ANTIBODIES TO SOMATIC L_3 ANTIGEN NOT PROTECTIVE AGAINST *BRUGIA MALAYI* INFECTION

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Abstract. Western blot analysis of infective larvae (L_3) antigen of *Brugia malayi* were performed on 200 sera from six groups of individuals: 36 samples from *B. malayi* microfilaremic individuals; 10 samples from individuals with elephantiasis; 50 and 20 samples from amicrofilaremic individuals in a *B. malayi* endemic area with no anti-filarial IgG4 antibodies (towards microfilaria and adult worm antigens) and samples with high titres of the anti-filarial IgG4 antibodies respectively; 50 samples from non-endemic normals and 34 samples from geohelminth-infected individuals. After protein transfer, PVDF membrane strips were successively incubated with blocking solution, human sera, monoclonal anti-human IgG4 antibody-HRP and developed with luminol chemiluminescence substrate. 28/36 (78%), 1/10 (10%) and 16/20(80%) of sera from individuals with microfilariae, elephantiasis and amicrofilaremic individuals with high titers of anti-filarial IgG4 antibodies respectively recognized L_3 antigenic epitopes; the dominant and consistent antigenic bands were of ~ MW 43 kDa, 14 kDa, 15 kDa and 59 kDa. The rest of the sera were unreactive. This study showed that microfilaremics may or may not mount a notable antibody response to somatic L_3 antigens, thus lending evidence that antibody response to this antigen is not protective against establishment of *Brugia malayi* infection.

INTRODUCTION

Lymphatic filariasis caused by *Brugia malayi* and *Wuchereria bancrofti* infect about 120 million people worldwide (Ottesen and Ramachandran, 1995). Third stage infective larvae (L_3) of the parasite enters the body through the bite of infected mosquitos. Identification of L_3 antigens that are involved in the host antibody response towards the invading parasite is important in the understanding of infection and immunity in lymphatic filariasis (WHO, 1992). This study was conducted to investigate *B. malayi* L_3 antigenic components that are recognized by endemic area individuals, in order to identify potential protective epitopes.

MATERIALS AND METHODS

Antigen preparation

Infected mosquitos were mass-dissected two weeks post-infection and *B. malayi* L_3 were collected, washed and frozen at -70°C. A final total of about 23,000 L3 were suspended in phosphate buffered saline (pH 7.2) containing protease inhibitors (Boehringer Mannheim, Germany). The larvae were homogenized, sonicated, freeze-thawed and concentrated. The protein content of the L_3 antigen was estimated to be 1,200 µg/ml by the Bio Rad assay.

Study population

Sera from a brugian filariasis endemic area in a state in North Eastern Malaysia was used for this study. Informed consent was obtained from all subjects before the blood sampling. A sandwich ELISA using soluble filarial worm antigen to detect anti-filarial IgG4 antibodies in these sera had previously been performed and the cut-off optical density value for detection of active infection was determined to be 0.420 (Rahmah *et al*, 1998). A total of 200 serum samples were divided into the following groups: 1) Individuals with circulating microfilariae (n=36). 2) Amicrofilaremic individuals with elephantiasis (n=10). 3) Amicrofilaremic individuals from the endemic area who demonstrated no anti-filarial IgG4 antibodies towards microfilaria and adult worm antigen *ie* optical densities obtained were zero or less (n=50). 4) Amicrofilaremic individuals in the endemic areas with high titers of the anti-filarial IgG4 antibodies (OD above 1.200; n=20). 5) Healthy city volunteers *ie* non-endemic normals (n=50). 6) Soil-

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transmitted heminth infected individuals living in non-endemic areas (n=34).

