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RESEARCH ARTICLE

Modulation of host's immune responses and parasite load in *Mastomys coucha* immunized with recombinant mitochondrial HSP60 of human lymphatic filarial parasite *Brugia malayi*

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ABSTRACT

Recently we have shown that mitochondrial HSP60 of *Brugia malayi* (mtHSP60bm) shows high degree of homology with *Escherichia coli* GroEL/ES and that the ATP binding pockets of HSP60 in humans, *E. coli* and *B. malayi* were structurally conserved. In the present study we investigated the immune responses to rmtHSP60bm in *Mastomys coucha* and the fate of infection in the immunized animals. The animals received 4 immunization doses of rmtHSP60bm and were subsequently exposed to *B. malayi* infection. Microfilaremia, adult worm status, nitric oxide (NO), Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-10 and TGF-β) cytokine release, cell proliferative response, levels of specific IgG and its subclasses, and the mast cell status in lymph nodes were assessed on day 135/136 post infection. Immunization with rmtHSP60bm and subsequent exposure to infection resulted in significantly high microfilaraemia but without any change in adult worm burden. Immunization with rmtHSP60bm increased IgG, IgG1 and IgG2b levels, and IL-2 and IFN-γ release and suppressed NO release and CMI responses, but without any change in IL-4 and IL-10 release. Exposure of immunized animals to infection enhanced the CMI responses and, NO and IL-10 release but decreased IgG1 levels and IL-2 and IL-4 release; however, IgG, IgG2a, IgG2b, and IFN-γ responses remained unaltered. Mast cells in the draining lymph nodes of immunized-infected animals showed significant degranulation but without any increase in cell count. However, no pathology was found in the lymph nodes. These findings indicate that mtHSP60bm may modulate and balance the host's immune responses to favor parasite survival without inducing any pathology.

KEYWORDS: Mitochondrial HSP60; *Brugia malayi*; IgG and its subclasses; cellular proliferation; cytokines; mast cell in lymph node.

INTRODUCTION

Human lymphatic filariasis (LF) is a mosquito-transmitted disease of the tropics caused by the nematode parasites *Wuchereria bancrofti, Brugia malayi* and *B. timori*. In areas endemic for this disease, about 1.34 billion people are at risk of the infection,120 million show infection and 40 million suffer from chronic disease manifestations like hydrocele, lymphoedema and elephantiasis (WHO, 2005; Taylor et al., 2010).

The adult worms live in the lymphatics, where they survive for prolonged periods. The female worms produce and release in to host's blood circulation millions of first-stage larvae (microfilariae; mf) which enter the mosquito vector during the latter's blood meal and develop into the third stage infective larva (L_3). The infection is re-initiated/transmitted by the mosquito during its next blood meal when L_3 enter the host and develop in to adult worms.

The adult worms evolve several anti-inflammatory strategies that safeguard their survival in the host. In endemic areas, the infected population can be categorized into putatively immune asymptomatics, asymptomatic microfilaraemics and those showing chronic pathology. This categorization is relatable to the mounting evidence that diverse arrays of both suppressive and stimulatory antigens are present in the parasite. Recently we reported that a proinflammatory fraction F6 (54.35-67.80 kDa) of *B. malayi* protects the host against homologous infection in *Mastomys coucha* (Sahoo et al., 2009) and even protects against *Leishmania donovani* infection in hamsters, via Th1/Th2 type and NO responses (Murthy et al., 2008). MALDI TOFF analysis of F6 showed 5 proteins of which 3 proteins: heat shock protein 60 (HSP60), elongation factor-2 (EF-2) and cytoplasmic intermediate filament (CIF) were found to be immuostimulatory (Sahoo et al., 2009). The present communication reports our studies on HSP60 of *B. malayi.*

Heat shock proteins (HSPs) are highly conserved and ubiquitously distributed proteins and play a central role in host-parasite interaction. They were considered to be major targets of immune responses in bacterial, fungal, protozoal and filarial infections (Young, 2009; Young et al., 1989; Kaufmann, 1990; Zugel and Kaufmann, 1999a, 1999b; Ravi et al., 2004). HSP60 elicits proinflammatory response from macrophages, vascular endothelial cells and smooth muscle cells *in vitro* (Kol et al., 1999; Chen et al., 1999; Tsan and Gao, 2004, 2009) and the proinflammatory signaling was shown to be via TLR4 (Ohashi et al., 2000).

