection by standard subcutaneous (s.c.) route to assess whether sensitization enhances the intensity of infection. BmAFI-sensitization permitted survival of L3 and their development to adult stage by day 60 p.i. in the p.c. of *M. coucha*; in non-sensitized animals L3 could molt to L4 only and no parasite could be recovered beyond day 30 p.i. In *M. coucha* that received infection by s.c. route, pre-sensitization with BmAFI enhanced the microfilaraemia and adult worm recovery. In sensitized Swiss mice L3 could successfully molt to L4 in p.c. with improved recovery of parasite. BmAFI sensitization upregulated TGF-β and IL-10 release, IgG1 and IgG2b levels, GATA-3 and CTLA-4 mRNA expression, suppressed the cellular proliferative response and downregulated Con A stimulated response, IgE, IL-13, IFN-γ and NO responses. Immunoblot analysis showed that the BmAFI antiserum also strongly reacts with some L3 molecules. The results show, for the first time, that sensitization with the anti-inflammatory BmAFI which shares some of its molecules with those in L3, facilitates parasite survival in the non-permissive p.c. of the permissive host *M. coucha*, render a non-permissive Swiss mouse partially permissive to infection and enhances parasite load in *M. coucha* receiving the infection through permissive s.c. route by evoking a modified Th2 type of response and anti-inflammatory milieu. In conclusion, the findings suggest that the anti-inflammatory BmAFI fraction facilitates survival of *B. malayi* infection even in non-permissive environment.

1. Introduction

Lymphatic filariasis (LF), a longstanding chronic infection caused by *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*, is prevalent in many parts of the tropics and sub-tropics of the world. Currently over 120 million people are affected by the infection with 40 million people showing chronic disease symptoms (Molyneux, 2003). Active modulation of the host's immune response is part of the parasites' strategies for long-term survival which is characterized by a marked cellular hyporesponsiveness and a shift of the cytokine balance toward a Th2/Th3 response (Doetze et al., 2000; King et al., 1993; Plier et al., 1996). It is reported that the down-regulation of inflammatory reactions in the host is induced by secreted products of the parasites (Allen and MacDonald, 1998; Hewitson et al., 2009; Whelan et al., 2000) by manipulating the cytokine network (Hartmann et al., 1997), signal transduction pathways (Harnett et al., 1998) or inhibitors of essential enzymes (Hartmann and Lucius, 2003) which eventually contribute to cellular hypo-responsiveness (Harnett and Harnett, 2008) and a possible pathogenicity factor essential for the

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persistence of parasite within its host (Lawrence, 2001; Maizels et al., 2004; Schonemeyer et al., 2001). Also, strong Th2 responses induced by the parasite molecules, even regulatory T cells (Taylor et al., 2007) or alternatively activated macrophages (Allen and MacDonald, 1998; Kreider et al., 2007) lead to blocking of protective immune effector responses, allowing parasites to survive in a "modified Th2" environment muting effector Th1 and Th2 responses (Hewitson et al., 2009; Maizels and Yazdanbakhsh, 2003).

Our earlier study has shown that BmAFI, a Sephadex G-200 eluted fraction of *B. malayi* adult worm extract, stimulated the release predominantly anti-inflammatory cytokine (IL-10) and facilitated survival of *B. malayi* adult worms instilled in to the peritoneal cavity (p.c.) in *Mastomys coucha* (Dixit et al., 2004).

In the present study, we used two rodent species: *Mastomys coucha* and Swiss mouse for determining the effect of sensitization with BmAFI on the survival and development of subsequently introduced infection and the hosts' immunological correlates for the observed fate of the infection. *M. coucha* is a highly susceptible host for *B. malayi* infection if the infection (L3) is introduced through subcutaneous route (a route through which humans get the infection) and the immunological profile of this rodent model of filarial infection is well characterized (Dixit et al., 2006; Sahoo et al., 2009; Tyagi et al., 1994, 1985). However, *M. coucha* does not support the infection if it is introduced in to the peritoneal cavity (Gupta et al., 2004; Murthy et al., 1997). This model is therefore unique in that it provides a non-permissive pocket (peritoneal cavity; p.c.) in an otherwise highly permissive host. As a result, by just choosing the appropriate route of initiation of infection, both parasite survival-facilitating (i.p. route) as well as survival-inhibiting or survival-enhancing potential (s.c. route) of sensitization could be studied in this model. The second rodent, Swiss mouse is a non-permissive host and the infection fails to develop whether it is introduced s.c. or i.p. Therefore, the non-permissive Swiss mouse is used for confirmation of the effects of sensitization on parasite load. An advantage of mouse is that it is a good model for studying short term immune response.

