Identification of 38kDa Brugia malayi microfilarial protease as a vaccine candidate for lymphatic filariasis

K N Krithika, Pankaj Dabir, Sandeep Kulkarni, V Anandaraman & M V R Reddy*

Department of Biochemistry & Jannalal Bajaj Tropical Disease Research Centre, Mahatma Gandhi Institute of Medical Sciences, Sewagram 442 102, India

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A FPLC purified 38kDa protease (Bm mf S-7) isolated from B. malayi microfilarial soluble antigen was identified. It showed pronounced reactivity with sera collected from 'putatively immune' asymptomatic and amicrofilaraemic individuals residing in an endemic area for bancroftian filariasis. Further the immune protective activity of Bm mf S-7 antigen was evaluated in susceptible hosts, jirds (Meriones unguiculatus) against B. malayi filarial infection. The antigen showed 89% cytotoxicity against mf and 87-89% against infective (L3) larva in in vitro antibody dependent cellular cytotoxicity Assay (ADCC) and in situ micropore chamber methods. Bm mf S-7 immunized jirds after challenge infection showed 81.5% reduction in the adult worm burden. The present study has shown that, the 38kDa microfilarial proteases (Bm mf S-7) could stimulate a strong protective immune response against microfilariae and infective larva in jird model to block the transmission of filariasis. Analysis of IgG subclasses against Bm mf S-7 revealed a significant increase in IgG2 and IgG3 antibodies in endemic normals. Lymphocyte proliferation to Bm mf S-7 was significantly high in endemic normal group as compared to that in clinical and microfilarial carriers. Significantly enhanced levels of IFN-γ in the culture supernatant of PBMC of endemic normals following stimulation with Bm mf S-7 suggest that the cellular response in this group is skewed towards Th 1 type.

**Keywords:** Filariasis, Microfilarial soluble antigen, Protease, Vaccine

Nematode infections still affect the majority of the human population. Lymphatic filariasis caused by Wuchereria bancrofti and Brugia malayi is a mosquito transmitted disease. The human filariasis affecting over 120 million people worldwide remained as a major cause of clinical morbidity in developing countries. Development of a suitable anti filarial vaccine will be an additional measure to the existing therapeutic and vector control methods in the elimination of this disease. In areas endemic for filariasis, almost all individuals are exposed to filarial parasites. However some of the residents grouped as ‘endemic normals’ are asymptomatic and have no current or past evidence of parasite burden. Such a subpopulation is believed to be ‘putatively immune’ to infection and this group is quite useful to identify host protective antigens. The molecules preferentially recognized by sera samples of this endemic normal population are expected to stimulate protective immune response against filarial parasites in the host.

There have been reports on the induction of protective immunity against filarial infection by immunizing with irradiated infective third stage larvae (L3) and larval/microfilarial extracts. Infective larval antigens being the parasite components exposed to the host at invasion stage are believed to be appropriate targets for filarial vaccine development. An alternative strategy would be to develop a ‘transmission blocking’ vaccine by targeting microfilarial (mf) stage for immunological clearance. There are indications that antibodies against mf components participate in the clearance of mf in experimental animals and presumably in human.

Development of such anti-filarial vaccines requires thorough searching and identification of purified filarial antigens. In the present study, we report the identification of a purified microfilarial protease as protective immunogen based on its pronounced seroreactivity with putatively immune endemic normal sera and its ability to induce protective immunity against B. malayi infection in jird model.

**Materials and Methods**

*Human sera:* Blood samples were collected from microfilaraemic carriers (positive by microscopic examination of night blood smear) and endemic normals residing in villages surrounding Sewagram and from patients visiting the out-patient department of Kas.
turba Hospital with clinical symptoms of filariasis. Asymptomatic, non-microfilaraemic individuals with no symptoms of the disease were considered endemic normals (EN). Non-endemic normal samples were collected from undergraduate medical students coming from non-endemic areas such as Himachal Pradesh and Jammu & Kashmir at the time of their admission. Informed consent was obtained from all the patients and students before collection of the blood samples. Sera were separated and stored at −20°C freezer with 0.1% sodium azide as preservative until use.

