Expression of different filarial parasite adult stage antigens during course of DEC treatment in mouse model of Brugian filariasis

Mohammad A. Khan*

Department of Medicine,
Stanford University,
Palo Alto, 94304, California, USA
E-mail: afzal@stanford.edu
*Corresponding author

M. Saleemuddin

Department Biotechnology,
Aligarh Muslim University,
Aligarh, 202 002, UP, India
E-mail: msaleemuddin@rediffmail.com

Puvadda K. Murthy

Department of Parasitology,
Central Drug Research Institute,
Lucknow 226 001, UP, India
E-mail: drkalpana@yahoo.com

Abstract: Filariasis is a tropical disease with high morbidity rate due to lack of permanent cure. Here, we investigated specific filarial parasite antigens useful in treatment strategies for filariasis in areas where the chances of constant stimulations are at high peak. This study highlighted alterations in antigen recognition in the people living in endemic countries and receiving the orthodox treatments with diethylcarbamazine. We found that reinfection with L_3 stage in pre-DEC treated mouse sera recognises 62 kDa, 45 kDa and 32 kDa antigens, which was not present in control animal sera. Further investigation shows that 62 kDa antigen was present in microfilaria while 45 kDa and 31 kDa was part of adult stage. These findings demonstrated that reinfection during the course of DEC treatment unmask specific parasite antigen, which do not express during initial stage of infections. Our findings argued that mapping of these new antigens would be helpful in the development of treatment strategies through appropriate vaccine.

Keywords: *Brugia malayi*; microfilaria; L_3; reinfection; diethylcarbamazine treatment; Brugian filariasis; parasite antigens.

Reference to this paper should be made as follows: Khan, M.A., Saleemuddin, M. and Murthy, P.K. (2011) 'Expression of different filarial parasite adult stage antigens during course of DEC treatment in mouse model of Brugian filariasis', *Int. J. Immunological Studies*, Vol. 1, No. 3, pp.272–284.

Biographical notes: Mohammad A. Khan is a Research Associate at Stanford University, USA. He carried out this work under the supervision of Prof. M. Saleemuddin at Department of Biotechnology, Aligarh Muslim University, Aligarh, India during his stay for PhD programme.

M. Saleemuddin is a Senior Scientist at Department of Biotechnology, Aligarh Muslim University, Aligarh, India. He is the Chair of Biotechnology programme at this centre.

Puvadda K. Murthy is working as a Senior Scientist at the Division of Parasitology, Central Drug Research Institute and thoroughly assisted this study up to standards of this publication.

1 Introduction

Filariasis is caused by *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* species. Worldwide, 1,100 million people are at risk and 78.6 million show microfilariae (Mf) or overt disease (Michael et al., 1996a, 1996b). Global estimates of LF reveals approximately 129 million people with *Wuchereria bancrofti* (90% of cases) and with *Brugia malayi* (10% of cases) are affected, with one billion people considered to be at risk of becoming infected (Michael et al., 1996a).

The somatic antigens have been fractionated earlier derived from a number of filarial parasites including *W. bancrofti* (Michael et al., 1996a); and *B. malayi* (Lal and Ottesen, 1988; Maizels et al., 1983a, 1983b; 1983c; Selkirk et al., 1986). A number of antigenic molecules viz. 44–14 kDa of *B. malayi* (Cheirmaraj and Harinath, 1991), 25 kDa (Kazura et al., 1986), 92–25 kDa (Parab et al., 1990) of Mf were earlier found to be immunoreactive using human bancroftian sera. Molecular weight < 200, 170–200, 40–44, 30–36, 23–28, 20–22 and 17–19 kDa of *B. malayi* adult induced protection against L_3 in Balb/c mice (Hammerberg et al., 1989; Hayashi et al., 1989), 97 kDa of *B. malayi* Mf and adults induced partial stage specific protection against *B. malayi* Mf in mice (Nanduri and Kazura, 1989).

Reduction of morbidity associated with the disease and interruption of transmission are the two general strategies being used in the control of human filariasis employing chemotherapy and vector control methods. In spite of constant efforts to treat patient from free of infection some of the Mf carriers show reappearance of Mf after treatment. Therefore, in view of re-infection control of lymphatic filariasis has been a challenging problem. The main problem is with the limited understanding on aftermath of drug treatment on host-parasite relationship. DEC and ivermectin (Campbell et al., 1983) are microfilaricidal with little or doubtful action on adult worms. In spite of repeated intake of the drug, the infection is spreading very fast.

However, irrespective of the altered approaches, the disease is persisting and the reasons for this are not readily evident. It is however, known that in the absence of an effective method for the eradication of the mosquito vector, the population in the endemic areas will continue to be infected through mosquito bites. What is not known is whether antifilarial therapy alters the infected host's immune system/responses and whether such alterations influence the host's susceptibility to reinfection in the endemic areas. Unfortunately, there have been no such studies pertaining to re-infection following antifilarial therapy. The major constraint faced towards this end has been primarily due to

non-availability of suitable laboratory model, which can largely simulate human situation.

