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Immunoprophylactic studies with a 43 kDa human circulating filarial antigen and a cross reactive 120 kDa *Brugia malayi* sodium dodecyl sulphate soluble antigen in filariasis

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Abstract. Bancroftian filariasis is a major public health problem affecting about 120 million people all over the world. Immunoprophylaxis may serve as an additional adjunct along with chemotherapy and anti larval measu[r]es for successful filaria control. Circulating filarial antigen fraction (CFA_2-6) containing 43 kDa antigen and adult *Brugia malayi* sodium dodecyl sulphate (S DS) soluble antigen fraction BmA-2 with a 120 kDa molecule were earlier shown to be reactive with endemic normal sera by immunoblotting and indirect ELISA techniques. BmA-2 was found to be highly cross reactive with CFA_2-6. Sera raised against both the antigen fractions showed about 9 0 % cytotoxicity to the parasites in the presence of jird peritoneal cells in *in vitro* as well as by *in situ* micropore chamber implantation technique. Further in *in vivo* studies using animal model, jirds CFA_2-6 and BmA-2 could [i]nduce about 90% protection to infection in immunized animals. In passive transfer studies of immunity it has been observed that BmA-2 induced protection is mainly antibody mediated.

Keywords. Filariasis; immunoprophylaxis; cytotoxicity; passive immunization.

1. Introduction

Inspite of advances in vector control methods and chemotherapy, the lymphatic filariasis particularly the infection caused by *Wuchereria bancrofti* continues to be a major cause of clinical morbidity in developing countries. The vaccine against filarial infection can be an effective additional adjunct to the existing methods in the control of filariasis. However the immunoprophylactic studies in bancroftian filariasis are hampered by many reasons that include the strict primate host specificity of *W. bancrofti,* the diversity of clinical manifestations of the infection in humans and the lack of a clear picture about host's immune response to the infection and naturally occurring immunity (Philipp *et al* 1988). The fully permissive rodent models available for maintenance of sub periodic strain *Brugia malayi* filarial parasite i.e., *Mastomys natalensis* (mastomys) and *Mariones unguiculatus* (jirds) are very useful for identifying protective immunogens in filariasis.

Earlier reports on immunoprophylactic studies related to filariasis are mainly on the use of either alive or attenuated *B. malayi* parasites (Denham *et al* 1983;

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Nakanishi *et al* 1989; Abraham *et al* 1989). Attempts have also been made to identify the protective antigens from different stages of filarial parasites (Kazura and Davis 1982; Kazura *et al* 1986; Freedman *et al* 1989). However the studies on the use of purified filarial antigens in prophylaxis are scanty. In this communication we present the comparative effectiveness of a circulating filarial antigen fraction CFA-6 containing a 43 kDa protein and a cross reactive *B. malayi* adult soluble antigen BmA-2 with a 120 kDa protein in protection against filarial infection using rodent animal models.

2. Materials and methods

2.1 *CFA_2-6*

CFA_2-6 was prepared from pooled plasma of *W. bancrofti* infected microfilaraemic cases by 34–75% saturation with ammonium sulphate followed by ultrogel ACA-34 gel filtration column chromatography as described by Reddy *et al* (1986). CFA_2 was further resolved by SDS Polyacrylamide gel electrophoresis (PAGE) on 10% non gradient slab gel as described earlier (Cheirmaraj *et al* 1991). The SDS-PAGE gels were sliced horizontally (into 12 slices) at 1 cm regular intervals and the proteins of 6th slice (from the top of the gel) which included a 43 kDa protein were eluted into 0·05 M sodium phosphate buffer (SPB), pH 7·2. The eluant was dialysed, concentrated and labelled as CFA_2-6. The protein content was estimated by the method of Lowry *et al* (1951).

