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Successful development of *Brugia pahangi* in T-cell deprived CBA mice

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Summary

CBA mice were thymectomized and treated with anti-thymocyte serum. Seven such mice were given 90–100 infective larvae of *Brugia pahangi* each by intraperitoneal (ip) injection and 5 given 99–100 larvae each by subcutaneous (sc) injection. From 62 days after infection 6 of 7 mice infected ip had microfilariae in their peritoneal cavities. Only one mouse infected by sc injection showed microfilariae in peripheral blood and this not until 98 days. At autopsy 5–45 adult worms were recovered from the ip infected mice. Only 2 of the 5 sc infected mice had adults and these only 3 each. No microfilariae or adult worms were detected in similarly infected unthymectomized CBA mice.

Key words: *Brugia pahangi*; CBA mouse; thymectomy; anti-thymocyte serum.

Brugia pahangi, a filarial nematode which in nature parasitizes many mammalian species in Malaysia and Indonesia, will successfully develop in jirds (*Meriones unguiculatus*) (Ash and Riley, 1970) and golden hamsters (*Mesocricetus auratus*) (Malone et al., 1974) but not in normal mice (Chong and Wong, 1967; Ahmed, 1967; Suswillo et al., 1980). It is very similar to *Wuchereria bancrofti* and *Brugia malayi* which are important pathogens of man.

Suswillo et al. (1980) and Vincent et al. (1980) found that *B. pahangi* developed to full maturity and produced microfilariae in athymic nude mice. In one experiment Suswillo et al. (1980) found microfilariae in 12 of 20 nude mice which had been inoculated with infective larvae of *B. pahangi*. In view of these results we decided to attempt to infect T-cell deprived mice with *B. pahangi* to determine whether the susceptibility of nude mice was due to their lack of a thymus or to some other factor.

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Materials and methods

The general parasitological methods, such as those for the production of infective larvae and enumeration of microfilariae, were those of Denham et al. (1972). The inbred CBA/H-*T6T6* mice were bred on site. 12 mice were thymectomized at 8 weeks of age using the method described by Law et al. (1963). On days 1, 3, 5 and 7 after thymectomy they were each given 0.25 ml of rabbit anti-mouse thymocyte serum (Levey and Medawar, 1966) by subcutaneous injection. 28 days after thymectomy 5 thymectomized mice and 3 intact mice were injected subcutaneously with 90–100 larvae of *B. pahangi* each and 7 thymectomized and 3 intact mice were injected intraperitoneally with 90–100 larvae each.

From 50 days after infection the mice were monitored for blood or intraperitoneal microfilariae (Suswillo et al., 1980) depending on route of inoculation. 165–167 days after infection the mice were killed. The peritoneal cavities were searched for adults and microfilariae. 1% Evans blue solution was used to dye the lymphatics which were dissected and searched for adult worms (see Denham et al., 1972 for details).

Results

Neither microfilariae nor adult worms were found in the intact mice. Microfilariae were first found in the peritoneal washings 62 days after infection of all the thymectomized mice which had been infected by ip injection. At autopsy microfilariae were found in tail vein blood from 5 of 6 animals tested. Only 1 of 5 thymectomized mice infected by sc injection became microfilaraemic and this was not seen until 98 days post infection.

The adult worm recoveries, and other details, are shown in Table 1. Percentage recoveries (i.e. adults recovered as percentage of larvae inoculated)

Table 1. Details of experiment in which infective larvae of *B. pahangi* were inoculated into T-cell deprived CBA/H-*T6T6* mice. No adult worms or microfilariae were found in intact controls

No. larvae and route of infection	Microfilariae		Number of adults			Percentage recovery
	in blood (100 µl)	in peritoneal cavity	in peritoneal cavity	in lymphatics	in heart or lungs	
94 ip	5	+	14♀, 13♂	0	0	29
100 ip	20	+	30♀, 15♂	0	0	45
100 ip	ND	+	32♀, 7♂	0	0	39
100 ip	0	—	5♀, 0♂	0	0	5
90 ip	4	+	8♀, 2♂	0	0	11
100 ip	5	+	26♀, 12♂	0	2♂	40
99 ip	2	+	6♀, 6♂	0	0	12
100 sc	0	—	0	0	0	0
99 sc	3	—	0	2♀, 1♂	0	3
100 sc	0	—	0	2♀, 0♂	1♀, 0♂	3
100 sc	0	—	0	0	0	0
100 sc	0	—	0	0	0	0

ip = intraperitoneal; sc = subcutaneous

ranged from 5–45 in the mice infected ip. Only two of the mice infected sc had adult worms which were found in the testicular lymphatics of both mice and in the heart of one. One mouse had female worms only which contained ova but no microfilariae and the other yielded 2 gravid females.

Discussion

The results with intact mice are similar to those obtained by Suswillo et al. (1980). The number of adult worms recovered from deprived mice infected by sc injection was of the same order as that obtained from nude mice but was much lower than that obtained from both T-cell deprived and nude mice infected by ip injection. The mean recovery of adult worms was 25.8% from the ip infected T-cell deprived mice which compares well with the 11.1% recovered from nude mice infected by the same route (Suswillo et al., 1980).

The ability of *B. pahangi* to develop in T-cell deprived mice strongly suggests that the failure of the same parasite to develop in normal mice is due to an immune response rather than to some innate physiological or biochemical insufficiency.

It should now be possible by a variety of reconstitution procedures to determine which components of the immune system are responsible for the death of *B. pahangi* in mice. This, in turn, might lead to a better understanding of the host-parasite relationship in other species of host in which the parasite survives well, but in which the immune response is as yet less amenable to manipulation than is that of the mouse. The T-cell deprived mouse has advantages over the nude mouse not the least of which are its greater hardiness and lower cost.

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