POPULATION DYNAMICS OF MICROFILARIAL PRODUCTION AND EOSINOPHILIC LEVELS IN SLOW LORISES INFECTED WITH BREINLIA SERGENTI, PETTER (FILARIOIDEA: DIPETALONEMATIDAE)*

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(Received 9 October 1973)

Abstract—Ho B.-C., SINGH M. and YAP E.-H. 1974. Population dynamics of microfilarial production and eosinophilic levels in slow lorises infected with Breinlia sergenti, Petter (Filarioidea: Dipetalonematidae). International Journal for Parasitology 4: 383–388. Observations have been made on microfilarial and eosinophilic levels in slow lorises infected with Breinlia sergenti. Animals given a single inoculation of 100–150 infective larvae exhibited three different patterns of microfilaraemia while superinfected animals showed enhanced microfilarial levels. It appeared that the number of inoculations as well as the interval between inocula are important factors in enhancing microfilarial levels. Two different types of incubation periods were seen, one at 110–120 days and the other at 200 days. The eosinophilic levels were investigated in some of the animals and an attempt was made to correlate these levels with the microfilaraemia. Cortisone injection appeared to promote a vigorous eosinophilia in some of the infected animals tested.

INDEX KEY WORDS: Breinlia sergenti; microfilarial levels; eosinophilia; slow lorises.

INTRODUCTION

Breinlia sergenti Petter is a filarial parasite of the slow loris (Nycticebus coucang). The adults live free in both the peritoneal and thoracic cavities. The males measure about 38 mm in length while the females are 77–93 mm long (Petter, 1958). Microfilaraemia is subperiodic type with a peak at 1800 h (Zaman & Chellappah, 1968). Its natural vectors are not yet known. However, studies on vector susceptibility showed that complete larval development of B. sergenti can occur in several species of laboratory-bred mosquitoes; both Aedes togoi (Theobald) and Armigeres subalbatus (Coquillett) are very good vectors (Zaman & Chellappah, 1968). We have successfully conducted transmission experiments by syringe inoculation of infective larvae of B. sergenti subcutaneously into uninfected slow lorises.

The present paper describes the population dynamics of microfilariae throughout the course of infections with B. sergenti in slow lorises, employing both single and multiple successive inoculations. We have also attempted to correlate microfilarial densities of B. sergenti with the eosinophilic levels in the infected animals.

MATERIALS AND METHODS

Preparation of larval inoculum

A laboratory-bred Singapore strain of Arm. subalbatus and a Taiwan strain of Ae. togoi were used for obtaining the infective larvae (L3) of B. sergenti. Batches of mosquitoes of uniform age were allowed to feed on an anaesthetized infected slow loris for 2 h, and all the fed females were removed and placed in 1 ft³ plastic cages. Honey and water were provided for these fed mosquitoes throughout the extrinsic period of larval development. The infected mosquitoes were dissected between 12 and 15th day after the infective blood feed. All active infective larvae were picked up individually from the mosquito tissues and were then transferred into saline solution in cavity blocks in groups of 50 L3 each.

Infection of slow lorises

Slow lorises employed for the experiments were obtained from local animal dealers. This animal is a tame animal and can be easily reared in captivity for years. They were fed bananas, papayas, eggs and sweet potatoes. Repeated blood smears of every animal host were examined carefully prior to the infections and only filaria-free slow lorises were used for the experiments. Only a very low percentage (5 per cent) of the animals...
were found to have a natural infection with *B. sergenti*. Thus far this is the only filarial parasite that has been detected in slow lorises.

For those slow lorises with a single inoculation, 100–150 L3 of *B. sergenti* were inoculated subcutaneously into an uninfected host. Several slow lorises were subsequently infected at intervals of about 5 months by 1–4 successive inoculations of 100 L3 of *B. sergenti* per inoculation. The experience of many investigators has shown that a dose of about 100 infective larvae produced high levels of microfilaraemia in different filarial models. We had selected a dosage of 100–150 L3 as the inoculum because this generally yielded high levels of microfilariae in the animals. At autopsy, we have found that about 90 per cent of the larvae inoculated had become adults.

In studies of the correlation of microfilarial fluctuations with the eosinophilic levels, 5 animals were each infected by inoculating 100 L3 of *B. sergenti* subcutaneously. The eosinophilic counts as well as the microfilarial counts were commenced immediately after infection and were observed for a full year. At the end of the 1-y period, 3 of the infected slow lorises were injected intramuscularly with cortisone acetate (11-dihydro, 17-hydroxy corticosterone-21-acetate, Scanpharm A/S, Denmark) at a dosage of 125 mg/kg body wt per day for a total period of 7 days. Both the microfilarial and eosinophilic counts of these cortisone-treated animals were performed daily for the first week and weekly for the following two weeks.

