Fate of infective larvae of *Brugia malayi* in the peritoneal cavity of *Mastomys natalensis* and *Meriones unguiculatus*

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Abstract. The fate of intraperitoneally inoculated infective third-stage larvae (L_3) of the nematode *Brugia malayi* Lichtenstein and the status of the peritoneal macrophage function were investigated in the susceptible rodent hosts *Mastomys natalensis* Roberts and *Meriones unguiculatus* Milne-Edwards (jird). Jirds and *M. natalensis* were inoculated intraperitoneally with 125 and 250 L_3 and the worm burden and peritoneal macrophage function in the two species were compared at different days post-inoculation (DPI). None of the infected *M. natalensis* had adult worms in the peritoneal cavity; very few degenerating L_3 surrounded by peritoneal cells were recovered 7 and 15 DPI. In contrast, all the infected jirds showed the parasite in different stages of development and the worm burden at different days PI was more in 250 L_3 dose group than in 125 L_3 dose group. The phagocytic function of peritoneal macrophages of normal *M. natalensis* was twice higher than that of jirds. This function was found significantly suppressed in both host species at 15 DPI; at 35 DPI, the activity was still at this low level in the jird, while that in *M. natalensis* reverted to uninfected age- and sex-matched control levels. These findings demonstrate that the peritoneal environment of *M. natalensis* is not conducive to the development of *B. malayi* and this is probably related to high macrophage activity in the peritoneum of this host compared to that found in the jird.

The jird (*Meriones unguiculatus* Milne-Edwards) and *Mastomys natalensis* Roberts are the two well established experimental rodent hosts for the lymphatic-dwelling human filariid nematode, *Brugia malayi* Lichtenstein (Ash and Riley 1970, Petranyi et al. 1975, Sänger et al. 1981, Murthy et al. 1983). In both models subcutaneous inoculation of infective third-stage larvae (L_3) leads to development and localization of adult worms in lymphatic and other organs. In addition, in jirds, intraperitoneal (i.p.) inoculation of *B. malayi* L_3, following the method of McCall et al. (1973) used for *B. pahangi*, results in the development of a large number of worms in the peritoneal cavity (Maizels et al. 1987, Hayashi et al. 1989). The jird model has therefore been widely used in different parasitological investigations as both adult worms and microfilariae can be conveniently recovered from the peritoneal cavity. Unfortunately, the jird adapts poorly to Indian climatic conditions. In contrast, *M. natalensis*, which is a prolific breeder, could be easily maintained in Indian climatic conditions and several reports document the suitability of this rodent with *B. malayi* infection for studies on host-parasite interactions in filariasis and for antifilarial screening (Murthy et al. 1986, Tyagi et al. 1986). However, whether intraperitoneal infection can also be established in *M. natalensis* is not known. The present study was therefore designed to find out if the development and localization of the worms within the peritoneum can be induced in the easily maintainable *M. natalensis*, so that not only a large number of adult worms and microfilariae can be harvested for different parasitological, immunological and biochemical investigations but it can provide a convenient model for evaluation of new compounds for adulticidal activity because of easy localization of parasites. In addition, a comparative assessment of the functional status of peritoneal macrophages was made in the infected and uninfected *M. natalensis* and the jird.

MATERIALS AND METHODS

Third-stage infective larvae (L_3) of *Brugia malayi* were produced in and collected from the susceptible vector *Aedes aegypti* Linnaeus mosquitoes as described elsewhere (Murthy et al. 1983). L_3 were washed 3 times in sterile insect saline (SIS) and checked microscopically for contamination before use. A total of 50 male *Mastomys natalensis* and 55 male jirds (*Meriones unguiculatus* 6–8 wk-old) of CDRI-NLAC animal colony were divided into 3 groups each. Groups I and II of each species received a single intraperitoneal (i.p.) inoculation of 125 and 250 viable L_3 (in SIS) per animal, respectively, while the third group of uninfected animals received saline only and served as controls. Peritoneal lavages were examined for presence of microfilariae on 90 days post-inoculation (DPI) and thereafter, at weekly intervals till 120 DPI. Batches of animals from each group were killed by an overdose of ether anaesthesia on 7, 15, 30, 120, 180 and 270–360 DPI. Developing stages and adult worms were recovered from peritoneal cavity of the animals by the method of McCall et al.

