Longitudinal humoral immune responses of Indian leaf monkey (*Presbytis entellus*) to *Brugia malayi* infection

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SUMMARY

Humoral immune responses of the Indian leaf monkey (*Presbytis entellus*) experimentally infected with *Brugia malayi* and exhibiting disease manifestations were studied. Microfilaraemia, filaria-specific IgG and circulating immune complexes (CICs) were determined in the monkeys at different time-points after inoculation of *B. malayi* 3rd-stage larvae. Sera were analysed for recognition pattern of adult parasite antigen molecules by immunoblotting. More than 60% of the infected monkeys developed episodic or persistent limb oedema with or without fever and with low or no microfilaraemia. While both CIC and filaria specific IgG levels were comparable in animals showing no disease symptoms (asymptomatics) and some animals showing symptoms (symptomatics), IgG levels peaked during pre-patent stage in symptomatics and during latent stage in asymptomatic animals. However, some of the symptomatic animals showed a low level of filaria-specific IgG as compared to asymptomatic and other symptomatic animals. The immunoblot analysis showed non-reactivity of 17 and 55 kDa antigens with sera of symptomatic animals. The results thus suggest that humoral immune responses as measured in the present study do not precede the development of the manifestations. However, 2 non-reactive antigen molecules identified by symptomatic sera need further study to establish their possible involvement, if any, in the development of acute disease manifestations in this model.

Key words: *Brugia malayi*, *Presbytis entellus*, immune response, antigen molecules, filariasis, symptoms.

INTRODUCTION

Filariasis is a chronic disease caused by prolonged exposure to antigenic stimulation of different stages of the parasite and both humoral and cell-mediated immune responses are involved in the pathogenesis of the disease. IgG is the predominant antibody isotype in the humoral response. Filaria-specific antibody levels are low in asymptomatic microfilaraemic patients, while intermediate and elevated levels of all isotypes were reported in acute and chronic patients, respectively (Philipp *et al*. 1989; Piessens, Wadee & Kurniawan, 1987). On the other hand, higher levels of filaria-specific circulating immune complexes (CICs) were found in chronic symptomatic patients compared to microfilaraemics and normal endemic cases (Prasad, Reddy & Harinath, 1983). However, the significance of these findings in relation to the pathogenesis of filariasis is uncertain as no systematic investigation was made on the chronological changes in antibody levels and CICs during different stages of the infection and during the development of disease manifestations. Such studies are not possible in human subjects for the obvious reason that the patients turn up for examination only when manifestations develop. Recently, we reported the successful transmission of the human subperiodic filarial infection, *Brugia malayi*, to the Indian leaf monkey, *Presbytis entellus*, and that the model also exhibits filarial manifestations such as fever and episodic attacks of limb oedema (Murthy, Tyagi & Chatterjee, 1990; Tyagi *et al*. 1996). Encouraged with these findings we have planned to investigate (1) the profile of filaria-specific IgG and circulating ICs in *P. entellus* at different time-points after exposure to infective larvae of *B. malayi* and (2) the reactivity of adult worm antigen molecules with sera of asymptomatic and symptomatic animals in order to know whether the responses in symptomatic animals are different from those of asymptomatic animals.

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MATERIALS AND METHODS

Animals

Twenty-one young wild-caught male Indian leaf monkey (*Presbytis entellus*) of 3–4 kg body weight obtained from local suppliers were used. Before use, the animals were quarantined for 45 days during which time they were dewormed and tested for tuberculosis (Mantoux test and chest X-ray), intestinal helminthiasis and microfilariae. They were found negative in all the tests and were included in the study.