The parasite development and burden in p.c. was determined in sensitized infected *M. coucha* and Swiss mice and the release of TGF-β, IL-4, IL-10, IL-13, IFN-γ, NO and cellular proliferative response to Con A and BmAFI and levels of IgG subclasses and IgE were determined in sensitized infected *M. coucha*. Cellular proliferative response to Con A and BmAFI, mRNA expression of GATA-3, CTLA-4 and T-bet were determined in sensitized Swiss mice. In addition, the parasitological parameter was also studied in BmAFI-sensitized *M. coucha* exposed to the infection by standard s.c. route to assess whether sensitization enhances the intensity of infection. Further, in order to find out whether BmAFI shares some antigens with those of L3, we compared SDS-PAGE profile of L3 with that of BmAFI and then immunoblotted L3 extract with anti-BmAFI sera from sensitized *M. coucha* with or without L3 infection.

2. Materials and methods

2.1. Animals

Healthy 8–10 week-old male *M. coucha* and Swiss mice (22–24 g) from the Institute's Animal Facility were used in the study in compliance with the Institutional Animal Ethics Committee guidelines. Throughout the study, they were housed in climatically controlled animal quarters (Temperature: 23 ± 2 °C; RH: 60% and photoperiod: 12 h light–dark cycles) and fed standard rodent chow supplemented with dried shrimps (*M. coucha*) and water ad libitum.

2.2. Isolation of parasites and preparation of parasite extract

Adult *B. malayi* parasites were recovered from p.c. of jirds (*M. unguiculatus*) infected with L3 4–5 months before (Murthy et al., 1997). Soluble extract of the adult parasites was prepared (Dixit et al., 2004) and protein estimated (Bradford, 1976).

2.3. Fractionation of B. malayi adult worm soluble extract by Sephadex G-200

Soluble extract of the adult parasites was fractionated by Sephadex G-200 (Pharmacia) as described earlier (Dixit et al., 2004). Three fractions obtained were designated as BmAFI, BmAFII and BmAFIII. BmAFI consisting of 42 to >180 kDa molecules which was earlier reported to show predominantly IL-10 stimulating potential (Dixit et al., 2004) was used in the present study.

2.4. Grouping and sensitization of animals with BmAFI

Grouping, sensitization and experimental schedule using *M. coucha* and Swiss mice are given in Table 1.

2.4.1. M. coucha

Male animals were divided in to groups of 5–7 animals each and used in two independent experiments. Initially a pilot experiment using 1, 2 and 3 dose schedule (subcutaneously administered) showed optimum response after 3-dose sensitization; therefore this dose schedule was used throughout the study. Two groups (Grs 1 and 2) received 3 injections of BmAFI (50 μg protein/animal)/PBS through s.c. at weekly intervals in Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA). The first injection was in FCA and the rest in FIA. The animals were sacrificed on day 7 post-last administration (p.l.s.) of BmAFI+FCA/FIA or PBS+FCA/FIA. Another two groups (Grs 3 and 4) received BmAFI/PBS with FCA/FIA in the same manner and one week after the last injection the animals received L3, by i.p. route; PBS+FCA/FIA injected animals served as sensitization control. A group (Gr 5) of normal healthy animals received L3 infection via standard s.c. route to compare parasite development at different time points. Two groups (Grs 6 and 7) of animals received BmAFI+FCA/FIA (sensitized)/PBS+FCA/FIA (non-sensitized) as above and subsequently exposed to L3 via s.c. route. Infection status (monitoring of microfilaraemia) in these animals was assessed for 115 days p.i. and then sacrificed on day 120 p.i. to determine the worm burden. Two groups (Grs 8 and 9) were sensitized either with BmAFI+FCA/FIA or PBS+FCA/FIA as described above and 7 days p.l.s dose, and given intravenous (i.v.) infusion of live microfilariae (mf). Microfilaraemia in these animals was monitored on day 2 post mf infusion (p.i.) and thereafter at weekly intervals till day 28 post infusion to determine the effect of sensitization on mf load and sacrificed on day 30 p.i.

2.4.2. Swiss mice

Groups of 5–7 mice/group were used in experiments carried out in replicates. One group was sensitized with BmAFI+FCA/FIA (25 μg protein/animal) as above. The 2nd group received PBS+FCA/FIA and served as sensitization control. These animals were exposed to L3 intraperitoneally and sacrificed on days 7, 15, 22, and 30 p.i. The 3rd and 4th groups received BmAFI/PBS with FCA/FIA, but were not exposed to infection; these were sacrificed on day 7 p.l.s. and expression of CTLA-4, STAT-3 and T-bet were determined. Fifth and sixth groups received BmAFI in FCA/FIA and PBS in FCA/FIA, respectively and sacrificed on day 7 p.l.s. and cellular proliferative responses were assessed.

One group each of *M. coucha* (Gr 10) and Swiss mice (Gr 7) were sensitized with BmAFI alone (i.e. without adjuvant) and sacrificed