**Brugia malayi microfilarial soluble (Bm mf S Ag) antigen—**B. malayi microfilariae were collected by the peritoneal lavage of infected jirds (Meriones unguiculatus, CDRI, Lucknow. All the animal experiments of the present study was part of the funded research projects that was cleared by our Institutional Animal Ethics Committee). The parasites were then homogenized in ice, sonicated and extracted overnight at 4°C in 0.05 M Phosphate buffer saline (PBS) and the homogenate was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was removed, concentrated by ultra membrane filtration and labeled as B. malayi microfilarial soluble antigen [Bm mf (S) Ag].

**Fast Protein Liquid Chromatography (FPLC) purification of B. malayi mf S antigen—**The B. malayi mf S Ag was fractionated by FPLC (Amersham Pharmacia Biotech Ltd.) using Resource Q anion exchanger column. The mf S antigen (250 µg/500 µl) was applied onto the column and the fractions were eluted using 0.02 M Tris buffer pH 8 with a gradient of 0 to 0.7 M NaCl at a flow rate of 1 ml/min and monitored at 280 nm. Fractions of 0.5 ml volume were collected and concentrated by Ultra Membrane Filtration Technique. The fractions were checked for protease activity using Azocoll as substrate\textsuperscript{15} and also assayed for their seroreactivity using samples from microfilaraemic, endemic and non-endemic normal groups.

**Seroreactivity of purified B. malayi microfilarial antigen fractions—**A total of 30 sera samples belonging to microfilaraemia, clinical filariasis and endemic normals were screened against FPLC purified Bm mf S antigen fractions by indirect ELISA as described by Reddy et al.\textsuperscript{14}. The optimal antigen concentration of FPLC fractions (50 ng/50 µl/well), 1:100 diluted sera samples, optimally diluted anti human IgG-HRPO conjugate (1:4000) and OPD substrate were used.

**SDS–PAGE and Immunoblotting—**Bm mf S-7 antigen was analyzed by SDS-PAGE on 10% gradient gels under reducing conditions\textsuperscript{15}. The method of Towbin et al.\textsuperscript{16} was followed for the electro transfer of proteins from polyacrylamide gels to nitrocellulose paper at 100 mA for 1 hr and blocked with skimmed milk powder for overnight at 4°C. The NCP strips were incubated individually with optimally diluted pooled sera (1:50) from endemic normals, microfilaraemics and non-endemic normals. The bound antibodies were probed with specific anti human IgG peroxidase conjugate and its precipitating chromogenic substrate containing 0.003%H₂O₂ and 0.05% 3 3′ Diaminobenzidine in citrate phosphate buffer.

**Immunization of jirds—**A total of 4 male jirds of 2 month old age were immunized ip, with Bm mf S-7. Each animal received a primary dose of 25 µg of Bm mf S-7 in complete Freund's adjuvant followed by 3 doses of same amount of antigen in incomplete Freund's adjuvant at weekly intervals. A similar number of jirds representing the control group received only 0.05 M PBS emulsified Freund's adjuvant. After final dose, the jirds were used in different in vitro, in situ and in vivo experiments to assess the protective immune response against B. malayi parasite.

**In vitro antibody dependent cellular cytotoxicity (ADCC) assay—**The cytotoxicity assay was carried out as described by Chandrashekhar et al.\textsuperscript{17}. Briefly, 100 mf or 10 L₁ larvae in 50 µl of RPMI-1640 medium were incubated with 50 µl of 2×10⁶ normal jirds PEC and 50 µl of normal or immune jirds serum in 96 well culture plates (Costar Inc, MA, USA) in an atmosphere of 5% CO₂ at 37°C. After 48 hr the samples were examined microscopically for cellular adherence and cytotoxicity to parasites. Percentage of cytotoxicity was expressed as ratio of number of immobile or dead parasites by adherence of effector cells to the number of parasites recovered within the experimental period.