Infection

Infective 3rd-stage larvae (L_3) of *B. malayi* were obtained from laboratory bred female *Aedes aegypti*

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mosquitoes fed on microfilaraemic *Mastomys coucha* as described previously (Murthy *et al*. 1983). Groups of monkeys were given 4–5 (Group I; $n = 10$) or 7–8 (Group II; $n = 8$) inoculations of L_3/animal at different time-periods; each monkey of Gr. I and II received a total of 500–1100 and 700–1400 L_3, respectively, in insect saline within a span of 90 and 145 days. A group of 3 animals (Group III) received inoculations of insect saline only in place of L_3, and served as control. All the inoculations were given subcutaneously in the ankle of 1 leg only. Microfilaraemia (between 21.00 h and 22.00 h) of infected animals was monitored by membrane filtration technique (Murthy *et al*. 1990) on days 60, 75 and 90 post-first L_3 inoculation (p.i.) and thereafter at monthly intervals till the end of the study. Infection was considered latent when microfilaria (mf) count was less than 5 % of the peak mf count.

Clinical examination

All the monkeys were examined daily for changes in body temperature and externally visible inflammation in the limb(s). Rectal temperature was recorded at regular intervals. Quantitative assessment of swelling of limb(s) was made as described by Tyagi *et al*. (1996). Briefly, peripheral (circumference) measurements of both affected and unaffected limbs were taken at different predetermined areas between the knee and the ankle. Any inflammation in the toes was also considered. The swelling ratio was determined between measurements of affected limb and unaffected limb.

Preparation of antigen

Adult worms were recovered from the peritoneal cavity of experimentally infected jirds (*Meriones unguiculatus*). Soluble somatic antigen of the worms was prepared following the method of Tandon *et al*. (1988) for use in enzyme-linked immunosorbent assay (ELISA). For sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE), the antigen was prepared by the method of Fletcher *et al*. (1986). Protein content of the antigen preparations was estimated by the method of Lowry *et al*. (1951) and the antigen was stored in 0·2 ml aliquots at −20 °C till use.

Enzyme-linked immunosorbent assay (ELISA)

Filaria-specific IgG was determined by ELISA broadly following the method of Voller, Bartlett & Bidwell (1976) with some modifications (Murthy *et al*. 1995). Briefly, ELISA plates (Nunc, Denmark) were coated with 1 μg/ml of the antigen in 0·06 M carbonate buffer (pH 9·6); animal sera (primary antibody) were used at 1:500 dilution and rabbit anti-monkey IgG-peroxidase conjugate (Sigma Chemical Co., St Louis, MO) was used at 1:2000 dilution. Absorbance was read at 492 nm in an ELISA reader (Multiscan).

Determination of circulating immune complexes

Circulating immune complexes (CICs) in the sera were determined by ELISA (Matsumara *et al*. 1986) using anti-human Clq (Sigma Chemical Co., St Louis, MO). Microtitre wells (Nunc, Denmark) were sensitized with 100 μl of anti-human Clq (1 μg/ml) in PBS. Sera were used at 1:500 dilution and rabbit anti-monkey IgG-peroxidase conjugate was used at 1:2500 dilution. The procedure for ELISA was the same as that followed for IgG determination.

Western blot analysis

B. malayi adult worm antigen was resolved by SDS–PAGE in a 7·5–12·5 % discontinuous gradient gel with 2·5 % stacking gel (Laemmli, 1970). Molecular weight standards used were those supplied in the kit by Sigma Chemical Co. 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 visualized by immunoperoxidase staining (Tsang, Peralta & Simons, 1983). Briefly, the blots were treated successively with: 3 % gelatin 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 % gelatin (90 min at 37 °C), TBS–T wash and, finally, with peroxidase conjugate of rabbit anti-monkey IgG (1:100; Sigma Chem. Co.). Bound peroxidase was visualized by incubating in the chromogenic substate medium consisting of 0·03 % 3,3-diaminobenzidine-4-HCl and 0·0003 % H_2O_2 in TBS. Control blots were incubated as above but omitting infected animal serum. Two representative control blots are included in Fig. 2B and F.

Data were analysed by Student's *t*-test and difference was considered significant if $P < 0·05$.

RESULTS

IgG and CIC profile during infection

The main thrust of our present study was to investigate humoral immune responses of Indian leaf monkey experimentally infected with *B. malayi* and to determine whether the responses in asymptomatic (Asym) animals (i.e. animals which never showed any clinical signs and symptoms) are different from those of symptomatic (Sym) animals (i.e. which showed symptoms such as limb oedema with or without fever, hydrocoele).