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Table 1. Parasite burden in peritoneal cavity of *Mastomys natalensis* and jirds after intraperitoneal inoculation of *Brugia malayi* infective larvae (mean ± SE).

Host	Inoculum	Live parasite burden in peritoneal cavity					
		7*	15	30	120	180	270–360
Mastomys	125	0	0	0	0	0	0
	250	0	0	0	0	0	0
Jird	125	39.0 ±12.3	46.7 ±16.9	61.0 ±15.8	48.4 ±5.5	36.2 ±13.9	10.6 ±1.7
	250	63.7 ±28.6	91.7 ±25.2	113.0** ±24.6	76.6** ±9.7	75.0** ±13.3	20.0 ±5.1

* days post inoculation (DPI)
** $p < 0.05$ (250 vs 125 inoculum group of jirds)

(1973). In addition, the heart, lungs, lymph nodes, lymphatics and testes were searched for the parasites by standard methods (Ash and Riley 1970, Petranyi et al. 1975, Murthy et al. 1983).

Phagocytic function of peritoneal macrophages was assayed in additional 12 infected (250 L_3) and 12 age-matched uninfected animals of both species on day 15 and 35 PI according to the method of Ross (1981). Glass adherent monolayers of macrophages prepared from peritoneal lavages were incubated with haemolysin (Sigma, St. Louis) – coated sheep red blood cells for 90 min at 37°C, washed in phosphate-buffered saline (pH 7.2), fixed in methanol-acetone (1 : 1) and stained with Giemsa. Phagocytosis was enumerated by counting at least 400 cells/animal and the results were expressed as percent phagocytosis.

The parasite recovery from peritoneal cavity and percent phagocytosis data were analyzed by Mann-Whitney U-test and Student's *t*-test, respectively.

RESULTS

Degenerating and disintegrating third-stage larvae (L_3) were found in the peritoneal cavity of *Mastomys natalensis* on 7 DPI in Group I (5.0 ± 2.0) and Group II (4.4 ± 1.5). One of the 3 animals of Group II had 3 inactive L_3 covered with cells on day 15 PI. One male and 1 female worm were recovered from the testes of one of the 3 animals of Group II on 120 DPI (data not shown). However, neither adult worms nor microfilariae could be found in the peritoneal cavity of the remaining *M. natalensis* of Groups I and II beyond 120 DPI.

Jirds of Groups I and II autopsied on 7, 15, 30, 120, 180 and 270–360 DPI had parasites in the peritoneal cavity; microfilariae appeared in the peritoneal cavity by 100 DPI (Table 1). Third, fourth and fifth-stage larvae were recovered on 7, 15 and 30 DPI, respectively, while fully grown adult male and gravid female worms were recovered on 120 DPI onwards. The parasite burden was significantly heavier in Group II than in Group I ($P < 0.05$) from 30 to 180 DPI. Most of the worms recovered at 270–360 DPI were found to be either calcified or undergoing calcification with peritoneal cells adhering to them. A few worms were also recovered from extra-peritoneal sites like lungs, heart, testes and lymph nodes (data not shown).

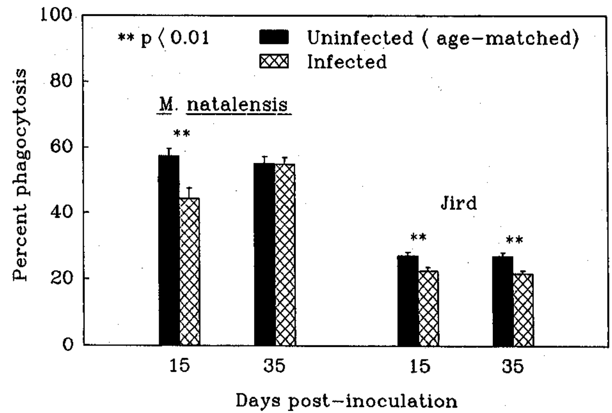

Fig. 1. Phagocytic function (mean ± SD) of peritoneal macrophages of *Mastomys natalensis* and jird after intraperitoneal inoculation of *Brugia malayi* infective larvae.