Our previous work has optimised mouse model of Brugian filariasis (Dixit et al., 2004; Khan et al., 2004), and its chemotherapeutic responses to DEC and IVM match with those reported for humans. Therefore, we successfully applied DEC treatment and reinfection approach to investigate changes in parasite antigens during course of DEC treatment. It is apparent from the above review of literature that various attempts have though been made to characterise antigens of different filariid species, the information is still patchy. In fact, not much concerted effort has been made to purify the identified immunogenic molecules of human filariid and to evaluate their immunogenicity or diagnostic efficiency. It therefore, appears necessary to identify such molecules in the target human filarial species and to evaluate their potential in the host parasite interaction.

2 Materials and methods

2.1 Infection

Infective third stage larvae (L_3) of *B. malayi* were obtained from laboratory bred female *Aedes aegypti* fed on microfilaraemic *Mastomys coucha* as described previously (Dixit et al., 2004; Khan et al., 2004).

2.2 Selection and grouping of animals

Mf positive animals (five months old infection) and were showing progressive rise in microfilaraemia were selected for study. These were grouped in to four (Table 1). Each group consisted of 8 to 16 animals. Two replicates for each experiment were carried out.

Table 1 DEC treatment in different experimental groups

Group	*No. of animals*	*Animals*
I	16	Infected (Inf)
II	8	Infected Treated (Inf-T)
III	8	Infected treated-reinfected (InfT-R)
IV	8	Infected-reinfected (Inf-R)
V	16	Normal (N)
VI	8	Normal-treated (N-T)
VII	8	Normal treated infected (NT-I)
VIII	8	Normal-infected (N-I)

2.3 Preparation and administration of DEC

Infected animals and normal animals were treated with DEC at 150 mg citrate/kg through i.p. for five consecutive days.

2.4 Serum isolation

Blood from animals was collected from retro-orbital plexus or heart and was allowed to clot (37°C for 1 hr.). Serum was isolated, centrifuged at 2,000 rpm for 10 min. in a refrigerated centrifuge and kept at –20°C, for immunoblot analysis.

2.5 Preparation of soluble somatic extract of adult worm

The antigen used was soluble somatic extract of *Brugia malayi* adult worms. It was prepared according to procedure adopted by Hamilton et al. (1981) with some modifications. Briefly, the adult worms collected from peritoneal cavity of experimentally infected Jirds were homogenised with 0.01 M phosphate buffered saline (PBS), pH 7.2, in a potter Elvejhn tissue grinder (A. Thomas Scientific, at 4°C. The homogenate was sonicated on ice at 10 Kc/ sec for 10 min with 60-sec intervals after every stroke of 60-sec. After sonication, the extract was centrifuged at 100,000 g for 1hr and filtered through a 0.2 μM syringe filter (Millipore, Bangalore, India). The protein content was measured by the method of Lowry et al. (1951) and the antigen was stored at –20°C until used.

2.6 Fractionation of B. malayi adult worm extracts by column chromatography

Fractionation of extracts of adult worms was performed according to the method of Egwang et al. (1988) using Sephadex G-200 column chromatography with some modifications. Sephadex G-200 was allowed to swell in 0.01M PBS pH 7.2 for overnight at 4°C. Elution volume was calibrated by adding Blue Dextran (2 mg/ml) prepared in 0.01M PBS. Flow rate was adjusted to 3 ml/15 minutes. The measured protein (16 mg/2 ml volume) of the extract was loaded and started collecting the fractions. Absorbance of the fractions collected was immediately read at 280 nm in UV-spectrophotometer. Three major peaks obtained were named as BmA-I (Peak I), BmA-II (Peak II) and BmA-III (Peak III) of BmA. The protein in peaks was estimated according to the method of Lowry et al. (1951). These fractions were kept at –20°C until further use [Figure 1(a) to (b)].

2.7 SDS-PAGE profile of BmA-crude extracts and purified fractions

SDS PAGE resolved components of BmA-I are shown in Figure 1. Loading concentration of protein used was 1.5 and 0.5 μg/ml for of BmA-I and A-II respectively. BmA-I, and A-II, resolved in to 16 and 12 bands, which ranged between 26.5–>180 kDa, 26.5–84 kDa, respectively [Figure 1(c)].

2.8 Western blotting of SDS-PAGE resolved components of BmA-fraction I

B. malayi adult worms were resolved by SDS PAGE in 12.5% continuous gel with 3.7% stacking gel (Laemmli, 1970). Molecular weight standards used were those supplied in the kit by Sigma Chemical Co. and molecular weight of the molecules were determined (Shapiro et al., 1967) Western blot transfer of antigen bands onto nitrocellulose paper (NCP; 0.22 μm; Millipore, India) was performed as described by Towbin et al. (1979). Blots in the NCP strips were visualised by immunoperoxidase staining (Tsang et al., 1983). Briefly, the blots were treated successively with 3% gelatine in 10 mM Tris-buffered saline (TBS; pH 7.4), TBS containing 0.01% Tween-20 (TBS-T), sera diluted (1: 50) in TBS-T containing 3% gelatine (90 min at 37°C), TBS-T wash and, finally, with peroxidase conjugate of antimouse IgG (optimum concentration; sigma Chem. Co.). Bound peroxidase was visualised by incubating in chromogenic substrate medium consisting of 0.03% 3; 3-diaminobenzidine-4-HCl and 0.0003% H2O2 in TBS. Control blots were incubated as above but omitted infected animal serum. The gel was subjected

to silver staining (Wray et al., 1981) for the visualisation of corresponding bands. Briefly, after completing the Western blot transfer the gel was immersed in 50% methanol and 10% acetic acid solution. After one hour the gel was immersed first in 5% methanol followed by immersion in staining solution (AgNO3 0.58M, 0.9M NaOH, NH3 8 M) for 15 minutes. The gel was washed three to four times with TDW and dipped in developer until the bands were visible (Figures 2 to 3).