A single inoculation of L_3 does not represent the type of exposure experienced by people living in endemic areas and it is unlikely that 1 exposure may lead to multiple episodic attacks. Therefore, in the

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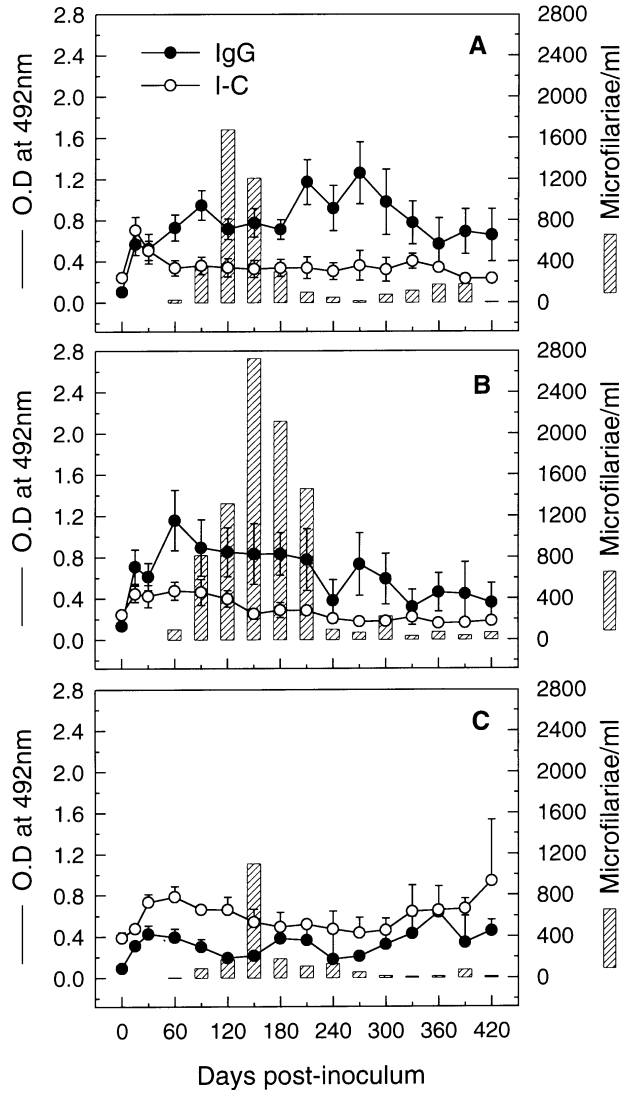

Fig. 1. Microfilaraemia, filaria-specific IgG and immune complexes in Indian leaf monkeys given multiple inoculations of *Brugia malayi* infective larvae (L_3). Vertical bars on data-points indicate S.E. of the mean of number of determinations. (A) Asymptomatic monkeys ($n = 7$) of Gr. II. (B) Symptomatic monkeys ($n = 7$) of Gr. II showing identical pattern of changes in IgG and immune complexes. (C) Symptomatic monkeys ($n = 4$) of Gr. II with a pattern different from other symptomatic monkeys of the same group.

present study, monkeys were exposed to repeated inoculations of L_3 to induce the disease manifestations following the method developed by us (Murthy *et al*. 1990; Tyagi *et al*. 1996).

All the animals of Gr. I and II became microfilaraemic between days 75 and 90 p.i. Microfilaraemia peaked between days 90 and 180 after the first inoculum following which the levels fell sharply and remained low.

