Litomosoides carinii and *Acanthocheilonema viteae* in rodents: The influence of nonspecific immunosuppression on establishment of filarial infection

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Prolonged incubation period and poor establishment rate of adult filarial parasites in experimental host have jeopardized much of laboratory investigational activities. Study was therefore planned with the objectives of shortening the prolonged incubation period of filarial infection and enhancing the establishment rate of macrofilariids with high peripheral microfilaraemia in host. It was observed that cortisone treatment in rodents at 10 mg/kg, subcutaneously from day –7 to day + 7 in two courses at week's interval, not only reduced the prepatent period of both *Litomosoides carinii* and *Acanthocheilonema vitae* infections by 10 days but also increased the adult worm recovery to a significant extent. Microfilarial level remained higher in cortisone-treated animals throughout the observation period. It was also interesting to note that length of adult male and female filariids (*A. viteae*) recovered from treated animals was significantly more in comparison to controls. The carbohydrate metabolism of recovered parasites from cortisone-treated hosts remained unaltered and thereby, remained suitable for various experimental purposes.

THE influence of immunosuppression of host on establishment of parasite infection is well documented[1–5]. Treatment with immunosuppressant leads to increased growth rate and size of parasites by counteracting the host's natural resistance towards parasite establishment mechanism. The present study was therefore designed with the objectives of lowering the prolonged prepatent period and increasing the intensity of microfilaraemia through higher establishment rate of adult filariids by suppressing the host's immune system with cortisone, an immunosuppressant[6–10]. The study was also extended to assess the metabolic status of recovered adult filariids from cortisone-treated animals to ascertain their physiologic status.

Two rodent filarial infections, *Litomosoides carinii* in cotton rat (*Sigmodon hispidus*)[11,12] and *Acanthocheilonema viteae* in *Mastomys coucha*[13,14] were used in the study.

Hydrocortisone (Sigma) was used initially at different dose levels (5, 10 and 20 mg/kg, s.c.) and courses (5 or 10 consecutive days or in two courses with a week's interval) in cotton rat and mastomys to determine the optimum dose level to bring about desired immunosuppressive effect as revealed by performing macrophage migration test[15,16]. The dose of 10 mg/kg × 5 days followed by another course preceded by a gap of one week was found optimum. Microfilaraemia was monitored till 75 of infective exposure of all treated and control animals by examining 5 cmm of tail blood. Animals were sacrificed on day 75 and worm burden was ascertained according to the method of Chatterjee *et al.*[12]. Length of recovered male and female parasites was also measured. The contents of uteri of female parasites were examined for any abnormality in developmental stages and microfilariae. The glucose metabolism[17–19] of parasites recovered from cortisone-treated hosts was measured by assessing *in vitro* uptake and lactic acid production capabilities.

In cortisone-treated animals, microfilariae of *L. carinii* and *A. viteae* appeared initially in peripheral blood of some animals on day 45 of L_3 exposure, i.e. 10 days earlier than control animals, as monitored by blood examination for microfilaria at 5 days interval since day 30 of L_3 exposure. The per cent positivity for microfilariae on day 45 was 97.5% in case of *A. viteae* and 66.0% in case of *L. carinii*-infected animals. On day 50, all the cortisone-treated groups of animals were microfilariae-positive. The intensity of microfilaraemia in treated animals was higher ($P < 0.001$) throughout the observation period (Figures 1 and 2). Per cent recovery of adult *A. viteae* was also higher (70%) in comparison to controls (32%) (Figure 2). In case of *L. carinii* infection, the number of adult parasites recovered from treated hosts was more (28 ± 3.5) in comparison to control animals (18 ± 3.0) (Figure 1). All female parasites (both *A. viteae* and *L. carinii*) contained normal and active developmental stages in the uteri. It was

