

HEMATOLOGICAL CHANGES IN SUBPERIODIC *BRUGIA MALAYI* INFECTION OF THE LEAF-MONKEY, *PRESBYTIS CRISTATA*

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Abstract. Hematological changes were monitored in the leaf-monkey, *Presbytis cristata*, infected experimentally with 200 subperiodic *Brugia malayi* infective larvae. Prepatent periods were 54-86 days and peak microfilarial geometric mean counts (GMCs) were 1324 per ml blood. Total leukocyte and differential counts were measured at pre-infection, and then at weekly intervals before and during patency. Blood eosinophil level increased to about thrice the initial level at 3 weeks post-infection and this was maintained for the next 13 weeks before it started to rise again, increasing to more than 5 times the initial level at 20 weeks post-infection. The observed pattern of eosinophilia is probably related to the level of microfilaremia and the destruction of microfilariae in the spleen. There was no significant change in the total leukocyte counts during the period of observation.

INTRODUCTION

Hematological studies in man and rodents infected with parasites are abundant in the literature (Mackenzie, 1980; Ackerman *et al*, 1981; Hague *et al*, 1981). However, there is comparatively little information on hematological changes in early filarial infection, especially during prepatency and the phase of increasing microfilaremia. As the subperiodic *B. malayi* infected leaf-monkey (*P. cristata*) is being used extensively for the tertiary screening of potential filaricides (Mak *et al*, 1990a; 1990b), we decided to study the hematological changes in relation to infection and in particular to microfilaremia in this model.

MATERIALS AND METHODS

Silvered leaf-monkeys, (*P. cristata*) each weighing 4-5 kg were caught from non-endemic areas and quarantined for 2-3 months in cages in mosquito-proofed rooms at the Institute for Medical Research. They were fed with monkey chow, sweet potatoes, long beans, green leafy vegetables, bananas and papayas. Water was given *ad libitum*. They were examined for malaria, filarial and intestinal

parasites and infections were treated with chloroquine and thiabendazole respectively.

At the end of the quarantine period, animals found to be negative for microfilaremia on repeated examination of a ml of blood by membrane filtration were entered in the study. Forty-eight monkeys (11 males and 37 females) were inoculated subcutaneously with about 200 infective larvae (L3) divided into equal doses in the upper medial aspects of the right and left hind limbs. The infective larvae of subperiodic *B. malayi* were obtained from mass dissection of *Aedes togoi*, fed two weeks previously, on an infected cat maintained at the Institute. Five ml of blood were collected in EDTA at pre-infection and then at weekly intervals thereafter, for total white and differential counts (neutrophils, lymphocytes, monocytes and eosinophils). One ml was used for the screening of microfilaremia by the membrane filtration method. Animals were anesthetized with ketamine hydrochloride during handling.

RESULTS

Infected animals became patent for microfilariae at 69.0 ± 8.0 days, microfilaremia first appearing

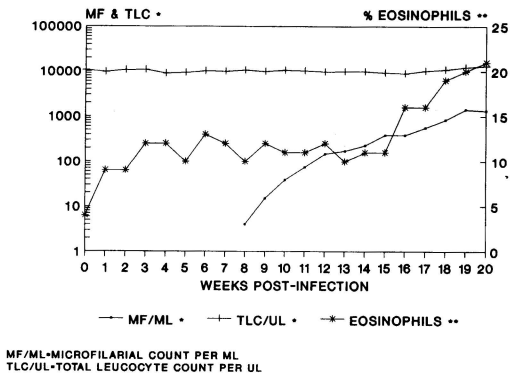


Fig 1—Relationship between microfilarial count, total leukocyte count and eosinophils in *P. cristata* infected with 200 *B. malayi* infective larvae.

at eight weeks post-infection, with a geometric mean microfilarial count (GMC) of 4 per ml, rising rapidly to 1324 per ml at 20 weeks post-infection (Fig 1). Eosinophils were elevated soon after infection, rising from an initial mean \pm SD of $4 \pm 4\%$ (479 ± 504 per μ l) to $12 \pm 6\%$ (1385 ± 974 per μ l) at three weeks post-infection and remaining at this level until 15 weeks, before rising further to reach $21 \pm 8\%$ (2843 ± 1237 per μ l) at 20 weeks. These levels are significantly higher than the pre-infection level ($t = 4.13$, $p < 0.01$ and $t = 8.65$, $p < 0.01$ respectively). The level at 20 weeks was also significantly higher than that at 3 and 15 weeks ($t = 2.96$, $p < 0.01$ and $t = 3.42$, $p < 0.01$ respectively). Total white blood counts were however, unchanged with mean \pm SD (range) of 10655 ± 4145 (4650 – 23550) per μ l at pre-infection and 13133 ± 2251 (10150 – 16600) per μ l at 20 weeks post-infection. Lymphocyte and monocyte levels remained constant whereas neutrophils decreased with increasing eosinophilia. At pre-infection the mean was 57 ± 13 (range 18–86)% and this decreased to 37 ± 10 (23–49)% at 20 weeks post-infection.

DISCUSSION

The total leukocyte levels in non-human primates vary widely. In *Macaca mulatta* this was reported to be 15155 ± 5981 (1200–43100) by Krise (1960). High leukocyte levels in monkeys could be due to natural infections or to stress induced by new

surroundings where they are undergoing primary investigations. In the present study, the leukocyte levels of *P. cristata* (which had undergone a “conditioning” period of 2–3 months prior to experimental infection with 200 L3), remained constant (Fig 1), but eosinophil levels increased fairly rapidly during the first three weeks post-infection to about thrice the initial level and these were maintained till the 15th week, when they started to increase rapidly to more than 5 times the initial level at the 20th week. Other than for the period between 3–15 weeks post-infection where the mean eosinophil level was stable at about 10–13%, there was a rapid rise in association with the level of microfilaremia. Khairul (1986) observed peak eosinophil levels between 3 and 5 weeks post-infection in jirds infected with *Dipetalonema viteae*, with these returning to normal by 12 weeks post-infection. In contrast, in our study, the high eosinophilia did not return to initial levels in *B. malayi* infection in *P. cristata*, but rose with increasing microfilaremia.

In filariasis, eosinophils are known to be involved in antibody-dependent cellular adherence and killing of *B. malayi* infective larvae in the presence of sera from patients with tropical pulmonary eosinophilia, elephantiasis and amicrofilaric symptomatic filariasis (Sim *et al.*, 1982). A similar mechanism is involved in the killing of microfilariae (Subrahmanyam *et al.*, 1978; Weiss and Tanner, 1979; Rudin *et al.*, 1980) and the role of toxic cationic proteins such as major basic protein (MBP), eosinophil cationic protein (ECP), as well as oxidative and other enzyme systems involved in such parasite killing, has recently been reviewed (Gleich *et al.*, 1989). Based on the previous observation that *B. malayi* infected *Presbytis melalophos* with high microfilaremia had huge granulomata in the spleen where microfilarial fragments engulfed by foreign body giant cells and surrounded with eosinophils were seen (Mak *et al.*, 1984), we believe that the high eosinophilia observed in the leaf-monkeys in the present study is associated with the destruction of microfilariae in the spleen and other areas.

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