A total of 11 out of 18 (61 %) infected animals of Gr. I (4/10) and Gr. II (7/8) developed oedema in 1 hind limb. Of these 11 animals, 9 showed pitting type oedema which appeared in 1 to 6 episodes, 1 animal showed oedema that progressed from pitting to non-pitting type which persisted for more than 300 days, and 1 animal developed both limb oedema and hydrocele (with mf in the scrotal fluid). In the animal that developed hydrocele, the oedema affected both legs alternatively. The severity of episodic oedema was almost identical among the animals but the duration for which each episode persisted varied greatly from 4 to 90 days. Some of these animals showed raised body temperature (1·2 to 1·5 °F above the normal temperature of 102·0 °F), but oedema was not always associated with fever. Animals which developed limb oedema showed low or no microfilaraemia.

CIC and filaria-specific IgG levels in Asym animals of Gr. I and II ($n = 7$) are shown in Fig. 1A. These animals showed a progressive increase in IgG levels reaching a peak between 210 and 270 days p.i.; thereafter the levels fell but remained higher than in uninfected controls (Gr. III) till 330 days p.i. ($P < 0{\cdot}006$–$0{\cdot}02$). These animals showed a sharp increase in CIC levels during the pre-patent periods i.e. on days 15 ($P < 0{\cdot}01$) and 30 ($P < 0{\cdot}02$) p.i.; the levels decreased thereafter and remained low till the end of the study period.

All the 4 Sym animals of Gr. I and 3 Sym animals of Gr. II showed an identical profile of IgG and CIC levels and therefore the data are combined and presented in Fig. 1B. IgG levels increased to maximum during the pre-patent period and remained elevated up to day 240 p.i. ($P < 0{\cdot}0005$–$0{\cdot}02$) but thereafter decreased gradually till the last day of the observation period. CIC level in these animals showed significant increase on days 30 ($P < 0{\cdot}05$) and 60 ($P < 0{\cdot}01$) p.i. followed by gradual decrease from day 120 p.i. onwards till the end of the observation period.

In the remaining 4 Sym animals of Gr. II, IgG levels were significantly low (up to day 270 p.i.; $P < 0{\cdot}05$) as compared to other Sym animals but progressively increased (statistically not significant) up to day 360 p.i. In contrast, CIC levels reached the first peak during the pre-patent period (day 60 p.i.; $P < 0{\cdot}01$) and declined gradually till day 270 p.i. After this period the levels increased progressively to slightly higher than the first peak ($P < 0{\cdot}05$–$0{\cdot}01$). In general, CIC levels were relatively higher than IgG throughout the observation period (Fig. 1C).

Peak microfilaraemia occurred between days 90 and 180 p.i. in all the infected animals; during this period specific IgG levels were apparently low as compared to the levels of other periods.

Antigen recognition pattern during infection

The antigen recognition pattern shown by sera of the infected monkeys at different periods of observation is shown in Fig. 2A–F and the overall recognition pattern in Asym and Sym animals is summarized in Table 1. In general, sera of both Sym and Asym