3 Results

3.1 Resolution of proteins of crude extracts of B. malayi adult worms (BmA), microfilariae (BmMF) and their fractions on SDS-PAGE

SDS PAGE resolved components of BmA, BmMf and fractions of BmA are shown in Figure 1. Loading concentration of protein used was 100 µg/ml for BmA, 1.5 μg/ml for of BmA-I, 0.5 μg/ml of BmA-II. Commercially available standard molecular weight marker (Sigma Chemical Co., St. Louis, MO) was used for determination of approximate molecular weight of the samples. The gels were stained with silver stain for localisation of bands. BmA and BmMf resolved into 40 (12–180 kDa) and 25 bands (1.5–>200 kDa) bands, respectively. BmA-I (Peak I), A-II (Peak II), and BmMf-I resolved into 16, 12 and 7 bands, which ranged between 26.5–>180, 26.5–84 and 6.5/17.5–> 97.4 kDa, respectively.

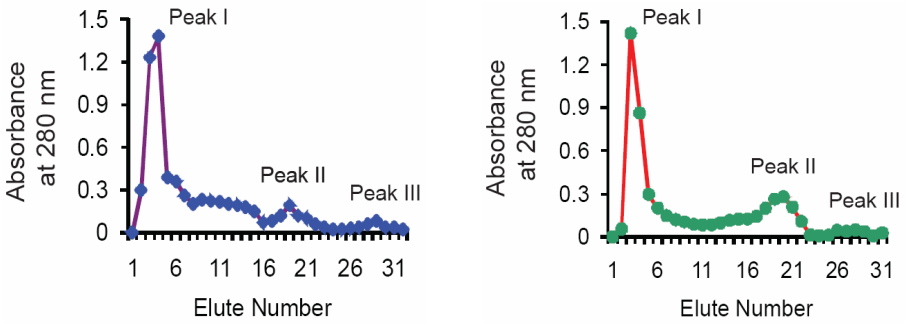
3.2 Effect of DEC treatment followed by re-infection with B. malayi: Immunoblot reactivity profile of BmA, BmMf, BmA-I and BmA-II with sera of M. coucha

The immuno-reactivity profile of SDS-PAGE resolved components of parasite extracts with sera of DEC treated and subsequently re-infected animals is illustrated in Figures 2 to 3 and Table 2. Normal animal sera did not react with any of the resolved components of BmA except 29 kDa. Sera of Inf animals reacted with a wide range of molecules, 14.2–>200 kDa of BmA. Inf-T animals reacted with a lesser number of bands (62 kDa with sera of one-month after treatment; 22, 160, 170 kDa with sera after two-months of treatment). However, range of reactive bands increased with sera of infected treated and re-infected group (InfT-R) animals (22, 32, 38, 44, 45, 70, 85, 97.4, 100, 200 kDa). Out of these, 45 kDa was strongly reactive. Sera of Inf-R (infected re-infected group) animals recognised lesser number bands (22, 28, 58, 180, 190 kDa) as compared to sera of InfT-R. N-T sera (one-month post-treated) reacted with two molecules (88, 100 kDa). However sera of second-month post-treated normal animals recognised more number of bands (24, 29, 56, 62 kDa). The reactive bands recognised by sera of NT-I animals ranged from 24 to > 200 kDa (24, 44, 56, 62, 64, 68, 70, 88, 97.4, 100, > 200 kDa). Sera of age matched N-I animals reacted with low to high molecular weight bands (6.8, 22.5, 29, 58, 66, 68, 70, 88, 97.4, and >200kDa).

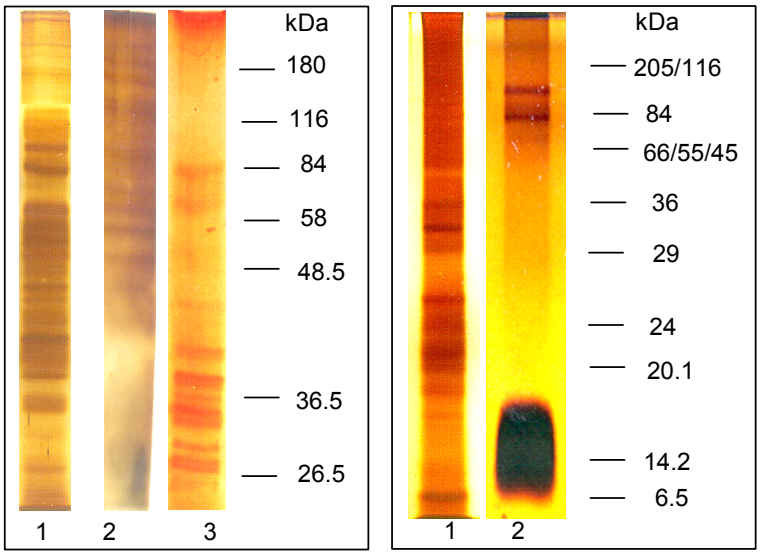
Inf sera reacted with 14.2, 29–32, 52, 60, 62, 66, 73, 97.4 kDa of BmA-I. Inf-T sera reacted with four molecules (62, 66, 73, 97.4 kDa). However, InfT-R sera showed reactivity with a large number of bands (29, 32, 45, 52, 58, 60, 62, 64, 73, 79, 97.4 kDa). In contrast, Inf-R sera identified 54, 68, 73, 97.4 kDa antigen molecules. Sera of normal