2.2 *BmA-2*

BmA-2 was prepared from *B. malayi* adult worms collected from peritoneal cavity of infected jirds (M. *unquiculatus)* as described elsewhere (Chenthamarakshan *et al* 1995). Briefly, freeze dried adult worms were homogenized, extracted in phosphate buffered saline (PBS) at 4°C and centrifuged at 50,000*g* at 4°C. BmA SDS soluble (BmA SDS S) antigen was prepared by extracting the pellet with a solution containing 5% SDS, 5% 2-mercaptoethanol and 8 M urea in 0.01 M SPB (pH 7.2). The supernatant containing BmA SDS-soluble antigen was further fractionated on SDS-PAGE and after electrophoretic run, the gels were cut at 1 cm intervals and the eluate collected from second slice which contained a 120 kDa protein band was labelled as BmA-2.

2.3 *Analysis of cross reactivity between CFA_2-6 and BmA-2*

Antibodies to filarial antigens CFA_2-6 and BmA-2 were raised in mouse ascites as described by Cheirmaraj *et al* (1990). The IgG fraction of mouse antibodies was separated using DEAE anion exchange (Whatman DE 52) chromatography by ionic gradient elution at pH 8. Indirect ELISA was carried out to screen SDS-PAGE fractions of CFA_2 and BmA (starting with initial concentration of each antigen fraction at 10 μg/well) using optimal dilutions of mouse anti CFA_2-6 and anti BmA-2 antibo dies, anti mouse IgG Peroxidase conjugate and O-Phenylenediamine substrate.

2.4 *Immunization of jirds with filarial antigens CFA_2-6 and BmA-2*

A total of 24 jirds were immunized intraperitoneally (i.p.) with filarial antigens CFA_2-6 or BmA-2 as described elsewhere (Chenthamarakshan *et al* 1995). Each animal received a primary dose of 25 µg of filarial antigen in complete Freund's adjuvant followed by three doses of same amount of antigen in incomplete adjuvant at weekly intervals. A similar number of jirds representing control group received only saline emulsified Freund's adjuvant. A week after the last dose, the jirds were used in different *in vitro, in situ* and *in vivo* experiments to assess the immuneprotective potential of filarial antigens.

2.5 *In vitro antibody dependent cellular cytotoxicity assay*

In vitro antibody dependent cellular cytotoxicity (ADCC) assay was carried out following the method described by Chandrasekhar *et al* (1985a, 1990). Briefly 10 *B. malayi* L_3 larvae were incubated with 1×10^5 normal jird peritoneal exudate cells (PEC) and 50 µl of jird serum in a final volume of 0·2 ml in 96 well culture plates (Costar Inc., MA, USA) at 37°C with 5% CO_2. The samples were examined microscopically after 48 h for cellular adherance and cytotoxicity to parasites.

2.6 *In situ cytotoxicity assay (Micropore chamber technique)*

Micropore chambers were assembled using 14 × 2 mm plexiglass rings (Millipore filter Corp., Bedford, USA) and 3 µm nucleopore membranes as described by Weiss and Tanner (1979). Micropore chambers loaded with 10 L_3 larvae in RPMI 1640 medium were implanted i.p. in CFA_2-6 or BmA- 2 immunized (4 in each group) and similar number of control group of jirds through an incission of 2–3 cm and the skin was sutured. After 72 h, the chambers were taken out and the contents were examined microscopically.

2.7 *Recovery of adult worms*

Two groups of jirds immunized with CFA_2-6 or BmA-2 (4 in each group) along with similar number of control groups of jirds were challenged each with 100 infective larvae. The jirds were checked for the development of patent infection by peritoneal lavage. After 90 days the jirds were sacrificed and adult worms were collected from peritoneal lavage and washings of abdominal organs.

3. Results

3.1 *Reactivities of CFA_2-6 and BmA-2 antigens with mouse antifilarial sera*

Analysis of reactivities of filarial antigens CFA_2-6 and BmA-2 was done against mouse anti CFA_2-6 and anti BmA-2 antibodies by indirect ELISA. While CFA_2-6 showed filarial antigen titres of 10,000 the BmA- 2 showed titres of 10,000 and 1,000 against anti CFA_2-6 and anti BmA-2 antibodies respectively with initial concentration of each

antigen at 10 µg/well. From the seroreactivity studies of different SDS-PAGE fractions of CFA_2 and BmA with mouse antifilarial sera, CFA_2-6 was found to be more immunogenic than BmA-2 (V Chenthamarakshan, K Cheirmaraj, M V R Reddy and B C Harinath, unpublished observations).