**Micropore chamber technique—**Immune response against filarial parasites in immunized and control jirds was analyzed by micropore chamber technique as described by Weiss and Tanner\textsuperscript{18}. Micropore chambers were assembled using 14x2-mm plexi glass rings and 3 µm nucleopore polycarbonate membranes. Membranes were attached to the plexi glass rings with cyanoacrylic adhesive and dental cement. The chambers were sterilized at 80°C for 10 hr and loaded with 10 infective larvae or 100 microfilariae in RPMI 1640 medium supplemented with 15% heat inactivated fetal
calf serum via an aperture (1 mm diam) at the side of plexi glass ring and then sealed with dental cement. The chambers were implanted i.p., into the immunized and control groups of jirds under anaesthesia through an incision of 2-3 cm and the skin was sutured. After 48 hr, the chambers were taken out, washed in normal saline, the contents were removed onto a glass slide and examined microscopically for cell adherence and cytotoxicity. The percentage of cytotoxicity was expressed as described above.

**Analysis of antibody response in immunized jirds**—Sera samples were collected from Bm mfS-7 immunized and control groups of jirds after the final dose of immunization for analysis of antibody response against immunized antigen. Indirect ELISA was carried out as described by Reddy et al.14. Briefly, Bm mf S-7 antigen (50 ng/50 μl/well) in 0.06 M carbonate buffer (pH 9.6) were coated in microtitre plates (NUNC) for 3 hr at 37°C. The free sites were blocked with 1% BSA in carbonate buffer for 2 hr at 37°C. The wells were incubated with optimally diluted (1:100 in PBST) sera samples of jirds and incubated at 37°C for 1 hr. The wells were washed thrice with PBS/T and then added with 50 μl of optimally diluted (1:5000) anti mouse IgG-HRPO conjugate for 1 hr at 37°C. The wells were washed 5 times with PBS/T and 50 μl of OPD substrate was added to each well. The reaction was stopped after 15 min with 5 N HCl and the OD values were recorded at 492 nm using ELISA Reader (Biotech).

**In vivo parasite clearance study**—Immunized and control groups of jirds (4 per group) were challenged by ip, infection of each animal with 150 infective larvae, at about 10 days after the administration of last immunization dose. The animals were monitored for development of patent filarial infection by examining the blood samples collected from cut end of the tails for mf19. After 120 days, the animals were sacrificed and the thorax and abdomen were opened to collect adult filarial worms from abdominal cavity, testes, heart, lungs and other organs. Number of adults worms recovered from each animal was recorded.

**Analysis of anti-Bm mf S-7 antibody levels during the course of infection**—Blood was collected from mastomys, prior to infection and on days 7, 15, 30, 60, 90 and 120 of post infection. Tail blood (20 μl) was collected from each of the mastomys on Whatman No.3 filter paper, dried at room temperature and stored at 20°C in moisture free conditions until use.

The blood samples from filter paper were eluted in 0.05 M PBS by gentle shaking for overnight at 4°C. The eluted blood samples were analyzed for antibodies by microtitre plate indirect ELISA14.

**Bm mf S-7 antigen-specific IgG isotype antibodies**—To analyze Bm mf S-7 antigen specific IgG isotype antibodies in sera samples of endemic normals, microfilaraemics and chronic clinical filarial patients, indirect ELISA was carried out as described by Dalai et al.30.

**Isolation of anti-mfS-7 antibodies from immune endemic normal sera using affinity chromatography**—Anti-Bm mf S-7 antibodies were isolated from pooled endemic normal sera by affinity chromatography31 using Bm mf S-7 antigen coupled A-H sepharose column. Endemic normal serum (200 μl) was applied onto the column and the column was then washed about 4 ml of 0.01 M SPB, pH 7.2. The antibodies bound to the antigen in the column were eluted out by passing about 4 ml of 0.2 M Glycine-HCl buffer (pH 2.5) and collected in 0.1 M Tris-HCl buffer (pH 8.6), to neutralize the eluant. The eluant was further concentrated, dialyzed against 0.01 M SPB, pH 7.2 and protein content was estimated.