For our studies on the role of *B. malayi* HSP60 in host's immune response against filarial infection, we have recently cloned the mitochondrial HSP60 of *B. malayi*, over expressed it and purified the recombinant (r) HSP60 (rmtHSP60bm). The gene expression was found in all life-stages of the parasite except in L_3 in which it could however, be induced several folds by heat-shock. rmtHSP60bm showed high degree of homology with *Escherichia coli* GroEL/ES and the ATP binding pockets of the protein were found to be structurally conserved in humans, *E. coli* and *B. malayi* (Misra et al., 2012). In the present study we characterized the immune responses in the filaria-susceptible rodent *Mastomys coucha* to rmtHSP60bm and the fate of infection given to the immunized animals. The immune responses studied were: levels of specific IgG and its subclasses, cell proliferative response, nitric oxide (NO) and Th1 (IFN-γ, IL-2) and Th2 (IL-4, IL-10 and TGF-β) cytokine release and mast cell status in lymph nodes.

MATERIALS AND METHODS

Animals

M. coucha and *jirds* (*Meriones unguiculatus*) were obtained from National Laboratory Animal Centre of the Institute and were handled and used in compliance with the Institutional Animal Ethics Committee guidelines. Throughout the study, they were housed

in polypropylene rodent cages [30cm(L) x 22cm(W) x 14cm(H); 3-4 animals/cage] in climate controlled animal quarters (Temp.: 23 ± 2 °C; RH: 60%; photoperiod: 12 h light-dark cycles). The animals were fed standard rodent "maintenance diet" prepared in-house (QC analysis: carbohydrates 58.3%, protein 21.1%, fat 7.2%, crude fibre 6.6%, moisture 6.8%) supplemented (for *M. coucha*) with dried shrimps; all animals had free access to drinking water.

Preparation of rmtHSP60bm

The primer (Forward: 5'-GAATTCAATGTTCCGTATCGGTGGAC -3' and Reverse: 5'-AAGCTTGTACATTCCTCCCATACCTC -3') was designed on the basis of the gene sequence retrieved from *B. malayi* draft genome database of TIGR (J. Craig Venter Institute; Gene bank accession No. GU062418.1, gi|262092495). Preparation of the primer, cloning of full length mtHSP60 gene of *B. malayi*, its over expression and purification and localization of the recombinant protein (rmtHSP60bm) in Western blot were carried out as described by Misra et al. (2012). A single ~65 kDa elution peak represented the purified protein. The purified rmtHSP60bm showed negligible endotoxin (<1 EU/mg) contamination (determined using E-toxate test kit, Sigma-Aldrich, USA).

Immunization of animals with rmtHSP60bm

Twenty four male *M. coucha* (9-11 weeks old) in four groups (6 animals/group in two experiments) were used in the study. Groups 1 and 2 were immunized with rmtHSP60bm whereas Groups 3 and 4 were given only PBS (non-immunized) in place of the r-protein. For immunization, rmtHSP60bm (50µg/animal) or PBS was mixed with Freund's complete adjuvant (FCA) and administered through subcutaneous (s.c) route on day 1 (first immunizing dose; f.i.d). This is followed by 3 subsequent s.c. injections each containing ½ the quantity of rmtHSP60bm (i.e, 25µg/animal) mixed with FIA on days 14, 28 and 35 f.i.d. Groups 2 and 4 received live L_3 on day 42 f.i.d. Groups 1 and 3 were killed on day 42 post f.i.d. whereas Groups 2 and 4 were killed on day 135/136 p.i., to assess parasite burden and immune responses.

Maintenance of infection in M. coucha and jird

Sub-periodic strain of *B. malayi* was maintained in *M. coucha* and *jirds* through black eyed susceptible stain of *Aedes aegypti* mosquitoes (Murthy et al., 1983, 1997). Each animal received 100 (*M. coucha*) or 200 (*jirds*) L_3 through s.c. and intraperitoneal (i.p.) routes, respectively. Microfilaraemia in *M. coucha* was assessed on day 90 post L_3 inoculation (p.i.) and thereafter at weekly intervals to select the microfilaraemic donor animals for maintenance of infection (Murthy et al., 1983). Microfilarial count was done in thick smears of peripheral blood (10 µl tail blood taken between 12:00 noon and 1:00 PM) stained with leishman stain (Gupta et al., 1990).

Isolation of adult filarial parasites from infected animals

Adult worms of *B. malayi* were isolated from peritoneal cavity of *jirds* as described by Murthy et al. (1997). The worms were washed in PBS and stored at -20 °C until used.

Exposure of animals to L_3

L_3 were freshly isolated from *A. aegypti* mosquitoes 90 days after they were fed on a blood meal from microfilaraemic *M. coucha* as described by Murthy et al. (1983). L_3 were washed thoroughly with sterile 1640 medium and injected subcutaneously into the animals (100 L_3/animal).

Assessment of parasite burden and fecundity

Microfilarial burden was assessed on day 90 post L_3 inoculation (p.i.) and thereafter at 7 days intervals till day 132 p.i.

