picked up only the conformational but not the linear epitopes. No definite explanation for different results of our laboratory from that of Gill *et al.*[7] can be rendered with ease, they but may be attributed to different immunization protocols and animal species used.

The present study conducted in the inbred Balb/c mice will serve as a prerequisite for the development of murine monoclonal antibody probes to the tick SGE and saliva antigens for future work on characterization and purification of tick immunogens for the development of subunit anti-tick vaccine and specific diagnostic kits to monitor anti-tick immune responses.

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Brugia malayi in Indian leaf monkey (*Presbytis entellus*) – Response to repeated exposures of infective larvae

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Longitudinal studies on the development of disease manifestations and alterations in eosinophil counts in Indian leaf monkey (*Presbytis entellus*) infected with *Brugia malayi* infection were carried out. Monkeys received multiple exposures of infective larvae during prepatency as well as during late patency. All the monkeys showed positivity for microfilaraemia (mf) after 75 days of infection. The period of patency was observed to be around 400 days. Following re-exposure with infective larvae there was development of stage-specific resistance against mf and the monkeys acquired resistance against adult parasites. Increased eosinophil counts coincided with decrease in mf counts and re-exposures with fresh harvest of L_3. The appearance of limboedema may or may not be associated with raised body temperature, however, low level of microfilaraemia coincided with the development of oedematous swelling.

OUR knowledge of genesis of lymphatic filarial diseases is scanty. In India, 45 million people are infected with this disease[1], of which 25 million harbour microfilarae in their blood and 19 million suffer from filarial disease manifestations such as swelling of limbs, hydrocoele, elephantiasis, etc[2]. In spite of certain startling revelations available today on differences in physiological and immunological parameters between carriers and symptomatic groups of subjects, the development of elephantiasis and the risk factors involved for the development of filarial disease is yet to be understood. The principal bottleneck in this area is the non-availability of a simulating model where longitudinal study could be carried out. Several rodents[3,4], canine[5,6] and feline[4,7] hosts infected with *Brugia* species have been used for studying the lymphatic filarial disease in recent years. However, the major drawback of these models is that the infection does not mimic the human disease in the anatomic localization of adult worms, in the symptomatology or in the immune effector mechanisms that may be involved. Moreover, the information generated so far with different experimental models is perhaps not completely translatable to human lymphatic filarial diseases. Nevertheless, the experimental studies conducted by Mak *et al.*[8] using non-human primate with *B. malayi* would probably explain the cause of filarial diseases. However, the setback in Mak's model is that the *Presbytis* species (with *B. malayi* infection) used does not produce any gross filarial manifestations (acute or chronic). The recent observations made in our laboratory show that Indian leaf monkey (*P. entellus*) when exposed to single or multiple doses of infective larvae (L_3) of *B. malayi* causes some of them to develop acute filarial manifestations[9]. Therefore, it is worthwhile to carry out longitudinal studies of alternations in various physiological parameters of host (*P. entellus*) harbouring *B. malayi* infection. We report here the results of experimental studies on development of disease manifestations, including alterations in absolute eosinophil counts in Indian leaf monkey, following single or multiple exposure with L_3 of *B. malayi*.

Six male monkeys (3–4 kg) were used for the study. Five of them were exposed to L_3 of *B. malayi* obtained from freshly dissected infected vectors (*Aedes aegypti*)[9]. One unexposed monkey served as an uninfected control.

Infective exposures were given subcutaneously. Out of the five infected animals, one received two exposures each of 500 L_3 administered on day 1 and 330 at right ankle, two animals received first exposure of 500 L_3 on day 1 in left ankle. The subsequent four exposures consisting of 100 L_3 each were given between day 240 and 315 of the first exposure in both right and left ankle regions alternatively at fortnightly intervals. The remaining two animals initially received four exposures each of 125 L_3 at fortnightly intervals. Later one animal received 500 L_3 on day 330 and the other animal received 2 inocula each of 100 L_3 on days 330 and 390 respectively in right and left ankles.

Night blood samples of all infected and control langurs were examined for the presence of mf using membrane filtration technique[10]. Briefly one ml of venous blood was drawn between 21.00 and 22.00 h initially on days 60, 75 and 90 post exposure (p.e.) and thereafter at monthly intervals till the termination of the experiment.