**In vitro ADCC assay using affinity purified anti-mf S-7 antibodies**—The cytotoxicity assay was carried out as described by Chandrasekhar et al.17. Briefly, 100 mf or 10 L3 larvae in 50 μl of RPMI-1640 medium were incubated with 50 μl of 2×10⁴ normal Peripheral blood mononuclear cells and 50 μl of anti-mfS-7 antibodies in 24 well culture plates (Nunc) in an atmosphere of 5% CO₂ at 37°C. γ-globulins isolated from chronic filarial sera and non-endemic sera were used in the place of anti Bm mf S-7 antibodies as positive and negative controls respectively. After 48 hr the samples were examined microscopically for cellular adherence and cytotoxicity to parasites. Percentage of cytotoxicity was expressed as ratio of number of immobile or dead parasites by adherence of effector cells to the number of parasites recovered within the experimental period.

**Determination of lymphocyte proliferation response in vitro**—The Peripheral blood mononuclear cells separated from each subject were adjusted to a final concentration of approximately 2×10⁶ cells/ml of the suspension in RPMI 1640 medium supplemented with 15% heat inactivated AB+ve serum. The cell suspension was then distributed into a 96 well tissue culture plate (Nunc, Denmark) so that, each well contained about 2×10⁵ cells/100 μl of cell suspension.
The cells in triplicate wells were stimulated by addition of 10 μg/well of Bm mf S-7 or 2.5 μg/well of ConA (mitogen). Unstimulated cell cultures in triplicate wells served as controls. Final volume of the culture fluid in each well was adjusted to 200 μl with RPMI 1640 (containing 15% AB+ve serum) medium. The plates were incubated for 72 hr at 37°C in a CO2 incubator (Forma Scientific Inc., Marietta, USA) with 5% CO2. The wells were then pulsed with 3H-Thymidine (0.5 μCi/well) and further incubated for 16 hr. Finally cells were harvested on glass fibre filters (Whatman, UK) using Cell Harvester (Nunc, Denmark). Uptake of 3H-thymidine by cells was measured in a liquid scintillation counter. The stimulation index (SI) was calculated by using the formula

\[
SI = \frac{\text{Mean counts per min (CPM) of stimulated cultures in triplicate wells}}{\text{Mean counts per min (CPM) of unstimulated cultures in triplicate wells}}
\]

Estimation of cytokines in culture supernatants of Bm mf S-7 antigen or mitogen stimulated PBMC of different groups—Cytokines interleukin-4 (IL-4), interferon-γ (IFN-γ) and IL-10 were measured in the culture supernatants of PBMC of different groups (microfilaraemia, clinical filarial and endemic normal individuals) stimulated with Bm mf S-7 antigen or ConA or no antigen as described above. The stimulated and unstimulated cell cultures in triplicate wells were maintained at 37°C and 5% CO2. Culture supernatants (50 μl) from each of the triplicate wells were collected at the end of 48 hr and 96 hr and pooled separately for the estimation of IL-4 or IL-10 and IFN-γ respectively. The cytokines were estimated using Duo set ELISA kits of R&D systems, Inc USA as per the protocols supplied with the kits.

Statistical analysis—The results are expressed as mean ±S.D. Student’s t test was used to determine the level of significance. P values of ≤0.05 were taken to be significant.

Results

Seroreactivity of Bm mf S-7—FPLC fractions of B. malayi microfilarial soluble antigen yielded seven protein fractions (Fig. 1) and the seventh fraction which showed significantly high reactivity with putatively immune endemic normals compared to its reactivity with sera of microfilaraemic and non-endemic normal groups. This fraction that also showed significant protease activity was labeled as Bm mf S-7. The mean absorbance value of filarial antibody levels against Bm mf S-7 in 10 endemic normals (1.098) was significantly higher (P<0.001) than in microfilaraemic cases (0.606) and non-endemic normal sera (0.444).

SDS-PAGE and immunoblotting analysis of Bm mf S-7—SDS-PAGE analysis of Bm mf S-7 run on 5-10% gel revealed that Bm mf S-7 antigen was a 38 kDa protein (Fig. 2). Seroreactivity by immunoblotting also showed the selective recognition of the 38 kDa protein band by pooled endemic normal serum (Fig. 3).