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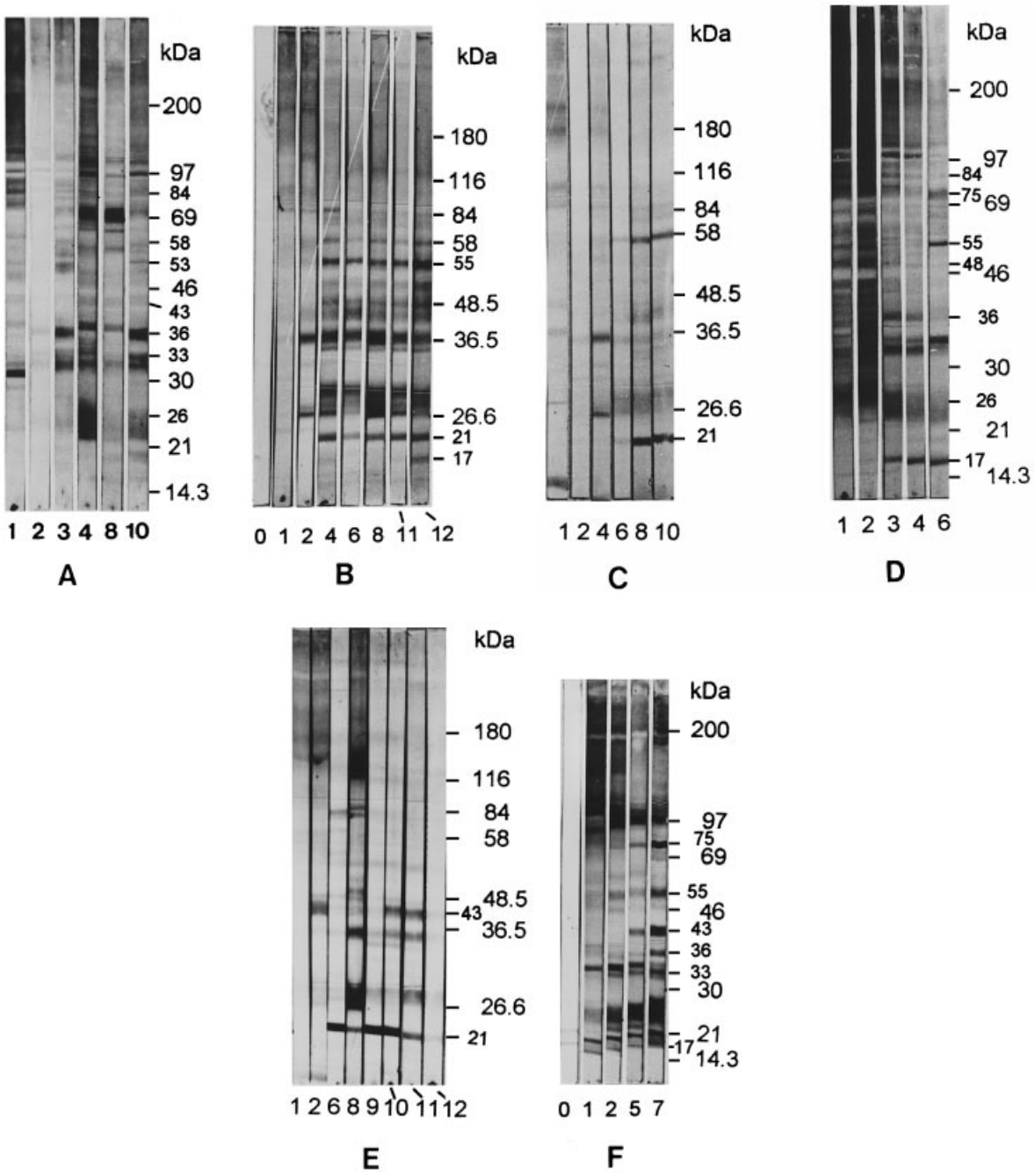

Fig. 2. Immunoperoxidase-stained Western blots of *Brugia malayi* adult somatic antigens reacted with sera of Indian leaf monkey harbouring different stages of *B. malayi* infection. (A) Symptomatic monkey (no. 62) of Gr. I. (B) Asymptomatic monkey (no. 68) of Gr. I. (C) Symptomatic monkey (no. 86) of Gr. II. (D) Asymptomatic monkey (no. 03) of Gr. II. (E) Symptomatic monkey (no. 41) of Gr. II. (F) Symptomatic monkey (no. 44) of Gr. II. Lanes: 0 = 0 day (before infection), 1 = 30 days p.i., 2 = 60 days p.i., 3 = 90 days p.i., 4 = 120 days p.i., 5 = 150 days p.i., 6 = 180 days p.i., 7 = 210 days p.i., 8 = 240 days p.i., 9 = 270 days p.i., 10 = 300 days p.i., 11 = 360 days p.i., 12 = 420 days p.i.

animals recognized almost the same molecular mass antigen proteins. However, sera of Sym monkeys recognized a comparatively lesser number of antigens. With the progression of infection, more and more antigens of molecular mass more than 97 kDa were recognized. However, sera of some Asym and Sym monkeys also recognized higher molecular mass (> 97 kDa) proteins.