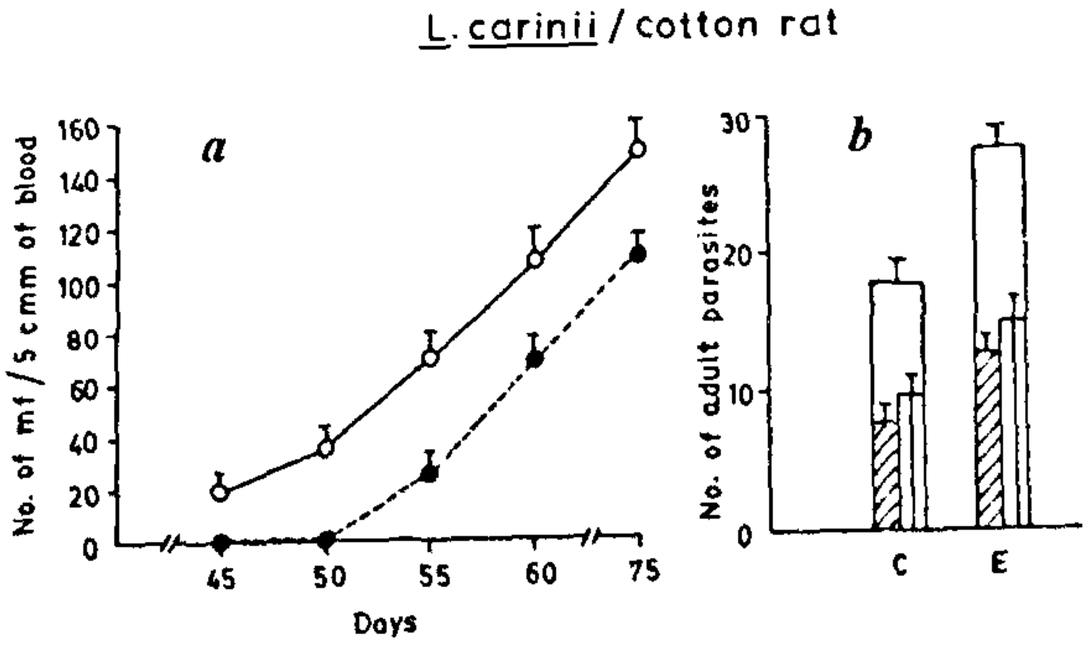

Figure 1. Microfilaraemia (*a*) and adult worm recovery (*b*) from cotton rats infected with *L. carinii*. o– – –o Cortisone treated; •– – –• Untreated control. ▨ Male, ▥ Female, □ Total. E, Experimental; C, Control.

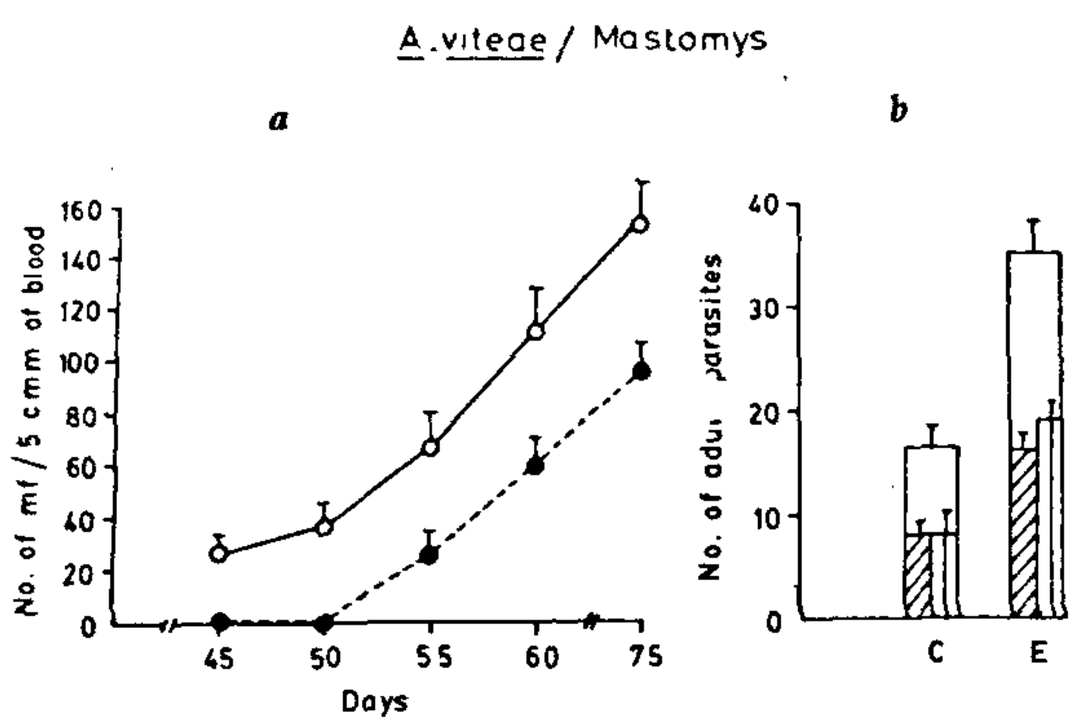

Figure 2. Microfilaraemia (*a*) and adult worm recovery (*b*) from Mastomys with *A. viteae* infection. o---o Cortisone treated; •---• Untreated control. ▨ Male, ▥ Female, □ Total. E, Experimental; C, Control.

interesting to observe that adult male and female *A. viteae* recovered from cortisone-treated animals were longer in length than their counterparts of control animals. Lengths of female and male parasites were 8.3 ± 3.0 and 3.4 ± 0.24 cm respectively whereas worms from untreated animals were respectively 5.4 ± 3.0 and 2.7 ± 0.22 cm. No such difference in length of *L. carinii* adults could be detected when recovered from cortisone-treated animals.

The glucose uptake of adult *L. carinii* and *A. viteae* from control animals was respectively 0.64 ± 0.06 and 0.49 ± 0.10 µmole/mg worm which was well comparable to worms obtained from their respective cortisone-treated hosts. Similarly there was no difference in the level of lactate production in cortisone-exposed parasites of both the species.