Anti-Bm mf S-7 antibody levels in immunized jirds—Jirds immunized with Bm mf S-7 antigen
showed significantly higher levels of specific antibody with mean absorbance values in ELISA at 0.838 compared to the levels in control group of jirds (0.268, P<0.001).

Anti-mf S7 serum induced in vitro cytotoxicity against B. malayi mf and L3 larvae—Sera from jirds immunized with Bm mf S-7 antigen promoted adherence of PEC to both mf and L3 larvae and induced significantly higher cytotoxicity (88.8%) against both mf and L3 compared to that induced by serum of control group of jirds which showed 5.8% cytotoxicity against mf and 8.1% against L3 (P<0.001) (Table 1).

In situ cytotoxicity against B. malayi mf and L3 larvae in micropore chambers—In micropore chamber method, the microscopic observation of chambers implanted in jirds immunized with Bm mf S-7 showed migration of host macrophages and polymorph nuclear cells into chambers leading to their adherence and killing of mf or L3 larvae within 48 hr. The cytotoxicity in the immunized jirds against L3 and mf was 87-89% that was significantly high (P<0.001) compared to the control group of jirds which showed cytotoxicity of 10% against mf and 8% against L3 (Table 1).

Adult worm recovery in Bm mf S-7 immunized jirds—Jirds immunized with Bm mf S-7 were challenged with 150L3 larvae and monitored for the development of parasite and establishment of infection. None of the jirds immunized with Bm mf S-7 showed microfilariae in circulation. Adult filarial worms were recovered from both the immunized and control jirds. However, the adult worms recovery in the jirds immunized with Bm mf S-7 was only 18.5% of that observed in the control jirds.

Anti-Bm mf S-7 antibody levels in mastomys during the course of B. malayi infection—The result of analysis of serum IgG antibody levels against Bm mf S-7 antigen of B. malayi parasite in mastomys with patent filarial infection are shown in Fig. 4. IgG antibodies against Bm mf S-7 antigen were detected on 15th day of post-infection. Thereafter there was a gradual decrease in the anti mf S7 antibody levels until the end of the monitoring period, whereas antibody levels against whole Bm mf S Ag showed a gradual increase and were maintained at high levels until the end of the monitoring period.

Bm mf S-7 specific isotype profiles in bancroftian endemic individuals—A total of 30 sera (10 sera each, belonging to mf carriers, chronic pathology and endemic normals) were screened for IgG subclasses and the results are shown in Fig. 5. Endemic normals showed significantly high levels of Bm mf S-7 specific IgG2 and IgG3 antibodies levels compared to
microfilarial cases and clinical cases (P<0.001). Analysis of IgG4 antibodies levels showed a significant increase in microfilaraemics (P<0.05) compared to endemic and clinical filarial cases. There was a significant increase of filarial IgG2 and IgG3 antibody levels in clinical cases also (P<0.05).

In vitro ADCC against mf and L3 larvae induced by affinity purified anti-mf S-7 antibodies—Affinity purified anti-mf S-7 antibodies from pooled endemic normal sera promoted adherence of PBMCs and induced significantly higher cytotoxicity against both mf (75%) and L3 (72.5%, P<0.001) compared to that induced by antibodies of non-endemic normals (negative control).

Lymphocyte proliferation against Bm mf 'S'-7—The lymphocytes from endemic normal individuals showed significantly higher (P<0.05) mean stimulation index (2.84) than that of microfilaraemics (1.23) or clinical filarial individuals (1.45). In contrast, against ConA, there was no significant difference in the proliferative responses observed in endemic normals (10.5) compared to that in microfilaraemics (11.13) and chronic filarial cases (10.8) (Table 2).