Sera of Asym monkey (no. 68) of Gr. I recognized several antigens of molecular mass ranging from 17 to 200 kDa during different stages of infection (Fig. 2B). Some high molecular mass antigens (97, 105, 141, 180 and 200 kDa) were detected on days 30 and 60 p.i.; additionally, day 60 p.i. serum reacted with low molecular mass (58, 55, 36·5 and 26·6 kDa) antigens. By day 120 p.i., there was further addition to the number of low molecular mass antigens recognized (84, 58, 52, 48·5, 43, 36·5, 33, 29, 26 and

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Table 1. Overall pattern of *Brugia malayi* antigen recognition by sera of asymptomatic (Asym) and symptomatic (Sym) Indian leaf monkeys with *B. malayi* infection

(+, Reactive; ±, feebly reactive; −, non-reactive; † non-reactive during symptomatic period only.)

Antigen molecule (kDa)	Asym monkeys		Sym monkeys			
	no. 68	no. 03	no. 62	no. 86	no. 41	no. 44
17	+	+	−	−	−	−†
21	+	±	+	+	+	+
24	−	+	−	−	−	+
26/26·6	+	+	+	+	+	+
29	+	+	+	−	+	−
32	−	−	−	−	−	+
33	+	+	+	−	−	+
34	−	+	−	−	−	−
36/36·5	+	+	+	+	+	+
43	+	+	+	−	+	+
48·5	+	+	+	−	+	−
52	+	+	+	−	−	−
55	+	+	−	−	−	−†
58	+	+	+	+	−	−
75	−	+	−	−	−	+
82	±	+	+	−	+	−
84	+	+	−	±	+	−
97	+	+	+	−	+	+
103/105	+	+	+	+	−	+
141	+	+	+	−	+	+
180	+	+	+	+	−	+
200	+	+	+	+	−	+
> 200	−	+	−	−	−	+

21 kDa) and this pattern persisted till day 360 p.i.; later, on day 420 p.i., a 17 kDa antigen became reactive. In the Sym animal (no. 62) in which limb oedema developed by day 240 p.i. and persisted till day 330 p.i., the antigen recognition pattern was almost identical to that shown by Asym animal.

Sera of 1 of the 3 symptomatic animals (no. 86; Fig. 2C) of Gr. II which showed 5 episodes of limb oedema after day 90 p.i., showed strong reactivity to 200, 180, 105, 58, 36·5, 26·5 and 21 kDa antigen molecules. Antigens of 200, 180, 105 and 84 kDa were recognized on day 30 p.i. but not on day 60 p.i. During peak microfilaraemia (day 120 p.i.), antigens of 36·5 and 26·6 kDa reacted strongly and some low and high mol. wt antigens reacted weakly. Recognition of 36·5 and 26·6 kDa antigens became weak from day 180 to 300 p.i. while that of 58 and 21 kDa antigens strongly reacted on day 240 to 300 p.i. (Fig. 2C). Since the pattern of antigen recognition shown by sera of > 400 days infection was identical to that of 240–300 days infection, these blots were not included in the figures.

Antigen recognition pattern of the sera in the remaining 2 symptomatic animals of Gr. II is shown in Fig. 2E and F. The pattern in Sym animal no. 41 which showed persistent limb oedema from day 96 p.i. was different from that of Sym animal no. 44 which showed episodic oedema. On day 60 p.i., the sera of animal no. 41 showed no reactivity with any of the antigen molecules and only 2 antigen molecules of 141 and 43 kDa were recognized on day 60 p.i. By far the largest no. of molecules (141, 97, 84, 82, 48·5, 43, 36·5, 26–29 and 21 kDa) were recognized on day 240 p.i. while comparatively fewer antigens were detectable on day 180 and 270 p.i. (21 kDa) and day 300 and 360 p.i. (43, 36·5, 26–29 and 21 kDa; Fig. 2E). No reactivity with any of the resolved antigens was obtained with sera of day 420 p.i.