Western blotting

Phast Electrophoresis System (Pharmacia, Sweden) was employed for running of precasted 10-15% gradient SDS-PAGE gels and for electroblotting. The soluble L3 antigen was mixed (2:1) with 2X sample buffer and 4 µl antigen per well was electrophoresed at 250V, 10.0 mA, 3.0W, 15°C, 65 Vh. The protein bands were transferred onto a PVDF membrane at 20V, 25 mA, 15°C, 5 Vh, 30 minutes; the membrane strips were then successively incubated with 1% blocking solution at room temperature (rt) for 30 minutes, human sera (1:100 dilution for 3 hours, rt) followed by mouse monoclonal anti-human IgG4 antibody conjugated to horseradish peroxidase (CLB, Netherlands) at 1:2,000 dilution for 30 minutes, rt. In between each incubation steps, the strips were washed twice using Tris buffered saline containing 0.05% Tween 20 (10 minutes/wash) followed by 2 times washing (10 minutes/wash) in 0.5 % blocking solution. Luminol chemiluminescence detection (Boehringer Mannheim) was then used to develop the blots. The approximate molecular weights (~ MW) of the antigenic bands were determined using a digital image analyzer (IS-1000 Alpha Innotech Corporation, USA).

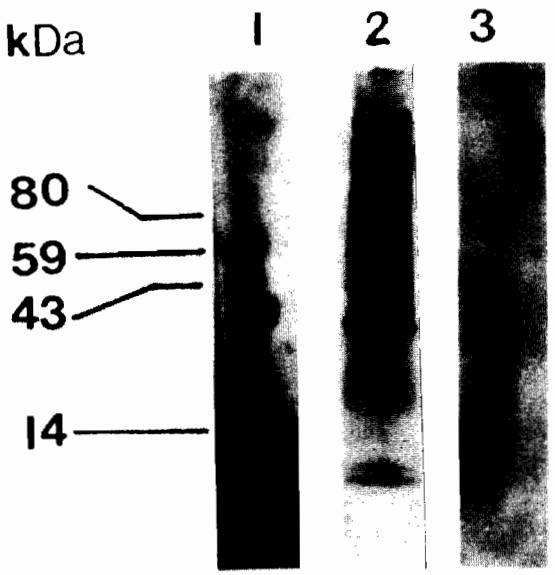

Fig 1–Western blots of *Brugia malayi* infective larvae antigen using representative sera from the two groups of microfilaremic individuals.
1, 2: positive blots using sera from two microfilaremic individuals.
3: negative blot using serum from a microfilaremic individual.

RESULTS

Representative sera from all groups of individuals were probed with IgG1, IgG2, IgG3 and IgG4 subclasses. The first three IgG subclasses were found to be either poorly reactive or reactive to sera of both infected and non-endemic normals.

The ~ MW of the antigenic epitopes displayed by the IgG4 positive blots were 191 kDa, 143 kDa, 80 kDa, 59 kDa, 43 kDa, 25 kDa, 15 kDa and 14 kDa. Out of these, the most dominant band observed was 43 kDa and the other prominent bands were 14 kDa, 59 kDa and 80 kDa. The most consistently observed bands was the 43 kDa and 14 kDa, followed by the 15 kDa and 59 kDa.

28/36 (78%), 1/10(10%) and 16/20 (80%) of sera from individuals with microfilariae, elephantiasis patients and amicrofilaremic individuals with high titers of anti-filarial IgG4 antibodies respectively demonstrated antigenic epitopes. 8/36 (22%), 9/10 (90%) and 4/20(20%) of the above sera groups respectively were unreactive. Sera from soil-transmitted helminth infected individuals, city dwellers and endemic areas individuals with no anti-filarial IgG4 antibody were not reactive. Fig 1 shows representative blots from microfilaremics who reacted and those that did not react to the L_3 protein blots.

DISCUSSION

Studies of antibody response towards L_3 antigens is important in our understanding of host response to filarial infection and in elucidating epitopes of potential protective value. Protective immunity against primary infection or protection againt repeated infections may occur in individuals living in an endemic area (Maizels and Lawrence, 1991; Day *et al*, 1991). Infective L_3 is thought to be the primary candidate in the search for a filariasis vaccine (Kurniawan-Atmadja *et al*, 1998). Due to the difficulty in obtaining sufficient amounts of infective larvae, to date few Westerm blot studies have been published on analysis of *B. malayi* L_3 antigenic epitopes (Maizels and Lawrence, 1991; Day *et al*, 1991; Kazura *et al*, 1986; Freedman *et al*, 1989).