The percent phagocytosis of peritoneal macrophages of uninfected *M. natalensis* was found to be 2-fold higher ($P < 0.01$) than that of the uninfected jirds. By 15 DPI, the percent phagocytosis decreased significantly ($P < 0.01$) in both *M. natalensis* and jirds. At 35 DPI, the activity was still at this low level in the jird, while that in *M. natalensis* reverted to uninfected age- and sex-matched control levels (Fig. 1).

DISCUSSION

The results of the present study demonstrate that although the jird and *Mastomys natalensis* are closely related, third-stage larvae (L_3) of *Brugia malayi* develop into adults in the peritoneal cavity in jird but not in *M. natalensis*. Increasing the size of inoculum from 125 to 250 did not to alter the response of *M. natalensis* except that in the higher inoculum group the infective larvae apparently migrated to extraperitoneal sites as 1 male and 1 female adult worm were recovered from the testes of one of the animals. In the jird, on the other hand, the worm burden was proportional to the size of inoculum.

In *M. natalensis*, the presence of dead L_3 covered with peritoneal cells, consisting predominantly of macrophages, suggested that the non-susceptibility of this rodent to i. p. infection is mediated by a marked cellular response against the L_3. Both the early attacking and late scavenging functions of macrophages were probably involved in the elimination of the L_3. In the present study the macrophage function demonstrated at 15 DPI represents, probably, the late scavenging activity

with the attacking activity occurring at 7 DPI (not studied). Using a smaller inoculum size of 50 L_3 and a shorter observation period of up to 48 hrs Chandrashekar et al. (1986) found adherence to and killing of L_3 by peritoneal cells in *Mastomys natalensis* and albino rat but not in jird. They also found that among the peritoneal cell population, macrophages were the most effective in adhering to and killing L_3 *in vitro*; eosinophils by themselves were much less effective, though eosinophil culture supernatants enhanced macrophage activity.

Earlier Hayashi et al. (1983) reported that the activity of macrophages was lower in jird than in cotton rat. In the present study, measurement of percent phagocytosis revealed that the activity was 2-fold higher in *M. natalensis* than in jird and it decreased significantly in both the species by 15 DPI. It was notable that in spite of the decrease, the macrophage activity in infected *M. natalensis* (44.5 ± 3.2) was still significantly higher than that in uninfected jird (27.0 ± 1.1). Moreover, by 35 DPI, the activity in *M. natalensis* recovered to uninfected level while that in the jird remained suppressed. This suggests that the activity of peritoneal macrophages in the infected *M. natalensis* was apparently neither naturally low enough nor suppressed for sufficient length of time by *B. malayi* infection to permit establishment of the infection. The mechanism of prolonged suppression of the peritoneal macrophage function in the jird and only transient suppression in *M. natalensis* and its significance in the differential susceptibility of the two hosts to intraperitoneal establishment of the infection remain to be studied. However, in addition to the macrophage functions such as phagocytosis and scavenging of dead parasite, other as yet unknown host factor(s) specific to and acting within the peritoneal cavity might be involved in the non-susceptibility of the host to peritoneal infection. The development to fully mature parasites (one male and one female) in the testes but not in the peritoneum in one of the intraperitoneally infected *M. natalensis* indeed suggests an interesting difference between peritoneal and extraperitoneal milieu; further studies are needed to test this hypothesis.

Thus, the results of the present study indicate that the peritoneal environment of *M. natalensis* is not conducive to the development of *B. malayi* L_3 to adult worms and that the high peritoneal macrophage activity may constitute one of the factors contributing to the refractoriness of this host to intraperitoneal establishment of infection.

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