### Microfilarial counts

Blood samples were taken in the mornings by drawing 20 mm³ of blood into a Sahli haemoglobin pipette by means of finger prick of the infected slow lorises. The blood was smeared on to a clean glass slide in three parallel streaks. Slides were dried in an incubator at 37°C for 24 h. Erythrocytes were lysed and microfilariae were stained with Giemsa. The microfilarial counts were performed monthly. All counts were made by the same technician throughout the period of observation of the prepatent and patent periods and, for several animals, a few months after the peripheral blood became negative.

### Eosinophilic counts

Blood for eosinophilic counts was obtained by means of finger prick of the infected slow lorises. These blood smears were prepared in the mornings between 10.00 and 11.00 a.m. Eosinophilic counts were calculated from differential counts of 200 cells in Giemsa-stained blood smears. A blood smear was taken from each experimental slow loris before infection to determine the pre-infection eosinophilic level of each animal host. Smears were then taken weekly between the 1st and 32nd week following the initial infection and then fortnightly for the remaining weeks of the 1-y observation period.

### RESULTS

**Microfilaraemia in animals infected with single and multiple successive inoculations**

A group of four slow lorises (A, B, C and D) were infected with a single inoculation of 100–150 L3 of *B. sergenti*. Curves of microfilarial density are shown in Fig. 1. The prepatent period for the infection was fairly consistent, being around 120 days. The number of microfilariae in the peripheral blood built up gradually and peaked between 200 and 260 days. The microfilarial levels tended to persist throughout the period of observations.

In the other group, three slow lorises (E, F and G) were given five successive inoculations of 100 L3 each (Fig. 2). These animals showed a similar period of prepatency (120 days) as in those that had received a single inoculation. However, as shown in
Fig. 2, microfilarial levels of these superinfected hosts rose sharply after 200 days and reached a higher level of microfilaraemia between 260 and 300 days. Microfilaraemia then fell rapidly over the ensuing 4-8 weeks after reaching peak levels. In slow loris G, following a decline at 320 days, microfilaraemia rose towards a second peak 160-200 days after the last two inoculations.

Figure 2 also shows a slow loris (H) which was infected with two inoculations of 100 L₃ each 150 days apart. The prepatent period for the infection in this host was relatively longer, being 240 days. Two peaks of microfilaraemia were observed. The first peak occurred 330 days after the initial inoculation. The second peak was seen 280 days after the second inoculation. Both peaks of microfilaraemia appeared to reflect the maturation period of the two populations of the infective larvae of B. sergenti.

**Animals with prolonged microfilaraemia**

A group of five slow lorises (J, K, L, M and N) were each infected with a single inoculation of 100 infective larvae of B. sergenti. Microfilarial levels were observed over a prolonged period until they became very low or negative.

The variation of microfilarial levels in the peripheral blood of three infected slow lorises (K, M and N) is depicted in Fig. 3. In these animals, microfilariae first appeared in the blood between 120 and 180 days after infection, rose sharply to peak levels between 150 and 280 days and declined rather sharply thereafter. The microfilarial levels tended to remain at low levels for further periods of 200-450 days before becoming negative.

Microfilarial levels were studied in two animals (J and L) over a period of 3-5 y. The microfilarial counts in the blood films of these two slow lorises showed longer prepatent periods of 195 and 225 days respectively (Fig. 3). The microfilarial levels tended to remain at a peak for about 200 days before gradually declining to lower levels. In these animals the low microfilarial levels persisted throughout the remaining period of observation.

**Eosinophilic response in infected slow lorises**

The eosinophilic response was studied in some of the infected animals. The results are shown in Figs. 4-8, in which both microfilarial levels and eosinophilia are plotted together. After inoculation of infective larvae, the eosinophilic levels remained fairly constant (0·3-3·8%) during the first two weeks before rising rapidly. In two of the animals (Q in Fig. 5 and C in Fig. 6), there was a sharp rise in eosinophilia (5·5 and 7·5%) during the 3rd and 4th weeks after infection. In all the animals, there was a maximal eosinophilia of 7·16% between 7th and 9th week after infection. After the peak level, the eosinophilia dropped to about 3-6%. There appeared to be a rise in eosinophilic level (5·1%) with the appearance of microfilariae in peripheral blood.
During the patent period, the eosinophilia fluctuated within a narrow range. The levels, however, were generally higher than preinfection levels. There seemed to be no correlation between the high microfilarial levels and eosinophilic levels as seen in animals P (Fig. 4) and Q (Fig. 5).

Cortisone acetate was injected intramuscularly into three infected slow lorises (C, D and R), (Figs. 6–8). There was a very marked and rapid elevation in eosinophilia in all three animals at day 4 after the start of drug treatment (Figs. 6–8). The peak levels attained were from 9 to 20%. The eosinophilia levels declined after cessation of cortisone treatment. However, the injections of cortisone had no significant effect on the microfilarial levels of the infected hosts.

Figs. 6–8. Correlation of eosinophilic and microfilarial levels in slow lorises (C, D and R) infected with a single inoculation of 100 infective larvae of Breinlia sergenti at day 0. The effect of 7 successive daily injections of cortisone (indicated by arrows) on the eosinophilic and microfilarial levels is shown.

