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Autor: Suswillo, R.R. / Owen, D.G. / Denham, D.A.
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¹ London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, England

² Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, England

Infections of *Brugia pahangi* in conventional and nude (athymic) mice

R. R. SUSWILLO¹, D. G. OWEN², D. A. DENHAM¹

Summary

AKR, BALB/c and CBA/Ca and T.O. mice were completely resistant to infection with third stage infective larvae of *Brugia pahangi*. Third, fourth and fifth stage worms transplanted from the peritoneal cavity of jirds into the peritoneal cavity of mice continued to develop. BALB/c mice were the most susceptible of the strains tested and adult worms were obtained after each type of transplanted infection. Congenitally athymic nude mice were much less resistant to transplanted worms and infective larvae developed to full maturity in most of them. Ten of 14 athymic mice infected by the intraperitoneal (ip) inoculation of infective larvae had microfilariae in their blood or peritoneal cavities. At autopsy a percentage recovery of adult worms of 0–38% (mean 11.1%) was obtained. Microfilariae were only found in the blood of 2 of 6 athymic mice infected by subcutaneous (sc) injection and at autopsy 0–19.1% (mean 6.1%) recoveries were obtained. The thymic littermates of the nudes were more resistant than those most of the other strains used.

Key words: *Brugia pahangi* in mouse; nude (athymic) mouse.

Introduction

Whilst *Brugia pahangi*, or the very closely related species *Brugia malayi* will develop in several rodent species, such as jirds, multimammate rats and hamsters, mice have been reported as being almost completely refractory to infection. Laing et al. (1961) and Ahmed (1967) found no signs of infection in mice inoculated subcutaneously with infective third stage larvae. Chong and

Correspondence: Mr. R. R. Suswillo, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, England

Wong (1967) inoculated infective larvae into 3 strains of mice and found adult worms in the lymphatics and hearts of two individuals although microfilaraemia did not develop.

The jird (*Meriones unguiculatus*) is a very good host for *B. pahangi* (Ash and Riley, 1970) but there is still a paucity of immunological information on this animal, and as neither inbred strains nor immunological reagents are readily available a proper analysis of its immunological response to infection is impracticable. There would be many advantages from the immunological viewpoint in being able to study *B. pahangi* infections in mice so we decided to attempt to infect different strains of mice with the macrofilarial stages of the parasite and also to study the infection in congenitally athymic nude (nu/nu) mice. Nude mice do not possess a thymus and therefore do not have T lymphocytes. Their littermates, which may be either nu/+ or +/+ possess a thymus and T lymphocytes but are otherwise genetically similar to the nudes of the same litter. In view of the susceptibility of nude mice to lethal viral infections, especially to mouse hepatitis virus, we used gnotobiotic mice for some of the experiments (Coates, 1968).

Materials and methods

The general parasitological methods such as those for production of infective material and enumeration of microfilariae were those of Denham et al. (1972).

Conventional inbred mice of the AKR, BALB/c and CBA/Ca were obtained from commercial sources as were outbred T.O. mice. Initially inbred nu/nu mice were also obtained commercially (Olac 1976 Ltd.) but in later experiments nude mice on an outbred background bred at MRC, Carshalton, were used. These mice were maintained under gnotobiotic conditions for the first experiment. These gnotobiotic mice were kept in plastic mouse cages inside a 5×2×2 foot plastic film isolator under strict germfree conditions (Coates, 1968). The diet and water were sterilized by irradiation before being introduced into the isolator.

For the second experiment nude mice were not kept gnotobiotic but were isolated in filter boxes. Mice were infected by intraperitoneal or subcutaneous inoculation of infective larvae derived from infected *Aedes aegypti* or by intraperitoneal implantation of third, fourth or adult *B. pahangi* obtained from the peritoneal cavities of jirds using the method of Suswillo and Denham (1977). Worms destined for gnotobiotic or isolated athymic mice were washed twice in 1 in 10,000 merthiolate in NCTC 135 and then twice in sterile NCTC 135. The containers of worms were sprayed with 2% peracetic acid and passed through the isolator entry port. All subsequent procedures were performed using the gloves attached to the portholes in the side of the isolators.