(N) identified 97.4 kDa, while N-T sera reacted with 54, 56, 62, 66, 68 kDa antigen bands. NT-I sera identified molecules from 30–97.4 kDa (30, 48, 54, 56, 62, 66, 68, 70, 73, 97.4 kDa). Age and sex matched N-I animal sera reacted almost same antigen bands. However, in addition to these bands the sera reacted moderately with 29, and 41.5–46.3 kDa antigen bands.

Figure 1 Fractionation of, (a) *Brugia malayi* adult (BmA) (b) microfilaria (BmMf) crude extract by sephadex G200 column chromatography (c) silver stained SDS-PAGE of BmA, BmMf and their fractions (see online version for colours)



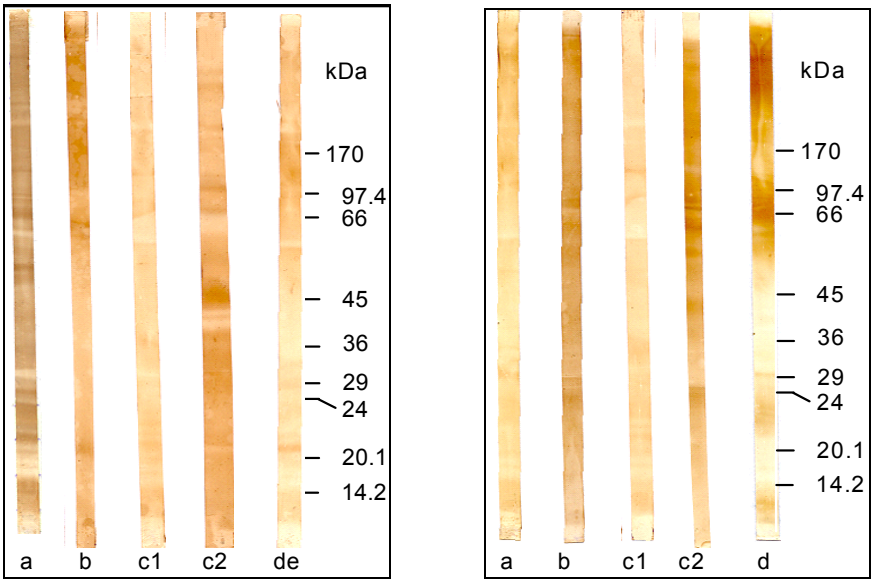
(a) (b)



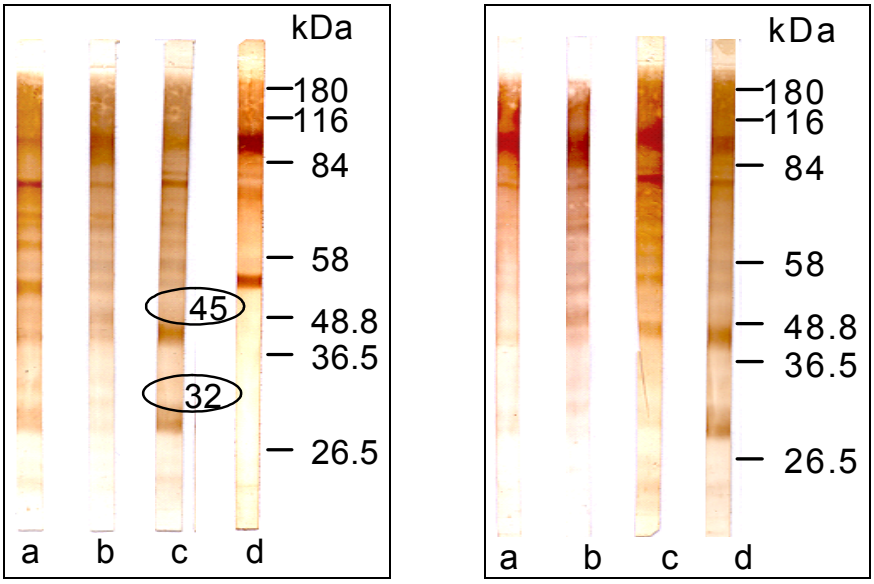
(c)

Lanes		*Lanes*	
1	BmA crude	1	BmMf crude
2	BmA-I	2	BmMf-I
3	BmA-II		

Figure 2 Reactivity of (a) BmA crude extract, and (b) BmF-I with DEC treated and re-infected *M. coucha* sera (see online version for colours)