Cytokine levels—Cytokines IL-4, IL-10 and IFN-γ were estimated in the supernatants of Bm mf S-7 or ConA stimulated PBMC cultures. Bm mf S-7 induced differential IL-4, IL-10 and IFN-γ secretion and no cytokine was detected in unstimulated cultures. The geometric mean (GM) level (Fig. 6a) of IFN-γ in endemic normals (108.52 pg/ml) was significantly higher than in microfilaraemics (21.66 pg/ml) or clinical filarial patients (23.45 pg/ml) (P<0.01). However, significantly enhanced secretion of IL-10 was observed (Fig. 6b) in microfilaraemics (49 pg/ml) than in endemic normals (27.5 pg/ml) and clinical filarial patients (12 pg/ml) (P<0.01). The GM levels of IL-4 in microfilaraemics (36 pg/ml) was significa-

![Graph](image)

**Table 2**—Stimulation indices against Bm mf S-7 and Con A stimulated PBMCs of different groups of bancroftian filariasis (values are mean ± SD from 10 samples in each group)

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<thead>
<tr>
<th>Group</th>
<th>Bm mf 'S'7</th>
<th>Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endemic normals</td>
<td>2.84± 1.0</td>
<td>10.5± 6.1</td>
</tr>
<tr>
<td>Microfilaraemic</td>
<td>1.23± 0.6</td>
<td>11.13± 8.1</td>
</tr>
<tr>
<td>Clinical filarial cases</td>
<td>1.45± 0.7</td>
<td>10.8± 5.2</td>
</tr>
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![Graph](image)
cantly higher compared to that in clinical filarial cases (24.7 pg/ml) (P<0.05) while there was no significant difference when compared with that in endemic normal group (30.2 pg/ml) (Fig. 6c).

Discussion

The normal individuals in the filarial endemic region with no microfilariae and no symptoms of disease or infection despite the evidence of continuous exposure to infection are generally believed to be putatively immune to filarial infection23,13. Analysis of humoral and cellular immune response against parasite antigens in putatively immune endemic normals in comparison with other infected groups has been the strategy adopted by many investigators to identify protective antigens3,4,24,25. Hence one of the approaches to identify protective antigens has been to assess their reactivity with the sera samples of endemic cases. A number of candidate antigens of prophylactic importance viz., a 43kDa antigen isolated from B. malayi microfilarial extract9 and 3 antigenic components (25, 58, 68 kDa) from B. malayi microfilariae24 have been shown to be recognized by endemic normal sera.

This strategy of what is called the “Rational screening” has been applied in the present study to identify purified B. malayi microfilarial antigen of immunoprophylactic potential. FPLC fractionation of Bm mf S Ag using Resource ‘Q’ anion exchanger column yielded 7 protein peaks labeled as Bm mf S-1 to Bm mf S-7. All the seven FPLC purified fractions of Bm mf S Ag were screened for their reactivity with microfilaraemics, endemic and non-endemic normals. One of seven fractions viz., Bm mf S-7 was highly reactive with sera of endemic normals and it was associated with high protease activity (Fig. 1). These results were further confirmed by screening ten individual sera of each of these groups for antibody levels against Bm mf S-7.

SDS-PAGE analysis followed by silver staining of Bm mf S-7 antigen showed it to be a 38 kDa protein molecule (Fig. 2). In immunoelectroblot analysis, the pooled endemic sera also recognized this 38 kDa band (Fig. 3). Based on high reactivity with endemic normal sera it was predicted that pre immunization of susceptible animals with Bm mf S-7 may be host-protective. Many investigators have adopted different approaches to explore the potential of an antigen in inducing protection against infection in animal models which includes in vitro ADCC assay17,26,27 and in situ micropore chamber method18,27 and in vivo challenge inoculation of either microfilariae or infective larvae9,23,29,31. The in vitro studies have been given importance for primary analysis and the in vivo studies have been considered as confirmatory methods.

In the present study, in in vitro antibody dependant cellular cytotoxicity (ADCC) assay, the anti sera raised against Bm mf S-7 promoted the adherence of peritoneal exudate cells (PEC) and induced cytotoxicity to mf and L3 larvae within 48 h. The cytotoxicity induced by the sera from jirds immunized by Bm mf S-7 was significantly high (P< 0.001) compared to that of control jirds. The cytotoxicity induced by jirds anti Bm mf S-7 serum against mf and L3 larvae was 87-89%. Both antibody and complement mediated effector mechanisms have been shown to be involved in inducing cytotoxicity to the mf and L3 larvae in vitro32,17,18,33. The involvement of different types of effector cells like macrophages, neutrophils and eosinophils in fastening the cytotoxic effect to filarial parasite has been reported by Higashi and Chowdhury34.