Adult worm burden was determined on day 135/136 p.i. The animals were killed by over dose of sodium pentothal and the worms were retrieved from heart, lungs, testes, lym-

phatics and lymph nodes and examined under microscope. The condition of the worms such as their motility, cell adherence on their surface, death and calcification of worms or their fragments were recorded. The number of worms recovered from immunized infected animals was compared with that in non-immunized infected animals and the percent recovery of worms was calculated (Gaur et al., 2007; Lakshmi et al., 2010). For assessment of sterilization of female worms, the worms were teased individually in a drop of saline and examined under microscope for any abnormalities in the uterine contents or distortion of embryonic stages and the percentage of worms showing abnormal embryos was calculated (Gaur et al., 2007; Lakshmi et al., 2010).

Determination of NO release

NO release was determined in the peritoneal macrophages of the animals broadly following the method of Thomas et al., (1997) with some modification to suit our condition (Dixit et al., 2006). Briefly, the animals were killed by an overdose of anesthesia. Under aseptic conditions macrophages were isolated from peritoneal cavity, washed in DMEM containing 5% EDTA, centrifuged (800-1000 rpm for 5 min) at 4 °C, checked for viability and counted. The cells were finally suspended in complete DMEM fortified with 10% fetal bovine serum (Sigma-Aldrich, India) and the final concentration of cells adjusted to 2 x 10^6/ml. The cells were incubated with rmtHSP60bm (1µg/ml), or LPS (1µg/ml) in sterile 24-well plates (Nunc-rosklide, Denmark) at 37 °C in 5% CO2 atmosphere for 48 h. NO release was quantified in 48 h culture supernatants using Griess reagent and employing NaNO2 solution (100mM NaNO2 serially diluted to 0.5mM) run simultaneously as standard. Absorbance was read at 550 nm and conc. of NO was calculated (Dixit et al., 2006).

Cellular proliferative assay

Lymphocyte transformation test (LTT) was used for assessing cellular proliferative response of splenocytes as described by Joseph et al. (2011). Briefly, splenocytes (0.4 x 10^6 cells/200µl) of non-immunized and rmtHSP60bm-immunized animals were plated in sterile 96-well plates (Nunc-rosklide, Denmark) and incubated in the presence of rmtHSP60bm (1 µg/ml) or Con A (10 µg/ml) for 72 h. The cells were pulse labeled with [3H]-thymidine (1µCi/well) and incubated for 18 h. Thymidine incorporation was counted in scintillation β counter (1217-Reckbeta, Finland) and the results were expressed as cpm.

Cytokine assay

Splenocytes of *M. coucha* immunized with rmtHSP60bm were plated (2×10^6/ml) in sterile 24-well plates (Nunc-rosklide, Denmark) and incubated in the presence of LPS (1 µg/ml) or rmtHSP60bm (1 µg protein/ml) for 48 h. The cytokines released were determined in 48 h culture supernatants using anti-mouse monoclonal antibodies of TGF-β, IL-4, IL-10, IL-2 (Pharmingen) and IFN-γ (Pierce Endogen, Rockford, IL, USA) in a paired antibody sandwich ELISA method following the manufacturer's instructions and absorbance was measured in a spectrophotometer (PowerWaveX, USA). Cytokine concentrations were calculated using standards of the respective paired antibodies obtained from the above sources (Joseph et al., 2011).

Determination of IgG and its subclasses

Antigen specific IgG and its IgG1, IgG2a, IgG2b and IgG3 subclasses were determined in optimally diluted sera (IgG: 1:100; subclasses: 1:50) of animals using optimum concentration of rmtHSP60 (0.1µg protein/ml), HRP conjugated goat anti-mouse IgG, IgG1, IgG2a, IgG2b and IgG3 (1:1000; Serotec, USA) and the chromogenic substrate O-phenylenediamine (OPD) as described by Joseph et al. (2011). Absorbance was read at 492nm in ELISA reader (PowerWaveX, Bio-Tek, USA).

Histopathology of lymph nodes

At the termination of experiments, animals were killed under deep ether anesthesia and the draining superficial inguinal and axil

lary lymph nodes were dissected out and fixed in 4% phosphate-buffered formaldehyde (PBF; pH 7.3) at room temperature. The nodes were processed for paraffin embedding and serially sectioned at 3 µm thickness. The sections were stained with Celestin blue–Iron alum-eosin and Toluidine blue for general histology and for mast cells, respectively. The no. of mast cells/mm2 of section and granularity of mast cells in lymph nodes were determined using digital microscopic images. The granularity of mast cells was graded, by two independent workers, on a visual scale of 0–2 where, 0 = nearly complete degranulation in the majority of cells, 1 = moderate (50%) degranulation in the majority of cells, 2 = rich granularity in almost all cells.

Statistical analysis

Results were presented as mean ± S.D. of 6 animals/group in two experiments and the data were analyzed in GraphPad Prism 3.03 using Student's 't' test and Newman Keuls multiple comparison tests. Differences with $P<0.05$ were considered significant.