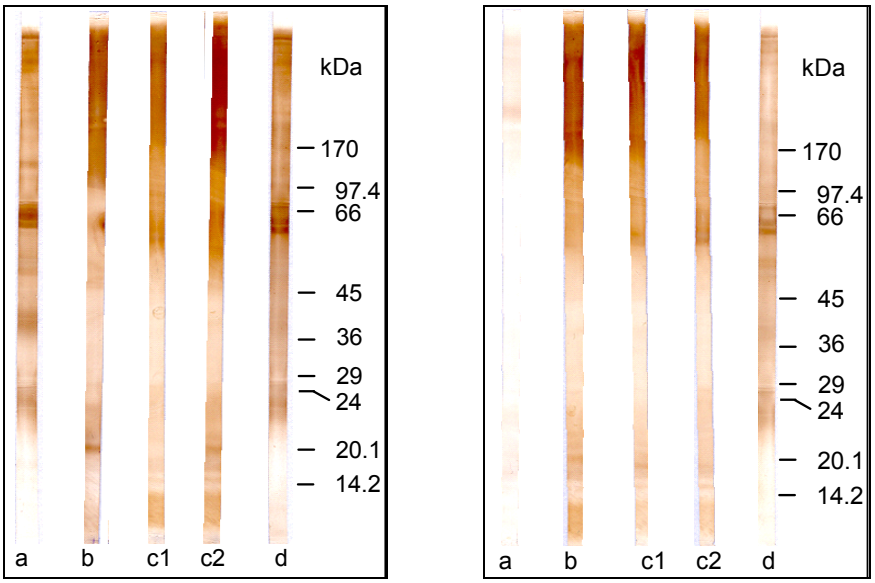


(a)

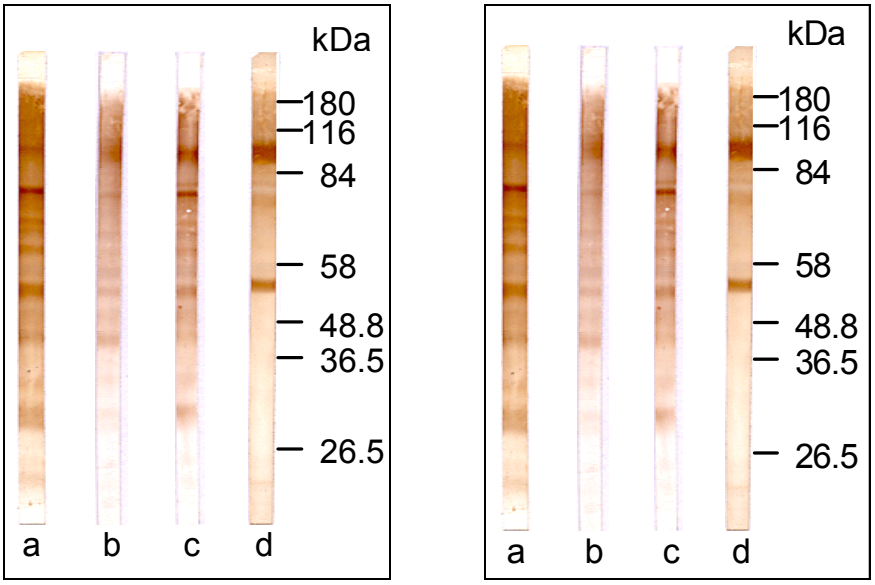


(b)

Figure 3 Immunoreactivity of (a) BmMf crude extract, and (b) Bm-FI with DEC treated and re-infected *M. coucha* sera (see online version for colours)



(a)



(b)

Lanes		*Lanes*	
a	Infected	a	Normal
b	Infected treated (1st month)	b	Normal treated (1st month)
c1	Infected treated (2nd month)	c1	Normal treated (2nd month)
c2	Infected treated re-infected	c2	Normal treated infected
d	Infected re-infected	d	Normal infected

Table 2 Immunoblot reactivity profiles of *B. malayi* antigens by sera of *M. coucha* treated with antifilarials and subsequently reexposed to L_3 of homologous infection

Group	*BmA*		*BmA-I*		*BmA-II*	
	Mol. wt (kDa)	*Reactivity*	*Mol. Wt (kDa)*	*Reactivity*	*Mol. wt (kDa)*	*Reactivity*
Inf	> 200, 170, 160, 97.4, 70, 66, 48, 29, 24, 22.5, 22, 14.2	++	97.4, 73, 66, 62, 52	++	38, 8.5	++
	62, 50, 46, 35, 34	+	60, 32–29, 14.2	+	36, 35, 24, 22	+
Inf-DEC	62	++	97.4	++	38, 30	++
	170, 160, 22	+	73, 66, 62	+	**28**	**+**
Inf-DEC-R	**45**	**+++**	**45, 32**	**+++**	32, 28	++
	100	++	97.4, 29	++	-	-
	200.97.4,85, 70, **44**, 38, 32, 22	+	79, 73, 64, 62, 60, 58, 52, 32	+	28, 17.5	+
Inf-R	**28**, **22**	**+++**	97.4, 54	++	**28**	**+++**
	190, 180, 58	+	73, 68	+	38, 30	++
					36.5, 8.5	+
N	29	+	97.4	+	38	+
N-DEC	100, 88, *24, 29, 56, 62*	+	68, 66, 62, 56, 54	+	38	+
N-DEC-Inf	44, 24	++	54	++	38, 30, 28	++
	> 200, 100, 97.4, 88, 70, 68, 66, 64, 62, 56	+	97.4, 73, 70, 68, 66, 62, 56, 54, 48, 30	+	-	-
N-Inf	22.5, 6.8	+	41.5–46.3, 29	++	38, 32	++
	> 200, 97.4, 88, 70, 68, 66, 58, 29	+	97.4, 79, 73, 68, 66, 62, 58, 54	+	-	-

Notes: +++ = strongly reactive; ++ = moderately reactive; + = weakly reactive. Number underlined = with 1st month treated sera; number italics = with 2nd month treated sera.