Sera of Sym animal no. 44 of Gr. II, which showed manifestations from days 82–89, 170–182 and 186–202 p.i., reacted with a large number of antigens. During the pre-patent stage (day 30 p.i.), serum recognized antigens of > 200, 200–97, 33, 32, 26 and 21 kDa, and day 60 p.i. serum recognized, additionally, 58, 55 and 46 kDa antigens. Patent-stage serum (day 150 p.i.) recognized 97, 55, 43, 33, 32, 24–26, 21 and 17 kDa antigens while latent stage (day 210 p.i.) serum reacted with a large no. of antigens (from 200 to 105, 97, 75, 55, 43, 36·5, 33, 32, 24–29, 21 and 17 kDa; Fig. 2F).

Sera of the Asym monkey (no. 03) of Gr. II recognized a wide range of antigens (17–> 200 kDa). On days 30 and 60 p.i., a feebly reactive 21 kDa

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antigen and many antigens between 26·6 and > 200 kDa were recognized, while during the progressive rise of microfilaraemia (day 90 p.i.), antigens of 103/105, 36, 33, 26·6, 24 and 17 kDa were strongly reactive. At day 120 p.i., antibodies to 103/105, 33 and 17 kDa antigens were predominant, while during the latent stage (day 210 p.i.), antigens of 75, 55, 34 and 17 kDa were strongly reactive (Fig. 2D).

DISCUSSION

The present study revealed that irrespective of the frequency of L_3 inoculation, the course of microfilaraemia following first larval exposure remained almost the same in both infected groups. Persistent or episodic/repeated limb oedema developed in 40% of Gr. I and 87·5% of Gr. II animals. Interestingly, 1 of the 7 Sym animals of Gr. II also developed scrotal swelling (hydrocoele). This low incidence of scrotal oedema compares well with the known low incidence of this manifestation in malayan filarial patients. As in patients showing acute and chronic manifestations, the Sym monkeys in our present study also showed low or no microfilaraemia. Though peak filaria specific IgG levels in Sym and Asym animals were attained at different times following first L_3 inoculation, the levels remained comparable between the 2 groups throughout the observation period. However, some of the Sym animals of Gr. II had relatively low levels of IgG compared with CIC levels. Qualitative analysis of antibody response to SDS–PAGE resolved adult antigens revealed that a lesser number of antigen molecules reacted with sera of Sym animals as compared to Asym.

While Sym animals of both Gr. I and II revealed a decline in filaria-specific IgG during the latent period, Asym animals showed peak IgG during this period. It is generally accepted that elevated filaria-specific IgG levels during latency could be due to destruction of mf. In the *B. pahangi*–cat model, Ponnudurai *et al*. (1974) demonstrated anti-mf antibody in amicrofilaraemic animals and Ottesen (1984) has proposed that clearance of mf is mediated by antibody. Thus, it was not surprising to note that both Asym and Sym animals of the present study developed low microfilaraemia almost at the same time following first larval inoculation and coincidence of comparable levels of specific IgG in them. We have earlier demonstrated that infected monkeys had calcified worms during latency (Murthy *et al*. 1990). Some of the Sym monkeys of Gr. II showed a comparatively low level of IgG. Possibly, these monkeys might have produced a comparatively high level of IgE that could be correlated to clinical manifestations of the disease but this was not examined due to non-reactivity of the human IgE kit in this model. An association has been suggested between disease manifestation in human filarial infection and presence of IL-5 induced eosinophilia with high levels of filaria-specific and non-specific IgE (King, Ottesen & Nutman, 1990). Studies with chronic limb oedema in dogs exposed to *B. pahangi* suggested that the oedema might be the result of an antigen non-specific inflammatory mechanism through macrophage activation (Schreuer & Hammerberg, 1993).