3.2 *In vitro and in vivo immune response*

In the *in vitro* serum dependent cellular cytotoxicity assay the anti CFA_2-6 and anti BmA-2 sera promoted the adherance of PEC and induced cytotoxicity to the infective larvae within 48 h (figure 1). The immune sera against both the antigen fractions induced more than 90% cytotoxicity to the parasites within 48 h.

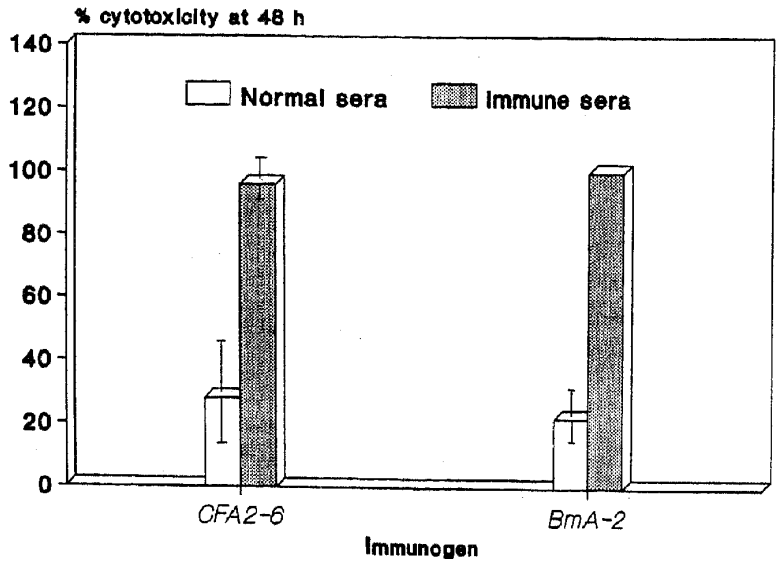

Figure 1. Anti CFA_2-6 and anti BmA-2 serum dependent cellular cytotoxicity to *B. malayi* L_3 larvae *in vitro* (bars represent mean + SD of the results obtained from four individual jirds sera).

In the micropore chamber method, the microscopic observation of chambers implanted in jirds immunized with CFA_2-6 and BmA-2 showed the migration of host macrophages and polymorphonuclear cells into chambers leading to their adherence and killing of about 90 % of the parasites within 72 h (figure 2). On the other hand in the chambers implanted in the control jirds, only 25–28% mortality was observed.

3.3 *Recovery of adult worms after challenge infection*

Jirds immunized with CFA_2-6 or BmA-2 were challenged with 100 L_3 larvae and monitored for development of parasite and establishment of infection. None of the immunejirds showed microfilariae in circulation and in contrast 75–88% control jirds turned microfilaraemic within 90 days. Adult filarial worms were recovered from both the immunized and control jirds. However, the adult worm recovery in the jirds immunized with both CFA_2-6 and BmA-2 antigens was only 12% of that observed in the control jirds (figure 3).

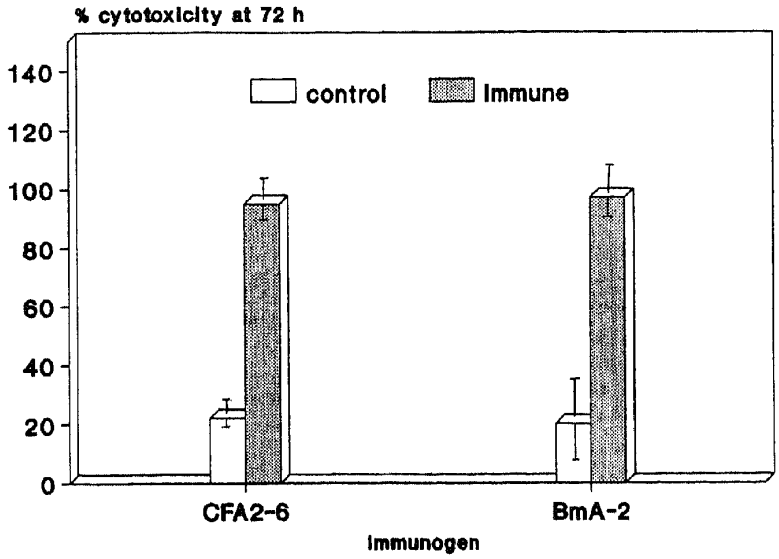

Figure 2. Cytotoxicity to *B. malayi* L_3 larvae in micropore chambers implanted into jirds immunized with CFA_2-6 or BmA-2 and control jirds (bars represent mean ± SD of the results obtained from four individual jirds).