Molecules of 38 kDa and below of BmA-II was reactive with different categories of sera. Lesser number bands were reactive with treated sera. InfT-R sera reacted weakly with 17.5, 28 kDa and moderately with 32 kDa molecules. While Inf-R sera reacted strongly with 28 kDa, the moderate reactivity was observed with 30, 38 kDa molecules and 8.5 and 36.5 kDa antigen bands reacted weakly. 38 kDa molecule was found to be commonly reactive with all the sera of animals belonging to the study groups.

Infected sera recognised lesser number of bands (23, 38, 40.5, 54, 56, 62, 64, 66, 70, 160, > 200 kDa) of BmMf as compared to BmA. One-month post-treated sera moderately reacted with 20.1, 62, > 170 kDa antigen bands. However, more reactive bands were identified with sera of second-month post-treated sera (62, 64, 66, 97.4, > 170 kDa). InfT-R sera reacted with 20.1, 22, 62, 64, 66, 97.4 and > 170 kDa of BmMf. Inf-R sera identified bands (moderately reactive: 23.5, 62–66, 97.4; and weakly reactive > 170 kDa). Sera of normal animal showed reactivity with 52 and 66 kDa molecules. N-T sera reacted with high molecular weight bands (> 170 kDa). However, NT-I sera reacted with 25, 62, 64, 97.4 and 100 kDa in addition to > 170 kDa antigen bands. Sera of age and sex matched N-I animals reacted with 23, 25, 40, 56, 62, 64, 88 kDa and > 200 kDa antigen molecules.

These findings showed that filarial infected sera moderately recognises vast array of adult stage antigens (Table 2) to crude extracts as well as purified fractionation.

While their DEC treated counterparts reacted with a lesser number of bands (62 kDa with sera of one-month after treatment; 22, 160, 170 kDa with sera after two months of treatment). Further analysis revealed that, sera from DEC treated infected and re-infected animals reacted strongly with 45 kDa (BmA), 45, and 32 kDa (BmA-FI) filarial antigens (Table 2).

Control mice sera with reinfection in already infected mouse strongly reacted with 28 and 22 kDa (BmA) out of these 28kDa was present in BmA-FII.

Further, we found that filarial infected mouse sera reacted with 14.2, 29–32, 52, 60, 62, 66, 73, 97.4 kDa of BmA-I while filarial infected and DEC treated mouse sera reacted with 62, 66, 73, 97.4 kDa. However, filarial infected and pre-DEC treated era showed reactivity with a large number of bands (29, 32, 45, 52, 58, 60, 62, 64, 73, 79, 97.4 kDa) in BmA-I fractions. In contrast, filarial infected and subsequent re-infected mouse sera identified 54, 68, 73, 97.4-kDa antigen molecules. Sera of normal (N) identified 97.4 kDa, while N-T sera reacted with 54, 56, 62, 66, 68-kDa antigen bands. NT-I sera identified molecules from 30–97.4 kDa (30, 48, 54, 56, 62, 66, 68, 70, 73, 97.4 kDa). Age and sex matched N-I animal sera reacted almost same antigen bands. However, in addition to these bands the sera reacted moderately with 29, and 41.5–46.3 kDa antigen bands.

4 Discussion

In humans it has been found that many treated patients appeared to have steady state of antibody levels (Laemmli, 1970). In Western blots studies of the recognition of specific antigens, DEC treatment led to recognition of new antigen specificities, defined by the appearance of new bands of 45 and 32 kDa. However, the significance of observation is not clear. The lack of appearance of new bands in some of the infected animals following treatment suggests that treatment per se does not necessarily lead to the expression of anti-microfilarial immunity. Consequently, direct evidence of increased cellular

responsiveness to filarial antigens in such treated animals might be likely to redevelop patent infection. Lammie et al. (1988a, 1988b) demonstrated 66 and 13 kDa molecules to be reactive with sera of some of the Mf positive patient on western blots and related to be dependent on microfilaraemia. However, they have also reported that in a large proportion of infected patients (but apparently have filarial antigen), specific responses were independent of microfilaraemia. However, Fletcher et al. (1986) have shown 22 and 18.5 kDa antigens present in adult and Mf of *B. pahangi*, were reactive with the sera of cats before the elimination of microfilaraemia, which became amicrofilaraemic thereafter.

