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 microfilaria 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 microfilaria. 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 microfilariaemia 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 microfilaraemia 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 microfilaraemia 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, microfilaraemia first appearing
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 ± SD of 4 ± 4% (479 ± 504 per µl) to 12 ± 6% (1385 ± 974 per µl) at three weeks post-infection and remaining at this level until 15 weeks, before rising further to reach 21 ± 8% (2843 ± 1237 per µl) 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 ± SD (range) of 10655 ± 4145 (10150-23550) per µl at pre-infection and 13133 ± 2251 (10150-16600) per µl 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 microfilaria. 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 microfilarialaemia.

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 microfilaricidal 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 microfilariaemia 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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REFERENCES