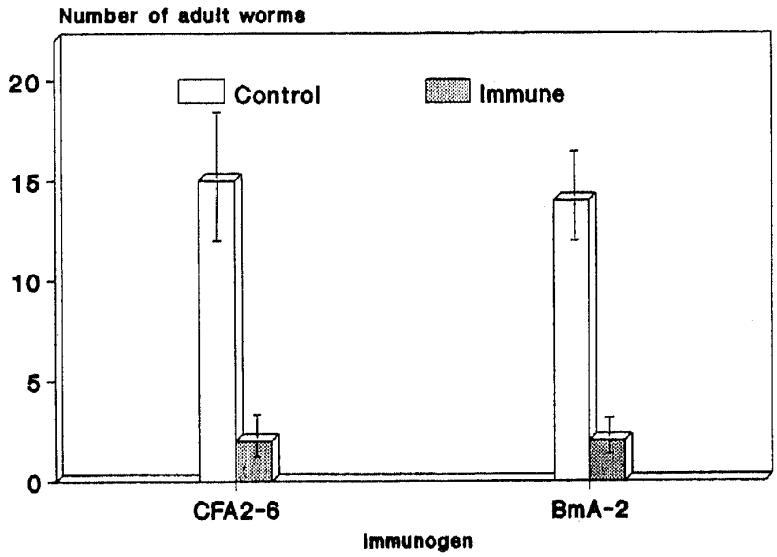

Figure 3. Recovery of adult worms from jirds immunized with CFA_2-6 or BmA-2 and control jirds after challenge infection with *B. malayi* L_3 larvae (bars represent mean ± SD of the results obtained from four individual jirds).

4. Discussion

A majority of the population in a filariasis endemic area shows neither microfilariae in circulation nor any clinical manifestations. This is heterogeneous group represented by a majority of those who are truely negative to the infection and some with either prepatent or occult filarial infections. Both cellular and humoral types of immune

responses have been shown to be significantly higher in endemic normal population compared to microfilaraemic cases (Ottesen *et al* 1977, 1982) and possibly a state of protective immunity is associated with this group. Hence one of the approaches to identify protective antigens has been to assess their reactivity with the sera samples of endemic normal cases. A number of candidate antigens ofprophylactic importance viz., a 43 kDa antigen isolated from *B. malayi* infective larvae (Freedman *et al* 1989), a 25 kDa molecule of*B. malayi* microfilarial extract (Kazura *et al* 1986) and 3 antigenic components (25, 58 and 68 kDa) from *B. malayi* microfilariae (Parab *et al* 1990) have been shown to be recognized by endemic normal sera. Earlier studies from this laboratory (Cheirmaraj *et al* 1991) had shown the two filarial antigens a circulating filarial antigen fraction from microfilariaemic plasma (CFA_2-6) containing a 43 kDa protein and a *B. malayi* adult antigen fraction (BmA-2) containing a 120 kDa protein to be more reactive with endemic normal sera (figure 4) and further the immunoprophylactic potential of BmA-2 was explored (Chenthamarakshan *et al* 1995). The present study reports the results of comparative analysis of CFA_2-6 and BmA-2 in *in vitro* and *in vivo* cytotoxic assays against *B. malayi* infective larvae.