In current study, 28 kDa and 22 kDa antigens were found to be strongly reactive in infected and L_3 reexposed animals. Balloul et al. (1987) reported that 28 kDa and 22 kDa antigens were protective in mice and rodents against schistosoma. Contrary to this 45 kDa antigen band, which was lighted up in, infected DEC treated and L_3 reexposed has been reported for use in monoclonal antibody production (Sutanto et al., 1985). Nevertheless, in our study we have found 29 kDa molecules to be weakly reactive with sera of all the animals. Nevertheless, parasite surface has received special attention for identifying prominent and potentially protective antigens. 29 kDa molecular weight protein of *B. malayi* has been found to bear epitopes cross-reactive between different stages and species of filariae (Kaushal et al., 1982; Maizels et al., 1983a, 1983b, 1983c). This antigen has close analogues in 29 to 30 kDa proteins from the adult worms of other lymphatic filariids, *B. pahangi*, *B. timori* and *W. bancrofti*. Interestingly, in the present study sera of normal and infected DEC treated animals also showed moderate reactivity with 29 kDa molecules. Possibly, the 29 kDa molecules were not only having filarial cross-reactive epitopes but might have epitope(s) directed towards DEC complexed host antigens, which were not identified with the sera of completely normal animals. 29 kDa molecules have been used for monoclonal antibody production (Maizels et al., 1985; Sutanto et al., 1985). Sera of infected DEC treated animals reacted strongly with 45 kDa (near to 43 kDa). Freedman et al. (1989) using *B. malayi* L_3 demonstrated reactivity of 43 kDa with endemic normal and suggested that this molecule may have prophylactic potential. As expected filarial infected sera recognised fewer numbers of bands with BmMf (microfilaria) extract as compared to BmA (adult) and none of the antigens were strongly reactive with any of the sera. It was suggested that the different pattern observed in the study and findings of Freedman et al. (1989) could have been due to differences in the strains of *W. bancrofti*. Hence, we may expect that 45 kDa molecule which was found to be strongly reactive may have protective potential. In conclusion, this basic strategy must be useful in future for Identification and expression of this entire antigen during reinfection will be helpful in vaccine development to prevent this disease from infestation.

Acknowledgements

Senior Research Fellowship (SRF) to M.A.K. from Council of Scientific and Industrial Research, India supported this work.

References

Balloul, J.M., Grzych, J.M., Pierce, R.J. and Capron, A. (1987) 'A purified 28,000 dalton protein from schistosoma mansoni adult worms protects rats and mice against experimental schistosomiasis', *J Immunol*, Vol. 138, No. 10, pp.3448–3453.

Campbell, W.C., Fisher, M.H., Stapley, E.O., Albers-Schonberg, G. and Jacob, T.A. (1983) 'Ivermectin: a potent new antiparasitic agent', *Science*, Vol. 221, No. 4613, pp.823–828.

Cheirmaraj, K., and Harinath, B.C. (1991) 'Humoral immune response to infective larval antigen in Brugia malayi infected Mastomys natalensis', *Acta Trop*, Vol. 48, No. 4, pp.305–312.

Dixit, S., Gaur, R.L., Khan, M.A., Saxena, J.K., Murthy, P.S. and Murthy, P.K. (2004) 'Inflammatory antigens of Brugia malayi and their effect on rodent host Mastomys coucha', *Parasite Immunol*, Vol. 26, No .10, pp.397–407.

Egwang, T.G., Akue, J.P. Dupont, A. and Pinder, M. (1988) 'The identification and partial characterization of an immunodominant 29–31 kilodalton surface antigen expressed by adult worms of the human filaria Loa loa', *Mol Biochem Parasitol*, Vol. 31, No. 3, pp.263–272.

Fletcher, C., Birch, D.W., Samad, R. and Denham, D.A. (1986) 'Brugia pahangi infections in cats: antibody responses which correlate with the change from the microfilaraemic to the amicrofilaraemic state', *Parasite Immunol*, Vol. 8, No. 4, pp.345–357.

Freedman, D.O., Nutman, T.B. and Ottesen, E.A. (1989) 'Protective immunity in bancroftian filariasis. Selective recognition of a 43-kD larval stage antigen by infection-free individuals in an endemic area', *J Clin Invest*, Vol. 83, No. 1, pp.14–22.

Hamilton, R.G., Hussain, R., Ottesen, E.A. and Adkinson, N.F. Jr. (1981) 'The quantitation of parasite-specific human IgG and IgE in sera: evaluation of solid-phase RIA and ELISA methodology', *J Immunol Methods*, Vol. 44, No. 1, pp.101–114.

Hammerberg, B., Nogami, S., Nakagaki, K. Hayashi, Y. and Tanaka, H. (1989) 'Protective immunity against Brugia malayi infective larvae in mice. II. Induction by a T cell-dependent antigen isolated by monoclonal antibody affinity chromatography and SDS-PAGE', *J. Immunol*, Vol. 143, No. 12, pp.4201–4207.

Hayashi, Y., Nakagaki, K., Nogami, S., Hammerberg, B. and Tanaka, H. (1989) 'Protective immunity against Brugia malayi infective larvae in mice. I. Parameters of active and passive immunity', *Am J Trop Med Hyg*, Vol. 41, No. 6, pp.650–656.

Kaushal, N.A., Hussain, R., Nash, T.E. and Ottesen, E.A. (1982) 'Identification and characterization of excretory-secretory products of Brugia malayi, adult filarial parasites', *J Immunol*, Vol. 129, No. 1, pp.338–343.

Kazura, J.W., Cicirello, H. and McCall, J.W. (1986) 'Induction of protection against Brugia malayi infection in jirds by microfilarial antigens', *J Immunol*, Vol. 136, No .4, pp.1422–1426.