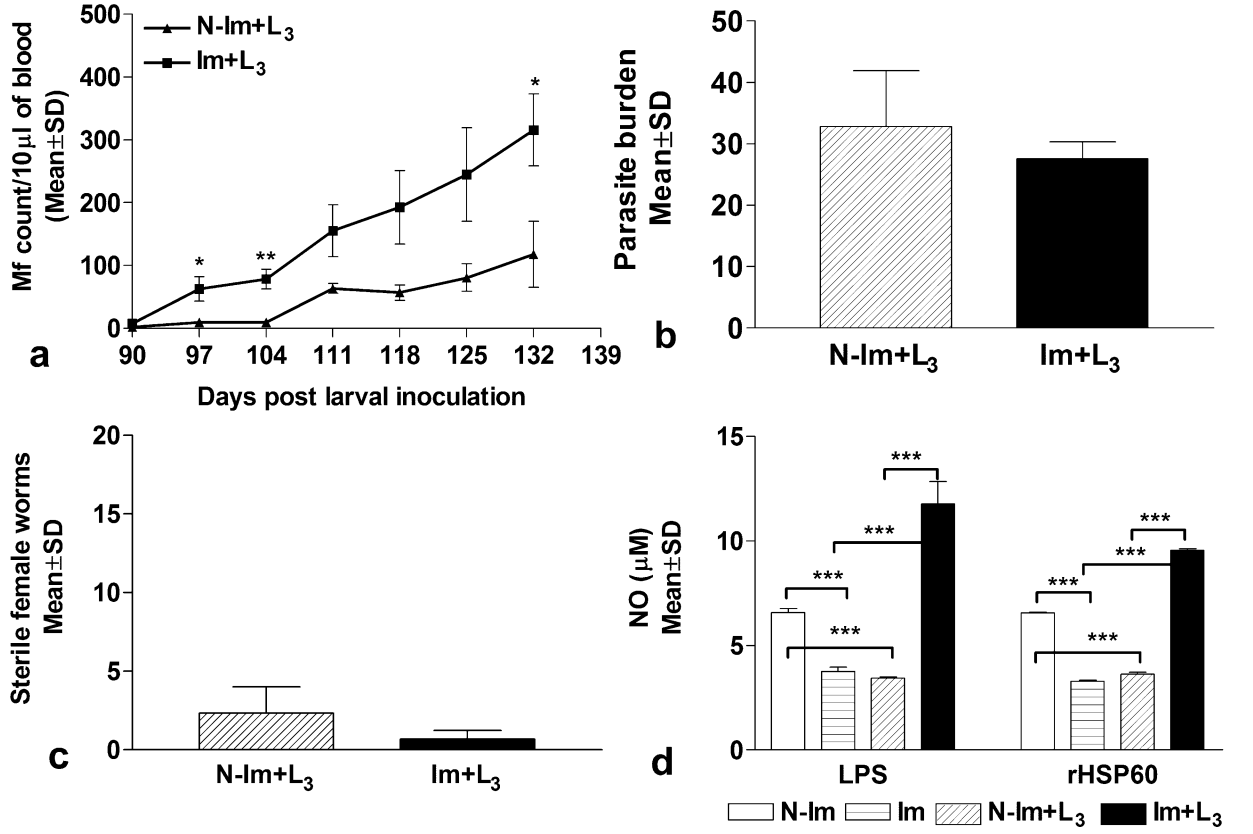

Figure 1. Parasite burden and nitric oxide (NO) response in *Mastomys coucha* immunized with rmtHSP60bm and subsequently exposed to L_3 of *Brugia malayi*. Group abbreviations: N-Im (Non-Immunized/Control), Im (Immunized), N-Im+L_3 (Non-Immunized+L_3), Im+L_3 (Immunized+L_3). The animals were killed on day 42 f.i.d. or day 135/136 p.i. (a) Weekly microfilarial count in infected animals from day 90 to 132 post larval inoculation (p.i.); (b) worm recovery; (c) No. of sterile female worms; (d) NO release assay was performed in peritoneal macrophages of all the animals in response to rmtHSP60bm and LPS stimulation *in vitro*. NO in the cell-free supernatants was quantified using Griess reagent. Values are from 6 animals in two experiments. Statistics: Student's *t* test and Newman Keuls multiple comparison tests. [Significance levels for data in (a) microfilaraemia: *P<0.05-0.01 (N-Im+L_3 vs Im+L_3); (b) worm burden: Difference between the two groups are not statistically significant; (c) No. of sterile female worms: Difference between the two groups are not statistically significant; (d) NO release, LPS/rmtHSP60bm stimulated: ***P<0.001 (N-Im vs Im, N-Im vs N-Im+L_3, Im vs Im+L_3, Im+L_3 vs N-Im+L_3).