When mice were infected by the intraperitoneal route they were tested for the production of microfilariae by aspirating samples of peritoneal fluid at regular intervals. Blood to test for the presence of microfilariae was obtained from the tail vein.

Experimental procedure and results

Infection of normal mice with infective (third stage) larvae. No sign of infection was found in the AKR, BALB/c, CBA/Ca or TO (Tuck strain) mice when they were autopsied 3 months after i.p. injection of 50 infective larvae. Microfilariae were never found in the blood or peritoneal fluid.

Table 1. Autopsy results of three experiments in which mice were infected by the intraperitoneal implantation of 4–5 day old *B. pahangi* third stage larvae from jirds

Mouse strain	Percentage adult recovery* at		
	2 months (infection 50 larvae each to 6 mice)	2½ months (infection 50 larvae each to 4 mice)	3½ months (infection 20 larvae each to 2 mice)
AKR	0	0	0
BALB/c	9	6	5
CBA/Ca	0	1.5	7.5
T.O.	0	–	–

* percentage recovery is $\frac{\text{number of worms recovered} \times 100}{\text{number of worms inoculated}}$

Table 2. Autopsy results of two experiments in which mice were infected by the intraperitoneal implantation of 17–19 day old *B. pahangi* fourth stage larvae from jirds

Mouse strain	Percentage adult recovery* at	
	2 months (infection 50 larvae each to 5 mice)	3½ months (infection 12 larvae to 2–3 mice)
AKR	3.6	0
BALB/c	2.4	12.5
CBA/Ca	14.0**	8.4**
T.O.	1.2**	8.3**

* as Table 1

** male worms only

Infection of normal mice with 4–5 day old (third stage) B. pahangi from jirds. Mice were infected by the intraperitoneal implantation of 20 or 50, 4–5 day old third stage larvae recovered from the peritoneal cavity of jirds which had been inoculated intraperitoneally with 400 infective larvae. The mice were autopsied 2, 2½ and 3 months after infection. The results are shown in Table 1.

Of the 4 strains of mouse inoculated the best recoveries were obtained from the BALB/c mice. The uteri of female worms recovered from the BALB/c mice 2 and 2½ months after implantation contained no microfilariae but did have ova. However, one BALB/c mouse had microfilariae in its peritoneal cavity from 3 months after implantation until it was autopsied 2 weeks later. Most male worms from BALB/c mice contained viable sperm. Only male adults were recovered from the CBA/Ca mice, and no worms were recovered from the AKR and TO mice.

Table 3. Autopsy results on mice infected by intraperitoneal implantation of 8 female and 4 male *B. pahangi* each obtained from jirds. Autopsies were made 3 months after infection

Mouse strain	No. of mice	Percentage recovery*	Percentage of mice with microfilariae i.p. at autopsy
AKR	4 f	25	25
AKR	4 m	43	75
BALB/c	4 f	56	100
BALB/c	4 m	52	100
CBA/Ca	4 f	72	50
CBA/Ca	4 m	49	25
T.O.	8 f	45	75

* as Table 1

f = female mice; m = male mice

Infection of normal mice with 17–19 day old B. pahangi from jirds. Fourth stage larvae obtained from the peritoneal cavities of jirds infected with 400 larvae 17–19 days previously were implanted into mice of the same strains as used above. Mice were autopsied 2 and 3½ months after infection. The results are shown in Table 2.

All strains harboured adult worms 2 months after infection but only males were recovered from the CBA/Ca and TO mice. Of the mice infected for 3½ months the AKR mice harboured no worms. Two of the 3 BALB/c mice had intraperitoneal microfilariae from 2½ months after infection. No other strain produced patent infections and again only male worms were recovered from the CBA/Ca and TO mice.