Our present study with experimental filarial infections using cortisone in rodent hosts indicates that nonspecific immunosuppression can promote significantly the per cent establishment rate of adult filariids, increase in size of worms and even causes reduction in the prolonged prepatency period. Hydrocortisone is well known to interfere with a variety of intra- and extra-cellular activities of mononuclear phagocytes, diminish monocyte chemotaxis and inhibit migration of leucocytes[20]. The decisive role of T-cell activity in establishment of helminth infections has been demonstrated earlier using athymic mice[21,22] or by transfer of immunity by T-lymphocytes[23,24] or hydrocortisone, a well-known T-cell suppressant[25]. Nevertheless, timing for application of immunosuppressor is important in case of filarial infection in which various developing forms take different time periods for maturity depending upon the species of parasite as well as host and the total incubation period may vary from 2 to 8 months. Keeping these in view, the course of treatment of cortisone is broadly divided into two time periods coinciding with the stages of larval forms, i.e. from L_3 to preadult stages so that all the developing forms may take the advantage of immunosuppressive state of the host. Thus, based on larval development cycle of *L. carinii* and *A. viteae*[26], two courses of cortisone, each of five days duration were administered to produce sustained immunosuppressive effect during the course of establishment period of parasites.

Both the species of parasites showed significantly higher per cent establishment rate in their respective hosts and in one of the species (*A. viteae*), the average length of females ($P < 0.001$) and males ($P < 0.01$) was also significantly higher than their counterparts from untreated control mastomys. Moss[2] has demonstrated that *Hymenolepis microstoma* in mice treated with cortisone grows larger than in control mice, and suggested that the increased growth of worms may be due to the combined effects of immunosuppression and growth promoter, which may be a hormonal growth-stimulating factor.

It is interesting to mention that whereas nonspecific immunosuppression of host helped in better establishment rate of adult parasites, immunostimulation on the contrary reverses the situation[6,7]. Thus, immunomodulation whether suppressive or stimulatory has in general a significant role to play in establishment of filarial infection in host.

The two other parameters as observed to be altered in both *L. carinii*/cotton rat and *A. viteae*/Mastomys systems following cortisone treatment are lowering of prepatency period of 10 days and increased intensity of microfilaraemia. Lowering of prepatency period appears to be due to increased growth rate of larvae in immunosuppressed host whereas higher intensity of microfilaraemia in cortisone-treated animals happens to be due to increased worm population.

Glycolysis is the major energy-generating pathway and both *L. carinii* and *A. viteae* recovered from cortisone-treated hosts, rapidly absorbed glucose from the medium and lactate was synthesized as the major end-product. Estimation of glucose uptake and lactate production of filariids showed parasites to be metabolically normal.

In conclusion, it may be stated that nonspecific immunosuppression of experimental host leads to lowering of prepatency period, higher intensity of microfilaraemia and increased population of physiologically normal filarial parasites. The data shown in this study would be of great help in laboratory maintenance of filarial infection and for meeting the growing demand of filaria parasite material for various experimental purposes.

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Block rotation in a part of eastern Dharwar craton of South India – A suggestion from aeromagnetic data interpretation

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Deformation in the brittle part of the earth's crust often gives rise to faults. It is believed that these faults tend to form conjugate fracture systems, and the rigid crustal blocks bounded by these fractures rotate about horizontal axes in the event of dip slip and about a vertical axis in the strike slip environment. Recent studies in the granite–greenstone terrain of a part of eastern Dharwar craton in South India (3.4–2.5 Ga) have suggested a tectonic environment in which the cross cutting NW, NE and ENE trending faults systems divided the crust into a mosaic of rigid crustal blocks which might have been rotated. Analysis of the aeromagnetic data of a part of this area suggests that (i) the NW–SE trending shears have strike-slip component, (ii) the rigid crustal blocks have rotated through an angle of 8.5° clockwise and (iii) the depth of the decoupling zone beneath the strike-slip faults is estimated to be 3.0 km, i.e. it is in the upper crustal level.

UNDER a variety of tectonic conditions the brittle part of the earth's crust is often prone to deformation. This deformation generally gives rise to faults. Simultaneous translation and rotation of fault-bounded crustal fragments, which are governed by kinematic constraints, are widely reported in varied geological and tectonic settings[1–4]. Fault-bounded crustal blocks rotate about vertical axes, where a fault slip has a strike parallel component[1,5,6], and on horizontal axes where dip slip occurs. Faults also rotate under continued deformation. These block rotations may occur (i) in discrete shear zones, (ii) zones of distributed shear probably caused by oblique subduction, (iii) during accretion, (iv) due to oroclinal bending, and (v) in overthrust faulting[2]. Such rotation may take place in different scales, starting from an outcrop to regional scale[5,7,8]. Recent studies on the granite–greenstone terrain of a part of Eastern Dharwar craton of south India, based on Landsat, aerial photos, age relationships of the structures such as faults, lineaments, dykes, etc. and on field evidences, suggested that tectonically this area is a composite mosaic of crustal blocks of various dimensions. These have rotated about vertical axes in geological time in response to various tectonic processes[9]. Several workers have reported palaeomagnetic, seismic and structural evidences in support of block rotation[2,4,10–17].