RESULTS

Responses of host to rmtHSP60bm

Filarial parasite burden

Animals immunized with mtHSP60bm and subsequently infected with L_3 showed a progressive rise in microfilaraemia from day 90 onwards ($P<0.05$-0.01; Fig. 1a) as compared to non-immunized infected animals. However, the worm burden in immunized-infected animals was comparable to that in non-immunized infected animals (Fig. 1b). Although the trend in sterilization of female worms was low in immunized infected animals compared to non-immunized infected ones, the difference was not statistically significant (Fig. 1c).

NO release

Immunization with rmtHSP60bm decreased the NO release from peritoneal macrophages of the animals in response to rmtHSP60bm ($P<0.001$) or LPS ($P<0.05$) challenge but introduction of L_3 infection in the immunized animals enhanced the release of NO ($P<0.001$; Fig. 1d). In summary, the results show that exposure of immunized animals to infection enhances the release of NO.

Cellular proliferative response

Proliferative response of splenocytes of immunized animals was enhanced by Con A whereas rmtHSP60bm downregulated ($P<0.05$) it (Fig. 2a). L_3 infection into these animals reversed the responses ($P<0.001$; Fig. 2a). Thus, immunization suppressed the specific cellular proliferative response but introduction of L_3 infection into these animals increased the response.

Cytokine response

Exposure to rmtHSP60bm significantly stimulated the release of Th1 cytokines IL-2 ($P<0.001$; Fig. 2b) and IFN-γ ($P<0.01$; Fig. 2c) from cells of immunized animals. L_3 infection in these animals decreased the IL-2 release ($P<0.001$; Fig. 2b) but not IFN-γ release (Fig. 2c). In contrast, rmtHSP60bm challenge did not stimulate release of Th2 cytokines IL-4 (Fig. 2d) and IL-10 (Fig. 2e) from cells of immunized animal, but L_3 infection in these animals inhibited IL-4 release from the cells ($P<0.05$; Fig. 2d) and enhanced IL-10 release ($P<0.05$; Fig. 2e). Immunization and infection showed no significant effect on TGF-β release in response to either rmtHSP60bm or LPS challenge (Fig. 2f).

IgG and its subclasses

Specific IgG, IgG1 and IgG2b increased ($P<0.01$-0.001; Fig. 3a,b,d) in sera of immunized animals, whereas IgG2a (Fig. 3c) and IgG3 (Fig. 3e) levels remained unaltered compared to non-immunized animals. L_3 infection in immunized animals decreased the IgG1 level ($P<0.01$; Fig. 3b) but not IgG (Fig. 3a), IgG2a (Fig. 3c), IgG2b (Fig. 3d) or IgG3 (Fig. 3e) levels. However, IgG2b level was higher in immunized infected animals ($P<0.05$; Fig. 3d) than in non-immunized infected ones; IgG2a level in immunized infected animals was lower than in non-immunized infected animals ($P<0.001$; Fig. 3c). In summary, immunization resulted in upregulation of IgG, IgG1 and IgG2b but L_3 infection downregulated IgG1.

Histopathological response of lymph nodes

No pathological changes were seen in lymph nodes of immunized-infected animals (data not shown).

Mast cells in the lymph nodes did not show any significant numerical increase in any of the groups. However, more than 75% of mast cells were degranulated in immunized-infected animals (Fig. 3f; $P<0.001$) as compared to non-immunized animals with or without infection.