Infection of normal mice with adult worms from jirds. Male and female mice of the same strains as above were implanted with 4 male and 8 female *B. pahangi* each from jirds which had been infected for 75 days. The mice were autopsied 3 months later. The results are shown in Table 3. Adult worms survived in all strains of mouse. The recovery of adults was roughly similar for the BALB/c and CBA/Ca mice but whereas all the BALB/c mice had microfilariae in their peritoneal cavities at autopsy only 37% of the CBA/Ca mice contained microfilariae. The sex of the host did not appear to affect worm recoveries.

Experiments with nude athymic (nu/nu) mice and their hairy heterozygous littermates

Preliminary experiments with “commercial” inbred nu/nu mice. Two mice were autopsied 3½ months after implantation of 4 day old worms from a jird but no worms were found. Two mice were autopsied 3½ months after implantation of twelve 18 day old larvae from a jird and a recovery of 64% obtained 3 months after infection. A mean recovery of 83% was obtained from 6 nude mice each of which was implanted with 8 female and 4 male adult worms. These

latter results, which were much better than those obtained with conventional thymic mice, prompted us to attempt to infect gnotobiotic nu/nu mice which could be expected to survive for longer and in better health than commercial stock maintained in an ordinary animal house alongside other mice.

Implantation of adult worms into gnotobiotic nude (nu/nu) and their hairy littermates. Five female nudes and 5 hairy littermates were each infected by the intraperitoneal implantation of 8 female and 4 male adult *B. pahangi*. All the mice had microfilariae in their peritoneal aspirates within 2 weeks of infection. The mice were autopsied 6 months after infection. The results are shown in Table 4.

The mean recovery of adult worms from the nu/nu mice was 66.7% and the female worms still contained normal microfilaria, ova and embryos and the male worms contained normal spermatozoa.

The hairy mice yielded a mean recovery of 18.1% and only a few of the female worms contained microfilariae. Where present microfilariae had a ragged 'crinkled' appearance and the ova and their contents were generally disintegrated. Spermatogenesis in the male worms appeared normal. Dead encapsulated worms were recovered from every hairy mouse. Microfilariae were present in the peritoneal exudates of all except one hairy mouse, which, paradoxically, yielded the highest number of adults in the group; another mouse had a few living microfilariae but no living adult worms.

Infection with fourth stage larvae. 24–30 14 day old fourth stage *B. pahangi* were injected i.p. into 5 male nude mice and 4 female hairy mice via 19 G needles.

The results of autopsies are shown in Table 5. In the hairy mice no adults or immature worms were recovered and dead, encapsulated worms were found in

Table 4. Number of *B. pahangi* recovered from peritoneal cavities of outbred athymic nude (nu/nu) mice and their heterozygous littermates six months after implantation of 8 female and 4 male adult worms

Nudes		Heterozygotes	
mfs i.p.	Adults (%)	mfs i.p.	Adults (%)
++	6f 2m (67)	+	1f 1m (17)
++	6g 3m (75)	+	0f 2m (17)
++	8f 2m (83)	+	0f 0m (0)
++	5f 3m (67)	+	2f 0m (17)
++	2f 3m (42)	–	4f 2m (50)
		+	1f 1m (8)
mean recovery = 67%*		mean recovery = 18%	

f = female; m = male

* see Table 1

only one mouse 66 days after infection. In the nude mice 23.9% of the larvae which had been inoculated were recovered as adults; the worms appeared healthy and fecund. The female worms recovered ranged from 3.4–4.4 cm (mean 3.8) and the male worms 1.4–2.0 cm (mean 1.6 cm) in length which is very similar to the size of the worms recovered from jirds.

Infection with third stage larvae from mosquitoes. In the first experiment 5 nude and 5 hairy mice each received between 86 and 100 larvae intraperitoneally and their peritoneal cavities sampled after 66 days which is the time that microfilariae are produced in jirds and cats. Results of autopsies are shown in Table 6.