Micropore chamber technique in which the mini chambers loaded with mf or L3 larvae are implanted in the immunized and control group of jirds is a useful technique to check whether similar ADCC reaction occurs in situ. In this technique, a closer physiological environment can be provided for the growth and survival of the parasite and thus more useful to assess the host effector mechanisms35. In the present study, the in situ cytotoxicity induced by Bm mf S-7 antigen was directed to both mf and L3. The cytotoxic effect was high and equally effective against both mf and L3 larvae (88.8%) in the jirds immunized with Bm mf S-7 antigen.

The effect of immunization on the development of B. malayi infective larvae to the adult stage was studied in jirds. The recovery of adult worms has been used as parameter to study the long-term protective effect of antigen30. Bm mf S-7 reduced the adult worm burden up to 82% in the immunized jirds compared to the control group of jirds.

Identification and analysis of filarial antigens is essential for understanding the functional immune responses of the host to the parasites. In the present study, an attempt was made to analyze the filarial antibody response against Bm mf S-7 antigen in mastomys during the course of infection. The sequential changes in antibody response against the Bm mf S-7 increased gradually from 15th day and then decreased
till the monitoring period. The decline in antibody level against Bm mf S-7 suggests hyporesponsiveness developed against this antigen during the course of infection resulting in patent infection in susceptible animals.

Analysis of IgG subclasses against Bm mf S-7 showed a significant increase in IgG2 and IgG3 antibodies in endemic normals as compared to other filarial groups (P<0.001). IgG3 has been implicated in protective roles on Onchocerciasis. 39, 40 IgG3 is known to play a role in ADCC and activation of the complement system. 41, 42

Analysis of lymphocyte proliferative response against Bm mf S-7 showed the endemic normals having significantly high proliferative response compared to that in microfilaraemics and chronic filarial cases (P<0.05). This response is filarial antigen specific, as the mitogen (ConA) - driven T cell proliferation is not significantly different between the studied groups. Dalai et al. 43 reported increased lymphocyte proliferation in endemic group in response to Seteria digitata antigens that had higher stimulation indices than those of microfilaraemics or clinical filarial cases. Piessens et al. 44 had also shown the asymptomatic and microfilaraemic individuals having specific cellular response to microfilarial antigens. Microfilarial and larval soluble antigen showing specific seroreactivity with endemic normals have also shown increased proliferation in endemic normals 45, 46

Comparison of the cytokine responses against Bm mf S-7 in the different patient groups showed copious secretion of IFN-γ by PBMCs of endemic normals as compared to clinical and microfilarial cases. IL-10 levels were higher (49 pg/ml) (P<0.05) in mf cases as compared to either chronic filarial patients (27.5 pg/ml) or endemic normals (12 pg/ml). These results seem to indicate that Bm mf S-7 induced immune response is of the Th1 type.

The antibody class switching to IgG2a is also promoted by IFN-γ which correlates with the type of humoral response seen in endemic normals. 47 Ravi-chandran et al. 48 have shown that Th1 cytokines predominate in putative immune individuals. Patients with elephantiasis, who have successfully cleared the parasite but have developed lymphoedema in the process, have a relative prominence of Th1 cytokines, similar to endemic normals. Thus, analysis of immune responses in different groups of bancroftian filarial cases in this study supports the perception that Interferon-γ induced activation of macrophages in endemic normal group led to a predominant Th1 type of immune response to Bm mf S-7 which is possibly be involved in immune protection.

Thus, in the present study, the analysis of seroreactivity of FPLC purified B. malayi mf soluble antigen helped in identifying a 38 kDa protease as immunopathogenic potential in filariasis. Further studies in the animal model jird have confirmed that immunization of jirds with Bm mf S-7 could induce anti-L3 and anti-mf effect and thus offer significant protection against B. malayi filarial infection.

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