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Short communication

Anti-inflammatory BmAFI of *Brugia malayi* modulates IgE, histamine and histamine receptor responses in *Mastomys coucha*

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ABSTRACT

We recently reported that BmAFI, an anti-inflammatory fraction of *Brugia malayi* adult worm supports parasite development in the hostile peritoneal cavity (p.c.) of *Mastomys coucha* through a modified Th2 type of response that includes IL-13 and IgE response and anti-inflammatory IL-10 cytokine milieu. In the present study we investigated IgE related responses such as histamine release and modulation of histamine receptors 1 and 2 (HR1 and HR2) by presensitization with BmAFI of *M. coucha* infected with *B. malayi*. Sensitization with BmAFI alone enhanced IgE, histamine and HR2, but decreased HR1. Exposure of these animals to infection produced an IgE response that was inversely related to the parasite burden, and decreased histamine conc., and HR1 and HR2 expression. However, there was an early small increase in HR1 expression for a short period after exposure to infection. As expected, BmAFI sensitization supported parasite survival and development in the hostile p.c. of the host. These findings further establish that BmAFI decreases inflammatory/Th1 response and modulates Th2 responses to favour survival and development of the parasite in the hostile p.c. of the host and that IgE and histamine play an important role in this.

1. Introduction

Lymphatic filariasis affects over 120 million people in the tropics with over one billion people at risk of developing the disease (Taylor et al., 2010). The parasites *Wuchereria bancrofti* and *Brugia malayi*, evade the host immune responses and survive for many years in the lymphatics (Maizels et al., 1993) releasing immune modulating molecules like the cystatins (Murray et al., 2005), serpins (Zang et al., 1999) transforming growth factor-β (TGF-β) (Gomez-Escobar et al., 1998) and excretory/secreted molecules (Moreno and Geary, 2008). These and other parasite molecules can stimulate IL-10 release which induces T-regulatory (Treg) cells (Satoguina et al., 2002) and alternatively activated macrophages (Loke et al., 2000) which in turn are able to suppress both Th1 and excessive Th2 responses (Gillan and Devaney, 2005) helping the larvae to survive and develop in to adult worms in the host. We have recently identified in adult worm of *B. malayi* extract 2 fractions BmAFI and BmAFII, which showed distinctly different cytokine release stimulating potential and equally different effects on the parasite in the host. BmAFI stimulated the release predominantly of anti-inflammatory cytokines (IL-10) and facilitated development and survival of parasite (Dixit et al., 2004) and also rendered a non-permissive Swiss mouse partially permissive to infection (Joseph et al., 2011). In contrast, BmAFII stimulated the release of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and inhibited the survival and development of infection (Dixit et al., 2004, 2006). In our studies to identify the components of Th1 and Th2 responses modulated by these fractions, we have recently shown that BmAFI modulates IL-13 and specific IgE response (Joseph et al., 2011). This prompted us to further examine IgE related responses such as histamine release and modulation of histamine receptors 1 and 2 (HR1 and HR2) by BmAFI.

Basophils and mast cells bind IgE through high affinity FcεRI receptors on their surface, degranulate and release histamine and other inflammatory mediators after cross-linking of these receptors (Ishizaka and Ishizaka, 1978; Turner and Kinet, 1999; Spencer et al., 2003). In filarial and other helminth infections, the release

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of histamine was proportional to the concentration of parasite antigen-specific IgE in serum (Gonzalez-Munoz et al., 1999). Histamine released from the cells bind to HR1 and HR2 in target cells to bring about its various pharmacological effect and immunological responses (Jutel et al., 2001; Wimazal et al., 2012).

The present study was therefore designed to see the effect of sensitization of *Mastomys coucha* with BmAFI and subsequent infection of the sensitized host with *B. malayi* 3rd stage larvae (L_3) on the total serum IgE, HR1 and HR2 mRNA expression and histamine levels in lymph node and the survival and development of L_3 in the non-permissive peritoneal cavity (p.c.) of the permissive host.

2. Materials and methods

2.1. Animals

All the experiments in animals were conducted in compliance with the Institutional Animal Ethics Committee guidelines of CSIR-CDRI for use and handling of animals. Healthy 8–10 week-old male *M. coucha* from the institute's animal facility were used. Throughout the study, they were housed in climate-controlled animal quarters (Temp.: 23 ± 2 °C; RH: 60% and photoperiod: 12 h light–dark cycles) and fed standard rodent chow supplemented with dried shrimps and water ad libitum.

2.2. Preparation and fractionation of parasite extract

Soluble extract of adult parasites isolated from p.c. of jirds (*Meriones unguiculatus*) experimentally infected with *B. malayi* L_3 (Murthy et al., 1997) was prepared and fractionated by Sephadex G-200 (Pharmacia) as described earlier (Dixit et al., 2004). Three fractions BmAFI, BmAFII and BmAFIII were obtained, of which BmAFI (consisting of 42 to >180 kDa molecules) was selected for the present study.