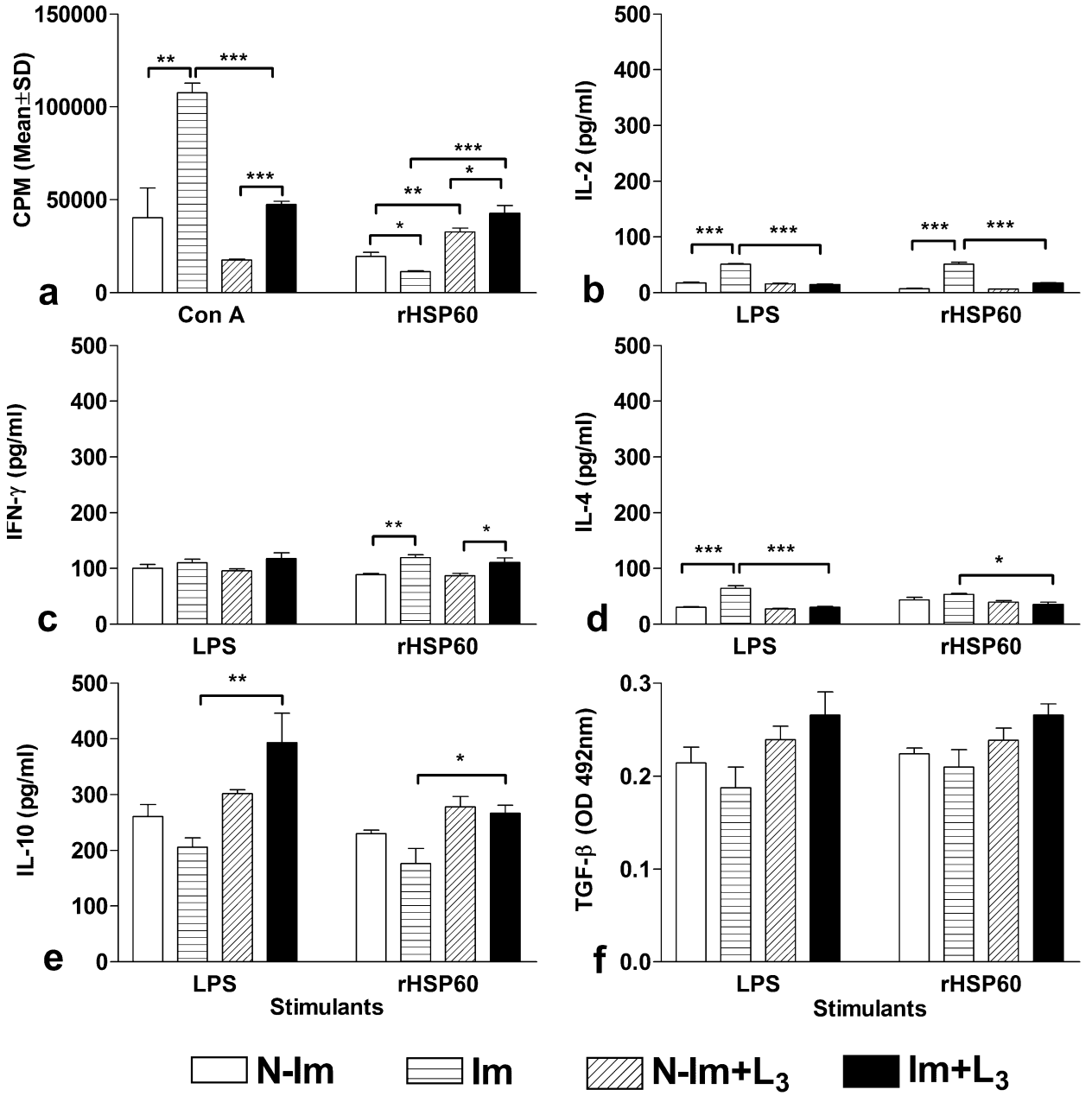

Figure 2. Proliferative response of and cytokine release from spleen cells in *M. coucha* immunized with rmtHSP60bm and subsequently exposed to L_3. The animals were killed on day 42 f.i.d. or day 135/136 p.i. Group abbreviations are same as in Fig. 1. (a) Proliferative response of cells challenged with rmtHSP60bm or Con A *in vitro*. Release of cytokines (b) IL-2, (c) IFN-γ, (d) IL-4, (e) IL-10, and (f) TGF-β from splenocytes of *M. coucha* in response to challenge with the rmtHSP60bm or LPS *in vitro*. Cytokines in the cell free supernatants were quantified by sandwich ELISA. Values are expressed in mean±SD of data from 6 animals in two experiments. Statistics: Newman Keuls multiple comparison tests. [Significance levels for data in (a) Con A stimulated: **P<0.05 (N-Im vs Im, N-Im+L_3 vs Im+L_3), ***P<0.001 (Im+L_3 vs N-Im+L_3); rmHSP60bm stimulated: *P<0.05 (N-Im vs Im, Im+L_3 vs N-Im+L_3) **P<0.01 (N-Im vs N-Im+L_3), ***P<0.001 (Im vs N-Im+L_3), ***P<0.001 (Im vs Im+L_3, N-Im+L_3 vs Im+L_3); (b) rmHSP60bm/LPS stimulated: ***P<0.001 (N vs Im, Im vs Im+L_3); (c) rmHSP60bm stimulated: *P<0.05 (N-Im+L_3 vs Im+L_3), **P<0.01 (N vs Im); (d) LPS stimulated: ***P<0.001 (N-Im vs Im, N-Im+L_3 vs Im+L_3), rmHSP60bm stimulated: *P<0.05 (Im vs Im+L_3); (e) rmHSP60bm/LPS stimulated: *P<0.05 (Im vs Im+L_3); (f) rmHSP60bm/LPS stimulated: No statistical difference was observed in TGF-β release from cells of animals between the groups].