DISCUSSION

Our experiments have revealed some interesting results with respect to fluctuations of microfilaraemia in slow lorises infected with B. sergenti. Slow lorises infected with a single inoculation showed a general trend in forming three types of patterns of microfilaraemia: (1) a progressive increase in the number of microfilariae with the microfilarial count tending to persist at low levels for a very long period of time (Fig. 1); (2) microfilarial levels rising sharply to a peak level, declining also sharply and thereafter remaining at low levels for a prolonged period, or, eventually becoming negative (Fig. 3); and (3) microfilariae in peripheral blood increasing gradually to a peak after a longer prepatent period, remaining at a plateau before gradually declining to low levels over a very long period of time (Fig. 3).

The microfilarial patterns in the infected animals reveal two different types of incubation period. In the majority of our experimental animals (11/16), microfilariae appeared quite consistently in the peripheral circulation, generally at about 110–120 days after infection. However, in five of our animals (J, K, L, M and N), a longer intrinsic incubation period (170–240 days) for B. sergenti was observed. In view of the longer incubation period seen in some animals, it becomes imperative to observe experimentally infected animals for periods longer than three months.

We have also found slow lorises which were completely refractory to infection. Two slow lorises had been reared in captivity in our department for nearly
four years. They were initially given an inoculum of 100 infective larvae subcutaneously. No microfilaraemia was detectable for one year. At the end of the year, 50 Ae. togoi infected with B. sergenti were allowed to feed on these animals. Again, no microfilariae were observed. A year later, a subsequent inoculum of 100 infective larvae was injected subcutaneously. Despite all these attempts, no microfilaraemia was seen in the peripheral blood of the animals. The resistance of some of these animals to infection could be a result of acquired immunity from previous infection. These animals would provide a useful model to investigate the immune response in this infection. Work in this direction is in progress.

Our results show that the levels of microfilaraemia, duration of pre-patency and patency in the infected animals may vary markedly even though these animals had been inoculated with a similar dose of larval inoculum. Variation in these parameters are clearly seen in Fig. 3. Indeed, such variations may occur in natural infections of slow lorises. The reasons for such differences are not understood, but, it is likely that these might be due to the immunological status of each individual animal. It must be borne in mind that these animals were obtained from local animal dealers, with no information on any prior infection in nature.

This study also shows that remarkably high peak levels of microfilaraemia were observed in superinfected slow lorises during the first few weeks after the initial detection of microfilariae. As seen in Fig. 2, it appears that the enhanced levels of microfilaraemia may be due to adults maturing from the first three inoculations given within a period of 8 weeks. Such an enhancement in the levels of microfilaraemia in superinfected animals had also been observed in Brugia pahangi infection in cats (Zaman & Chellappah, 1970; Denham et al., 1972).

In the superinfected slow loris G (Fig. 2), a second peak of microfilarial density was observed at 160–200 days after the 5th and 4th inoculations respectively. It appears that not only the number of inoculations, but also the interval between inocula are the important factors in enhancing the levels of microfilaraemia in experimental animals.

It is well known that helminthic infections induce high eosinophilia (Manson-Bahr, 1960; Basten, Boyer & Beeson, 1970). Human patients naturally infected with filariae may give rise to allergic phenomena manifested by persistent hyper eosinophilia (Danaraj et al., 1966). These authors proposed that continuous accumulation of microfilariae in the lung tissues may be one of the main factors stimulating the high eosinophilic levels. We have investigated this phenomenon in slow lorises infected with B. sergenti. Eosinophilia was at a low level during the first 2–4 weeks after inoculation. In two of these animals there appeared to be a sharp rise in eosinophilia during the 3rd and 4th weeks. A more pronounced rise was seen in all the animals from the 4th week onwards, reaching a maximal level around the 7–9th week. This rise in eosinophilia may result from the moulting of the larval stages within the host. It has been shown that the moulting fluid that is released at each moult evokes a strong response from the host (Schacher & Sahyoun, 1967).

Our results indicate that it may not be unreasonable to assume that the antigen released during moulting stages in the developing stages provide the impetus for the increase in eosinophilic levels. The release of microfilariae from the adult worms into the blood circulation also seems to provide a stimulus for maintaining the eosinophilia at levels higher than at pre-infection. This is illustrated in Figs. 4–8 where eosinophilia seems to increase at the time when microfilariae are first detectable in the peripheral blood.

We have attempted to investigate the possible role of the immune response in influencing both the levels of microfilaraemia and eosinophilia in the later stages of infection. Daily injections of cortisone seems to have no effect on the microfilarial levels. The continuous injections of cortisone provoked a vigorous eosinophilia as shown in Figs. 6–8. It is well known that cortisone suppresses the inflammatory response and cell-mediated immunity. How this influences the eosinophilic levels requires further investigation.

Acknowledgements—We appreciate the excellent technical assistance of Mrs. Evelyn Goh and Miss Kang Kim Lian.

REFERENCES