In the initial part of our study we investigated Western blot reactivities of L_3 soluble antigens when probed with all four IgG isotypes. Anti-human IgG4 was found to be reactive with pooled microfilaremic sera and at the same time exhibited no cross-reactions with non-endemic normals or geohelminth infected individuals; thus this antibody probe was used for the rest of the study. IgG1 was found to be also reactive with sera of some non-endemic normals and geohelminth infected individuals. A previous study (Kurniawan-Atmadja *et al*, 1998) using ELISA showed that antibodies reactive to somatic extracts of infective larvae are equally represented by IgG1 and IgG4. The difference in the technique (*ie* ELISA versus Western blots) and the kinds of sera employed might explain the differing observations.

In the present study we observed the existence of two groups of microfilaremics with regard to antifilarial IgG4 response to L_3 antigens. The majority (78%) are reactive to the L_3 antigens with dominant antigenic epitopes of ~ MW 80 kDa, 43 kDa, 14 kDa, and 59 kDa. It is interesting to observe that 20% of microfilaremics were unreactive to L_3 epitopes although many prominent antigenic bands were observed with all microfilaremic sera in IgG4 Western blots using adult soluble antigens (data not shown). There was no correlation observed between the L_3 Western blot results with age or with microfilaria density. Similar results were also seen in the group of amicrofilaremics with very high levels of antifilarial IgG4 antibodies to adult soluble antigens and thus are most likely to be actively infected (Rahmah *et al*, 1998; Haarbrink *et al*, 1995). However we could not ascertain whether the results may be correlated with duration of infection or the strain of *Brugia malayi* (*ie* subperiodic or periodic). In the group of chronic elephantiasis patients, 90% of the patients were unreactive to the L_3 antigen; this is not unexpected since it has been shown that antibody response to IgG4 is reduced in chronic stages of the disease (Hussain *et al*, 1987).

The 22% microfilaremics who did not appear to mount an IgG4 antibody response to L_3 antigens may had been less exposed to L_3 invasion due to a shift to a better house or the use of physical protections against vectors such as bed nets, mosquito coils or insecticides. It has been previously reported that one needs to be exposed to about 10,000 L_3 in order to induce anti-L_3 surface antibodies (Day *et al*, 1991). The other possibility is that in this group of individuals a much lesser numbers of L_3 exposure (which are not sufficient to elucidate a detectable antibody response to the antigen) may be adequate to establish active infection.

This study demonstrated that the establishment of active infection, as evidenced by the circulating microfilaria, is not correlated with recognition of soluble L_3 epitopes. Therefore the somatic antigen of L_3 did not seem to confer protection against establishment of *B. malayi* infection and may only be an indication of the degree of exposure to L_3 antigens. Our results are consistent with those of Steel *et al* (1996) and Day *et al* (1991). The former demonstrated that protective immunity to lymphatic filariasis is probably mediated by T-cell response and not by antibody responses to the invading parasite. The latter showed that the antigens on the L_3 surface showed age-dependent variation in recognition and thus may contain protective epitopes.

In a study on bancroftian filariasis (11), 43 kDa larval antigen of *B. malayi* was found to be recognised by 7/7 (100%) endemic normals and 1/12 (8%) of asymptomatic microfilaremics. The antibody response to the 43 kDa infective larval epitope was thought to probably induce protection against infection by *W. bancrofti*. However in our study L_3 antigens was not recognized by endemic normals and 43 kDa was recognized by 80% of microfilaremics and thus do not seem to have protective value against *B. malayi* infection. This difference in Western blot results may be attributed to the various differences inherent in these two studies *ie* kinds of infection sera, size and grouping of samples and type of second antibody probe employed.

In conclusion, our results showed that a proportion of microfilaremic individuals did not seem to recognize L_3 somatic antigens. This was also observed in amicrofilaremics with strong evidence of active infection. This study demonstrated that IgG4 antibody response to somatic L_3 does not seem to be protective against establishment of *Brugia malayi* infection. Thus as postulated previously (Maizels and Lawrence, 1991; Day *et al*, 1991), in the attempt to elucidate infective larvae protective epitopes against filarial infection, the focus should probably be on investigations of surface and not somatic antigens of L_3.

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