Any disease manifestations, especially swelling of inflammatory response in limbs, were observed and monitored carefully. For quantitative assessment of swelling of the limbs, peripheral (circumference) measurements of both affected and corresponding unaffected ones were taken at different predetermined areas between the knee and the ankle. The swelling ratio was determined by the formula:

$$\frac{\text{measurement of affected limb in cm}}{\text{measurement of unaffected limb in cm}}.$$

Rectal temperature of each animal was also recorded at regular intervals.

Blood sample (1 ml) from each infected as well as uninfected leaf monkey was collected, in a test tube containing ethylenediaminetetraacetic acid (EDTA), from femoral vein before L_3 exposure (i.e. on day 0) and thereafter at monthly intervals following exposure (on day 1) till the termination of the experiment for estimation of total leucocyte (TLC) and differential leucocyte counts (DLC). The TLC estimations were made by the standard method using Neubauer counting chamber[11] whereas DLC was estimated in the blood film stained with 5% Giemsa. Absolute eosinophil count (AEC) of an individual monkey was estimated with the help of TLC and per cent eosinophilia obtained from DLCs.

Out of the 5 exposed animals, only one (exposed with 900 L_3) died on day 378 of infection and postmortem examination could not be conducted due to excessive putrefaction of tissues. Nevertheless, the course of microfilaraemia and other parameters were monitored for all the animals.

All the monkeys irrespective of frequency and site of infective larval inoculum, became microfilaraemic by day 75. Progressive rise in peripheral microfilaraemia (mf) with peak attainment between 3 and 4 months of first exposure was observed, with one exception where the peak was attained around the sixth month (Table 1). The peak mf count in infected animals varied between 350 and 12,500 mf/ml of blood. In most animals, mf level came down steadily from 4th month onward and maintained at a low level up to 11 months. Re-exposures at late patency (between 8 and 11 months of infection) caused reversal of lowering mf level in 3 animals (receiving 500 L_3 initially). In the remaining two animals (receiving multiple exposures at prepatent stage), re-exposure at late patent stage caused no resurgence of mf.

It is evident from Table 1 that in spite of massive exposure of L_3 (700–1000) recovery of live adult worms was significantly low (<10). Nevertheless, calcification of lymph glands with debris of dead worms was observed in most of the monkeys.

Of the five infected animals, three developed oedematous swelling of pitting type in the lower part of one of the hind limbs (Table 2). There was no predictable timing for appearance of swelling. In the two monkeys (receiving single inoculum of 500 L_3 initially) swelling appeared on day 150 and 240 post exposure, whereas in the third monkey (receiving multiple exposures at prepatency) swelling appeared on day 420. Though intensity of swelling (ratio 1.1–1.3) did not differ significantly, the frequency of occurrence (1–4 episodes) and duration of persistence (4–90 days) differed to a great extent in individual monkeys.

Rectal temperature of all the exposed monkeys during the course of observation period was recorded. Whereas most of the symptomatics and asymptomatics did not show any alteration in body temperature (102°F), only one animal (receiving 700 L_3) while displaying 4 episodes of oedematous swellings had raised body temperature which was more pronounced during later attacks (Table 2). The highest temperature recorded in this monkey was 103.8°F around day 540, coinciding with the last episode of swelling.

The AEC of individual animals, just before filarial exposure varied from 56 to 334 eosinophils/cmm. The level of AEC of unexposed control animal varied between 56 and 426 eosinophils/cmm. It was observed that irrespective of size of inoculum and frequency of administration, the pattern of AECs during the course of infection did not differ much amongst exposed animals. During early patency with rising trend in microfilaraemia, the level of AEC remained within normal range. The elevated level of AEC apparently coincided with downward trend in mf level mostly during later stage of infection. The increased AEC level at any time varied between 3 and 5 times the individual's own initial level, with one exception where the level remained normal even after re-exposures (Table 2).

Lymphatic filariasis displays a wide spectrum of clinical manifestations ranging from acute attacks of lymphangitis to the development of grotesque deformities like elephantiasis. Our knowledge regarding the factors that generate and modulate the sequence and intensity of

Table 1. Course of *Brugia malayi* infection in *Presbytis entellus*

Number of monkey used	Total no. of L_3 inoculated	Prepatent period (days)	Peak mf counts/ml	Peak attainment (days)	Latency attained (days)	Day of resurgence of mf post re-exposure	No of adult worms* recovered		Total	Calcification observed in
	1000 (2)		350 (1)	90–120 (4)	200 (2)		3–6	2	5–8(2)	Popliteal inguinal and Axillary lymph glands (3)
		75 (5)				60 (3)				
5	900 (2)		1385 (1)	150.180 (1)	300 (1)		0	0	0 (2)	
			5000 (2)		500 (2)					
	700 (1)									
			12,500 (1)							