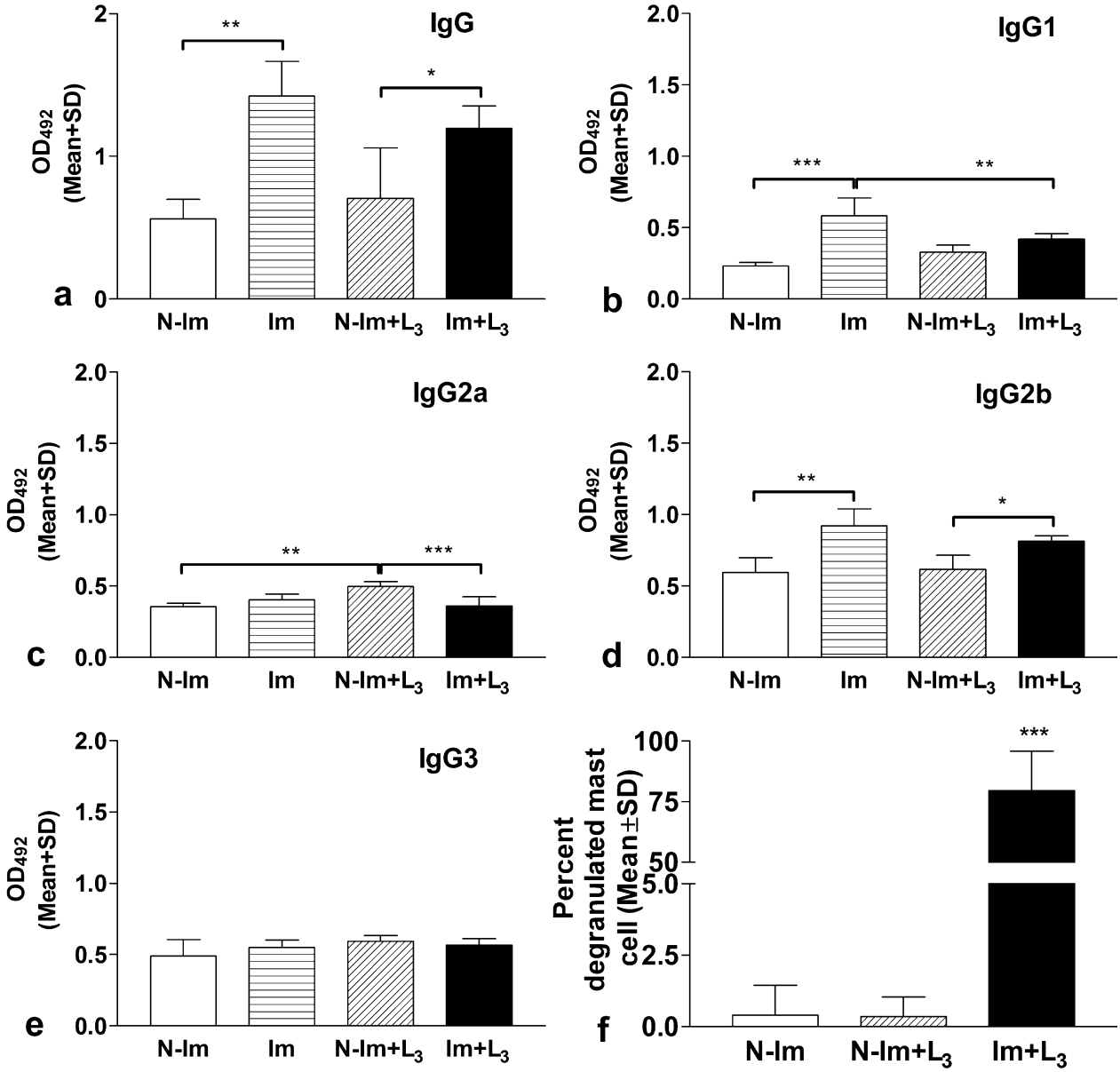

Figure 3. Specific IgG and its IgG subclasses in rmtHSP60bm immunized *M. coucha* and subsequently exposed to L_3. The animals were killed on day 42 f.i.d. or day 135 or 136 p.i. Group abbreviations are same as in Figure 1. Specific antibodies (a) IgG, (b) IgG1, (c) IgG2a, (d) IgG2b and (e) IgG3 were determined by ELISA in serum of the animals using commercially available probes (goat anti-mouse-IgG and its subtypes conjugated with horseradish peroxidase). (f) Percent degranulated mast cells in lymph nodes of rmtHSP60bm immunized animals receiving L_3 (Im+L_3), PBS in place of the rmtHSP60bm (N-Im) and non-immunized animals receiving L_3 (N-Im+L_3). The lymph nodes were collected on the same days as mentioned above and processed for mast cell staining. Values are from 6 animals in two experiments. Statistics: Newman Keuls multiple comparison tests. [Significance levels for data in (a) IgG: *P<0.05 (N-Im+L_3 vs Im+L_3), **P<0.01 (N-Im vs Im); (b) IgG1: **P<0.01 (N-Im+L_3 vs Im+L_3), ***P<0.001 (N-Im vs Im); (c) IgG2a: **P<0.01 (N-Im vs N-Im+L_3), ***P<0.001 (N-Im+L_3 vs Im+L_3); (d) IgG2b: **P<0.01 (N-Im vs Im), *P<0.05 (N-Im+L_3 vs Im+L_3); (e) IgG3: No statistical difference was observed between the groups; (f) Percent degranulated mast cells: ***P<0.001 (Im+L_3 vs N-Im+L_3/N-Im)].