Two distinct patterns of humoral responses were found in symptomatic animals. During the prepatent period, some Sym animals of Gr. I which developed 1–4 episodes and 1 animal of Gr. II which showed scrotal swelling, had relatively high IgG levels than CIC levels, while animals (Gr. II) which showed 3–6 episodes of oedema or persistent oedema had a reverse profile of IgG and CIC levels, i.e. relatively low IgG and high CIC levels. McGreevy *et al*. (1980) and Simonsen (1985) have demonstrated high antibody levels in clinical cases of filariasis. The reason for lower IgG than CIC levels in some of the Sym animals of Gr. II in which microfilaraemia was also low, is not readily apparent. Immune destruction of parasites and consequent low microfilaraemia and increase in CICs was also demonstrated in onchocerciasis patients (Mackenzie *et al*. 1985). Nevertheless there are conflicting reports on CIC levels in human patients. Filaria-specific CIC levels were found to be higher in chronic symptomatic patients, than in asymptomatic microfilaraemics and normal endemic cases (Prasad *et al*. 1983). On the other hand, Das *et al*. (1987) reported higher CICs in microfilaraemic carriers than in acute and chronic filarial patients.

It has been suggested that CICs may play a role in inflammatory reactions associated with the development of manifestation either directly or by interfering with the host's immune responses (Karavodin & Ash, 1982). However, the results of the present study do not support this hypothesis, as there was no marked difference between the levels of CICs in Sym and Asym animals. Hence, other factors may likely have influenced the clinical outcome of the infection.

Adult antigens of 21, 26/26·6 and 36/36·5 kDa were reactive with sera of both Asym and Sym animals, whereas 2 antigens of 17 and 55 kDa were almost completely non-reactive with sera of Sym animals of both the infected groups except 1 animal (no. 44) which recognized this antigen only during asymptomatic periods of infection. In jirds, antibody responses to the 17 kDa antigen corresponded with the development of intralymphatic thrombi (Farrar *et al*. 1991) which in humans is suggested to precede the development of symptoms. Reactivity to this antigen shown by the Asym animals in the present study may therefore, be associated with lymphatic pathology even in the absence of symptoms. In fact, these animals revealed pathological changes in the lymphatics and lymph nodes similar to those found

in symptomatics (unpublished observation). Similarly, in asymptomatic microfilaraemic patients, considerable lymphatic damage was detected by lymphoscintigraphy (Dissanayake, Watawana & Piessens, 1995). However, a direct correlation between specific antibody response to this antigen and pathogenesis of lymphatic lesions in filariasis has not yet been established. Recently, Kurniawan-Atmadja *et al*. (1998) found a high and preferential IgG_4 reactivity to a set of low molecular weight *B. malayi* antigens including a 17 kDa molecule, in microfilaraemics than in endemic 'normals' or in elephantiasis cases of malayan filariasis. As IgG_4 is implicated in blocking IgE-mediated hypersensitivity reactions, the predominance of this isotype may be considered to be inversely related to the appearance of symptoms. Sera of Malayan patients with chronic lymphatic disease or lymphadenopathy are known to recognize a 75 kDa antigen (Selkirk *et al*. 1986). In the present study, only 1 of the Sym animals (no. 44) showed reactivity to this antigen. However, more than 50% of the infected animals recognized a 43 kDa antigen. The recognition pattern shown by other antigens was identical in Asym and Sym animals, and antibodies started appearing irregularly at different stages of the infection. It was also observed that Asym animals had antibodies directed against a large number of antigen molecules as compared to the Sym animals. The reason for recognition of fewer antigen molecules by Sym sera could not be explained. However, identification of antigens in circulating ICs and tissue-fixed ICs may provide some information regarding implication of CICs in the development of disease manifestation in this model. Nevertheless, Hussain & Ottesen (1985) have reported different antigen recognition patterns in filarial patients with different clinical symptoms.

Thus in conclusion, the lack of definite correlation between clinical signs of the disease and parameters of humoral immune reactivity as measured in the present study suggest that humoral responses do not lead to the development of oedema or are required to sustain limb oedema. Investigations on the other arms of the immune system such as cytokines and inflammatory mediators may likely provide information on the precipitation of acute manifestation in this model. Further studies on the 2 antigen molecules of 17 and 55 kDa, which were identified to be non-reactive with sera of Sym animals, are needed to establish their possible involvement, if any, in the development of acute disease manifestations in this model.

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