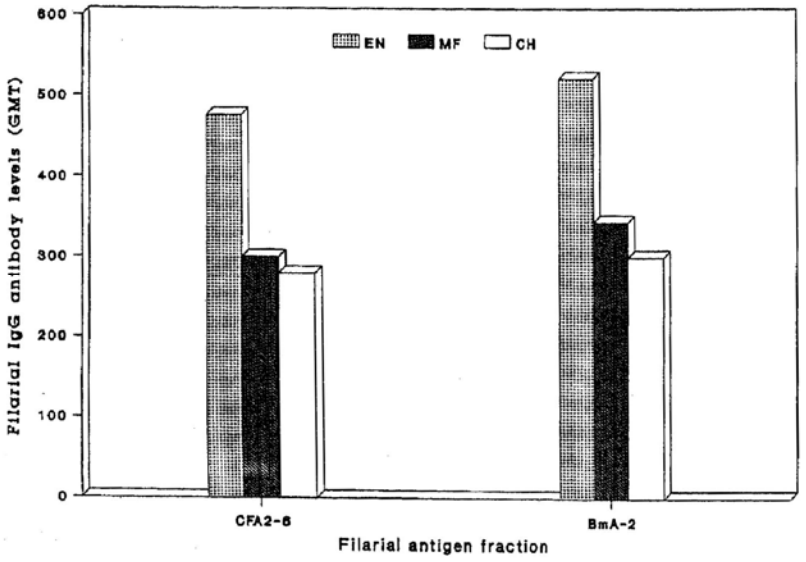

Figure 4. Geometric mean titres (GMT) of filarial IgG antibodies in different groups of endemic sera using CFA_2-6 and BmA-2 [EN, Endemic normal sera (10); MF, microfilaraemic sera (10); CH, clinical filarial sera (10)].

Analysis of reactivities of SDS-PAGE fractions of CFA_2 and BmA SDS-S antigen against mouse anti CFA_2-6 and anti BmA-2 antibodies showed CFA_2-6 and BmA-2 to be highly cross reactive with each other. In *in vitro* ADCC reaction the antisera raised against both CFA_2-6 and BmA-2 induced cytotoxicity to *B. malayi* infective larvae within 48 h (figure 1). However the anti BmA-2 serum induced stronger adherence of peritoneal cells mainly macrophages against *B. malayi* L_3 larvae compared to the cellular adherence observed with anti CFA2-6 serum. Both antibody and complement mediated effector mechanisms have been shown to be involved in inducing cytotoxicity to the microfilariae and infective larvae *in vitro* (Sim *et al* 1982; Chandrashekar *et al* 1985a, b; Weiss and Tanner 1979; Mehta *et al* 1980).

The micropore chamber technique in which more closer physiological environment can be provided for the parasite growth and survival has been used by many investigators to analyse whether a similar ADCC reaction could occur *in vivo.* The chambers loaded with filarial larvae and implanted in peritoneal cavity provoked infiltration of cell populations into the chamber and within 72 h of observation period, majority of the larvae were billed by cytotoxic effect. Chandrashekar *et al* (1990) showed macrophages to be the predominant cell type involved in *in vivo* ADCC to *B. malayi* larvae. The involvement of macrophages in mediating cytotoxicity reaction has also been demonstrated against other filarial parasites viz., *Dipitelonema setariosum* adults (Worms and McLaren 1982) and *Litomosoides carinii* microfilariae (Nelson *et al* 1976).

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 in jirds immunized with CFA_2-6 and BmA-2 was only 12% of that observed in the control jirds. None of the immunized jirds turned to be microfilaraemic. Chusattayanond and Denham (1986) reported that immunization with attenuated *B. phangi* L_3 larvae resulted in fewer and smaller adult worm recovery. In another study by Kajura and Davis (1982) immunization of jirds with microfilarial antigens induced a 50% reduction in the adult worms recovered.

As it was reported earlier from this laboratory (Chenthamarakshan *et al* 1995) increased resistance to worm burden in BmA-2 immunized jirds was confirmed by passive immunization experiments. While passive transfer of immune sera from jirds immunized with BmA-2 to naive jirds resulted in 71% reduction in adult worm recovery, the passive transfer of non-adherent spleen cells from immune jirds did not show any significant effect on the development of parasite. These results suggest that the protective immunity induced by BmA-2 is mainly antibody mediated. Thus, the *in vitro* and *in vivo* cytotoxicity studies against *B. malayi* filarial parasites have helped in identifying CFA_2-6 and BmA-2 as antigens of immunoprophylactic potential in filarial infection.

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