DISCUSSION

rmtHSP60bm has only recently been cloned and expressed by us and virtually nothing is known of its role in host's immune responses to filarial infection. In general, HSP60s are known to be immunogenic with the potential to evoke immune responses through T cell and have therefore been projected as vaccine adjuvants targeted to infections and cancers (Segal et al., 2006). In the present study rmtHSP60bm immunized animals that received L_3 infection showed a progressive rise in microfilaraemia till termination of the experiment. Although the reasons for this are not clear, immunization with rmtHSP60bm appears to improve the fecundity of the worms (8% worms in rmtHSP60bm immunized infected animals and 32% worms in non-immunized infected animals were found sterile, although this is not statistically significant) without changing the worm recovery.

rmtHSP60bm immunization resulted in changes in both Th1 and Th2 immune responses. L_3 infection in immunized animals upregulated the cell proliferative response to rmtHSP60bm challenge. In addition, there was an upregulation of Th2 response as evidenced by increase in IL-10 in these animals. NO, which is known to be involved in eliminating filarial parasites (Rajan et al., 1996; Taylor et al., 1996), was found to be decreased after immunization with rmtHSP60bm. This appears to be at least partly, due to the increasing trend in the iNOS-suppressing (O'Connor et al., 2000) counter-regulatory cytokine IL-4 (although this trend was not statistically significant) in these animals. This interpretation gains support from the observation that L_3 infection in these animals augmented the NO response and down regulated the IL-4 release. In our earlier study (Misra et al., 2012) we reported that HSP60 gene expression was virtually undetectable in L_3 stage but heat-shock could induce it several folds. HSP60 present in L_3 gets induced after entering into the host (after entry of L_3 stage from mosquito to warm blooded mammalian host) and stimulates the cells to release NO. This finding supports our present finding that temperature rise may have induced the expression of *B. malayi* HSP60 gene in L_3 which in turn augmented NO response. Our findings show that immunization-infection- induced low IL-4 response favors microfilaraemia and fecundity of adult worms (as evidenced by lower % sterilized worms). This is supported by Volksman et al. (2001) who found that Litomosoides sigmodontis worms in IL-4 gene knock-out mice displayed a high degree of fertility and microfilaraemia. In the present study both IL-2 and IFN-γ release was increased in immunized animals of which the increase in IFN-γ was probably due to decreasing trend in the counter-regulatory IL-10. Considerable degranulation, without any increase in mast cell number, was seen in the majority of lymph node mast cells of rmtHSP60bm-immunized animals receiving L_3 but not in non-immunized or non-immunized+L_3 infected animals. We have earlier reported similar absence of any significant numerical increase in lymph node mast cells after sensitization with anti-inflammatory BmAF1 fraction of adult worms (Dixit et al., 2004). This is not surprising because it is slowly becoming evident that mast cells can have both positive and negative immunomodulatory functions as seen in different mouse models of cutaneous hypersensitivity (Galli et al., 2005; Tsai et al., 2011) and in a mouse model of DTH to ovalbumin where impairment of antigen-specific T-cell responses were mediated by mast cells (Depinay et al., 2006). Recently, Kalesnikoff and Galli (2011) have shown that such positive and negative im

munomodulation may be mediated at least in part by histamine. Joseph et al. (2013) have also shown that histamine plays an important role in facilitating infection in animals sensitized with the anti-inflammatory BmAF1 fraction. The mechanism by which mast cell products facilitated parasite survival in the present study is, however, not clear. Further, immunized animals receiving L_3 did not show any pathology in the draining lymph nodes. This suggests that mtHSP60bm is different from and functionally unrelated to HSP60 of filarial symbiont Wolbachia since the latter is reported (Suba et al., 2007) to be associated with chronic filarial disease manifestations.

CONCLUSION

In conclusion the findings show that immunization with rmtHSP60bm modulates both Th1 and Th2 responses and facilitates microfilaraemia without altering parasite burden. Mast cells in the lymph nodes of immunized-infected animals showed significant degranulation but without numerical increase and the draining lymph nodes did not show any pathology. These findings indicate that mtHSP60bm may modulate and balance the host-immune responses to favor parasite survival without inducing any pathology.

AUTHORS' CONTRIBUTIONS

PKM conceived and designed the experiments. AKV SKV RV and VK performed the experiments. PKM SKV and WAS analyzed the data. PKM contributed reagents and materials. PKM and AKV wrote the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ROLE OF THE FUNDING SOURCE

The study was an institutional project and no funding was received from any external funding agency.

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