2.3. Grouping and sensitization of animals with BmAFI and their exposure to L_3

A total of 60 male *M. coucha* were used in the study. Grouping of animals and experimental design of the study are given in Table 1. Two groups of 30 animals each received 3 injections of PBS (Gr. 1) or 50 μg BmAFI/animal/occasion (Gr. 2) by subcutaneous (s.c.) route at weekly intervals in Freund's complete adjuvant (FCA; 1st injection) or Freund's incomplete adjuvant (FIA; 2nd and 3rd injections). Six animals each of Gr. 1 and Gr. 2 animals were killed on day 7/8 post last sensitizing dose (p.l.s.) of PBS + FCA/FIA or BmAFI + FCA/FIA for determining immunological parameters. The remaining 24 animals each of Gr. 1 and Gr. 2 were designated as Gr. 3 and Gr. 4, respectively, and received live L_3 (100/animal) by i.p. route 1 week after the last sensitization injection. The L_3 used were freshly isolated from *Aedes aegypti* mosquitoes as described elsewhere (Murthy et al., 1983). Six animals each of Grs. 3 and 4 were killed on days 15, 30, 45 and 60 post L_3 inoculation (p.i.) for determination of parasite burden and immunological parameters.

2.4. Assessment of parasite burden

Parasite burden in L_3 inoculated animals was determined as described earlier (Joseph et al., 2011).

2.5. IgE determination

Total serum IgE was determined using the mouse IgE ELISA Quantitation kit (Bethyl laboratories, Montgomery, TX, USA) following the manufacturer's instructions and using the standard obtained from the same source.

2.6. Determination of histamine conc.

Histamine concentration in the celiac, mediastinal, superior mesenteric, and periportal lymph nodes was estimated following the method of Shore et al. (1959). Briefly, the lymph nodes were homogenized in 10% (w/v) of 0.4 N perchloric acid, alkalinized and histamine was extracted into *n*-butanol. The extract was washed with 0.1 N NaOH, returned to 0.1 N HCl solution and condensed with o-phthalaldehyde (OPT) to yield a product with strong and stable fluorescence at 450 nm on excitation at 360 nm. The fluorescence was measured using a Varian Cary Eclipse fluorescent spectrophotometer (Varian, Australia). Histamine dichloride was used as the standard and histamine concentration was presented as μg/g tissue.

2.7. RT-PCR for histamine receptors

For determination of HR1 and HR2 expression, lymph nodes were collected as above, a cell suspension prepared and RNA extracted (Chomczynski, 1993). A total of 5 μg RNA was used for cDNA preparation using First Strand cDNA Synthesis Kit (Fermentas life Sciences, India). Reverse transcriptase PCR was performed to assess the mRNA expression of HR1 and HR2 in cells (Parungo et al., 2007). The primer sequences used to amplify their respective cDNA are given in Table 2. β-Actin was used as house keeping control.

One μl of cDNA was amplified by PCR (Joseph et al., 2011). The products were run on 1.5% agarose gel and visualized using gel documentation system and analyzed by Quantity one® software (BioRad, USA).

2.8. Statistical analysis

Data were analyzed by ANOVA followed by Newman–Keuls multiple comparison test. Student's '*t*' test was used to compare between the two groups. All statistical analyses were performed using GraphPad Prism 3.0 software. Data were presented as mean ± S.D. of 6 animals from two experiments and differences were considered significant, if $P < 0.05$.

3. Results

3.1. Effect of sensitization on parasite survival and development

As expected BmAFI-sensitized animals inoculated with L_3 showed 48% ($P < 0.01$) and 29% ($P < 0.001$) increase in recovery of developing worms (L_4 and L_5) in the p.c. on days 15 and 30 p.i. respectively, as compared to non-sensitized infected animals; 15% young adults were recovered on day 45 p.i. and 5% adults by day 60 p.i. (Fig. 1A). Non-sensitized infected animals showed 11% recovery of the L_4 stage on day 15 p.i. which decreased to 1% by day 30 p.i. L_4 could not survive to develop into adults after day 30 p.i. In summary, the findings demonstrate that BmAFI sensitization supports survival and development of L_3 to adult stage in the p.c. of *M. coucha*.

3.2. Sensitization upregulates total IgE and histamine conc.

Sensitization with BmAFI increased ($P < 0.001$) the total serum IgE levels in *M. coucha*. However, subsequent L_3 inoculation to these animals decreased the level by >15 times on day 30 p.i. ($P < 0.001$), but thereafter the level increased 6.8 times by day 60 p.i. compared to non-sensitized infected animals (Fig. 1B). In other words the total IgE level was inversely proportional to the parasite burden. In summary, sensitization with BmAFI caused increase in total IgE level. Sensitized infected animals showed inverse correlation between the level of IgE and parasite burden.