Khan, M.A., Gaur, R.L., Dixit, S., Saleemuddin, M. and Murthy, P.K. (2004) 'Responses of Mastomys coucha, that have been infected with Brugia malayi and treated with diethylcarbamazine or albendazole, to re-exposure to infection', *Ann Trop Med Parasitol*, Vol. 98, No. 8, pp.817–830.

Laemmli, U.K. (1970) 'Cleavage of structural proteins during the assembly of the head of bacteriophage T4', *Nature*, Vol. 227, No. 5259, pp.680–685.

Lal, R.B. and Ottesen, E.A. (1988) 'Characterization of stage-specific antigens of infective larvae of the filarial parasite Brugia malayi', *J Immunol*, Vol. 140, No. 6, pp.2032–2038.

Lammie, P.J., Eberhard, M.L., Leiva, L.E., Lowrie, R.C. Jr. and Katz, S.P. (1988a) 'The effect of diethylcarbamazine treatment of Bancroftian filariasis on the immunological reactivity of microfilaraemic individuals', *Trans R Soc Trop Med Hyg*, Vol. 82, No. 5, pp.726–729.

Lammie, P.J., Leiva, L.E., Ruff, A.J., Eberhard, M.L., Lowrie, R.C. Jr. and Katz, S.P. (1988b) 'Bancroftian filariasis in Haiti: preliminary characterization of the immunological responsiveness of microfilaremic individuals', *Am J Trop Med Hyg*, Vol. 38, No. 1, pp.125–129.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) 'Protein measurement with the Folin phenol reagent', *J Biol Chem*, Vol. 193, No. 1, pp.265–275.

Maizels, R.M., Meghji, M. and Ogilvie, B.M. (1983a) 'Restricted sets of parasite antigens from the surface of different stages and sexes of the nematode parasite Nippostrongylus brasiliensis', *Immunology*, Vol. 48, No. 1, pp.107–121.

Maizels, R.M., Partono, F., Oemijati, S., Denham, D.A. and Ogilvie, B.M. (1983b) 'Cross-reactive surface antigens on three stages of Brugia malayi, B. pahangi and B. timori', *Parasitology*, Vol. 87, No. 2, pp.249–263.

Maizels, R.M., Partono, F., Oemijati, S. and Ogilvie, B.M. (1983c) 'Antigenic analysis of Brugia timori, a filarial nematode of man: initial characterization by surface radioiodination and evaluation of diagnostic potential', *Clin Exp Immunol*, Vol. 51, No. 2, pp.269–277.

Maizels, R.M., Sutanto, I., Gomez-Priego, A., Lillywhite, J. and Denham, D.A. (1985) 'Specificity of surface molecules of adult Brugia parasites: cross-reactivity with antibody from Wuchereria, Onchocerca and other human filarial infections', *Trop Med Parasitol*, Vol. 36, No. 4, pp.233–237.

Michael, E., Bundy, D.A. and Grenfell, B.T. (1996a) 'Re-assessing the global prevalence and distribution of lymphatic filariasis', *Parasitology*, Vol. 112, No. 4, pp.409–428.

Michael, E., Meyrowitsch, D.W. and Simonsen, P.E. (1996b) 'Cost and cost effectiveness of mass diethylcarbamazine chemotherapy for the control of bancroftian filariasis: comparison of four strategies in Tanzania', *Trop Med Int Health*, Vol. 1, No. 4, pp.414–426.

Nanduri, J. and Kazura, J.W. (1989) 'Clinical and laboratory aspects of filariasis', *Clin Microbiol Rev*, Vol. 2, No. 1, pp.39–50.

Parab, P.B., Rajasekariah, G.R., Carvalho, P.A. and Subrahmanyam, D. (1990) 'Differential recognition of Brugia malayi antigens by bancroftian filariasis sera', *Indian J Med Res*, Vol. 91, Nos. 5–6, pp.138–143.

Selkirk, M.E., D.A. Denham, F. Partono, I. Sutanto, and R.M. Maizels (1986) 'Molecular characterization of antigens of lymphatic filarial parasites', *Parasitology*, Vol. 92, No. 1, pp.S15–S38.

Shapiro, A.L., Vinuela, E. and Maizel, J.V. Jr. (1967) 'Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels', *Biochem Biophys Res Commun*, Vol. 28, No. 5, pp.815–820.

Sutanto, I., Maizels, R.M. and Denham, D.A. (1985) 'Surface antigens of a filarial nematode: analysis of adult Brugia pahangi surface components and their use in monoclonal antibody production', *Mol Biochem Parasitol*, Vol. 15, No. 2, pp.203–214.

Towbin, H., Staehelin, T. and Gordon, J. (1979) 'Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications', *Proc Natl Acad Sci U S A*, Vol. 76, No. 9, pp.4350–4354.

Tsang, V.C., Peralta, J.M. and Simons, A.R. (1983) 'Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis', *Methods Enzymol*, Vol. 92, No. 1, pp.377–391.

Wray, W., Boulikas, T., Wray, V.P. and Hancock, R. (1981) 'Silver staining of proteins in polyacrylamide gels', *Anal Biochem*, Vol. 118, No. 1, pp.197–203.