Figures in parentheses indicate the number of animals.
*Postmortem examination of one animal was not done

Table 2. Disease manifestations in *Presbytis entellus* with *Brugia malayi*

Number of monkeys showing swelling	No. of episodic swelling	Persistence of swelling in days	Temperature (°F) during swelling	Number of monkeys showing eosinophilia	No. of episodic elevated AEC	Period of episodic elevated AEC
	1(2)	90 (1)	102 (2)	4	2 (3)	150–180 and
3	4 (1)	30 (1)	103.8 (1)		1 (1)	300–420 (3)
		4–15 (1)				300–420 (1)

Figures in parentheses indicate the number of animals.

filarial disease manifestations is fragmentary. It is interesting to note that the percentage of people developing chronic disease manifestations (elephantiasis) is rather low but the occurrence of episodic adenolymphangitis (ADL) with or without fever amongst infected persons is very high. In India, the annual incidence rate of chronic filarial pathology is reported to be 0.5% whereas the occurrence of ADL goes up to 50% (ref. 4). The major cause of filarial morbidity, leading to a huge loss of man-hours, is primarily due to episodic ADL. However, factors governing the development of various disease manifestations, specially the ADL, are still not clear.

In the present study repeated exposures of L_3 of *B. malayi* were given to monkeys with the objective of simulating the situation prevailing in the endemic areas. A critical analysis of the parasitological parameters indicates that the prepatent period of all monkeys, irrespective of quantum and frequency of exposure, was around 75 days. However, the intensity and course of microfilaraemia varied greatly in individual monkeys. Though in most of the monkeys, peak mf appeared around 100 days of infection, in the solitary case it was extended to 150 days displaying 12,500 mf/ml. Thus, *P. entellus* displayed far better performance as a host for *B. malayi* when compared with two other species *P. cristata* and *P. melalophos*[8] where highest mf densities were 182 and 65.8 mf/ml of blood respectively.

Apparently, re-exposures with various inoculum sizes did not alter the course of mf significantly. Nevertheless, resurgence of mf was observed following re-exposure in most of the monkeys. It may be noted that relapse of microfilaraemia occurred within 60 days of the first re-exposure. In our earlier[9] and the present studies, the prepatent period of *B. malayi* in *P. entellus* was observed to be 75 days. Thus reappearance of mf (within 60 days) following re-exposure with L_3 could be due to L_3-induced immunosuppression, resulting in appearance of mf released from adult parasites from initial exposure. The other conjecture could be due to shortening of prepatent period through host–parasite interaction following reinfection. It is difficult to explain why one monkey (exposed with 700 L_3) did not show any relapse of mf even after re-exposures. Nevertheless, this monkey received least number of L_3 (700 L_3 only). The recovery of adult parasites from the autopsied monkeys was extremely poor. As none of the monkeys was sacrificed during early stage of patency, it is difficult to ascertain the per cent worm establishment in individual monkeys. In our earlier study[9], we have observed wide variations in the recovery of adult *B. malayi* (0–120 worms) with calcified lymph glands. The per cent recovery of adult parasites from two other *Presbytis* species was also poor[8]. The heightened eosinophilic response following decrease in mf density or re-exposure in most of the monkeys support the role of eosinophils in the removal of antigen[12] and the presence of functional immune (T-cell) response[13]. Appreciable increase in eosinophil counts in experimental filarial infection has been observed earlier by several workers[7,14,15].

As stated earlier, episodic ADL is the major cause of loss of man-hours in human filariasis and, unfortunately, no suitable laboratory model is available to work out the

causation of such a syndrome. It was interesting to observe that the *P. entellus–B. malayi* model mimics to an extent the picture as observed in acute brugian filariasis in human beings[4]. The presence of low microfilaraemia as observed in all symptomatic monkeys also simulated the picture as observed in human beings[4]. Moreover, it has been established that the development of oedematous swelling in the present model appears not to be due to concomitant bacterial infection, as antibiotic (Terramycin at 50 mg/kg) failed to reduce inflammatory reactions. The aetiology of human episodic ADL is yet to be fully understood. However, a recent study[16] conducted with human subjects indicates that such episodic attacks are due to periodic release of filarial antigen rather than bacterial infections. Thus, *P. entellus* with *B. malayi* infection opens up better possibility for the investigation on the causation of filarial manifestations in human subjects which is a major problem in filariasis.

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