After 66 days no animals were patent and only 2 adult worms were recovered, both from a nude mouse; the one female worm recovered contained ova only. After 3 months, microfilariae were found in peritoneal exudates of

Table 5. Number of adult *B. pahangi* recovered from outbred athymic nude mice and their heterozygous littermates after inoculation of fourth stage larvae

Time to autopsy	Nudes		Heterozygotes	
	<i>i.p.</i> mfs	Adults recovered	<i>i.p.</i> mfs	Adults recovered
66 days	+	2m 6f	–	0
66 days	+	4m 1f	–	0
5 months	+	3m 2f	–	0
5 months	–	0	–	0
5 months	+	5m 10f		
		mean recovery = 23.9%*		0%

* see Table 1

Table 6. Recoveries of *B. pahangi* from outbred athymic nude mice and their heterozygous littermates after inoculation with infective larvae

Time to autopsy	Nudes		Heterozygotes	
	<i>i.p.</i> mfs	Adults recovered	<i>i.p.</i> mfs	Adults recovered
66 days	–	1m 1f	–	0
66 days	–	0	–	0
5 months	+	3m 6f	–	0
5 months	–	0	–	0
5 months	+	2m 6f	–	0
		mean recovery = 4.0%*		0%

* see Table 1

Table 7. Details of experiment in which infective larvae of *B. pahangi* were inoculated into nude, athymic mice

Mouse No. and sex	No. larvae and route of infection	Microfilariae		No. adults		Percentage recovery
		in blood	in peritoneal cavity	in peritoneal cavity	in lymphatics or lungs	
1f	81 sc	0	0	0	0	0
2m	99 sc	0	0		1f	5.1
3f	79 sc	0	0	0	0	0
14m	84 sc	0	0	0	1f	4.8
15m	90 sc	+	0	0	6f 1m*	7.8
16m	94 sc	+	0	0	11f 7m*	19.1
4f	99 ip	0	0	0	0	0
5f	98 ip	0	+	2f 1m	0	4.1
6f	54 ip	0	0	0	0	0
7f	100 ip	+	**	24f 11m	0	38
8f	100 ip	+	+	19f 12m	0	4
9f	95 ip	+	+	17f 6m	0	24.2
10f	99 ip	+	+	19f 3m	0	22.2
11f	100 ip	+	+	2f 3m	1	6
12f	99 ip	+	+	8f 4m	1f 2m	15.2
13f	97 ip	+	+	11f 2m	0	13.4
17m	100 ip	+	+	1f 1m	0	6
18m	98 ip	0	0	1f	0	1
19m	100 ip	0	+ but dead	13f	1f	14
20m	99 ip	0	0	6f 1m***	0	7.1

* One female had intrauterine microfilariae.

** More than 1000 microfilariae per ml of heart blood.

*** No sperm in male.

2 nude mice and were still present 5 months after infection when the remaining mice were autopsied. Worms were recovered from only the patent mice; one male worm was found in the heart of one mouse and no worms were seen in the lymphatics. There was a 4% mean recovery. Worm embryogenesis and spermatogenesis was still normal and the size range of female worms was 2.7–4.5 cm (mean 3.6 cm) and of the males 1.2 cm to 1.6 cm (mean 1.4 cm). Encapsulated dead worms were recovered from both nude and hairy mice but approximately twice as many were found in the nude mice.

In the second experiment 20 nude mice were inoculated with 54–100 infective larvae. Six mice (3 of each sex) were injected s.c. in the left groin and 14 (10 males and 4 females) were injected i.p. These 20 mice were subsequently kept in filter boxes under specific pathogen free conditions but not gnotobiotic. The mice were autopsied 5 months after infection. The results of this experiment are shown in Table 7.

Two of the 6 mice into which larvae had been injected subcutaneously had microfilariae in their blood and reasonable numbers of adult worms in their lymphatics. No worms could be found in 2 mice at autopsy.

Of the 14 mice infected by the injection of larvae intraperitoneally 8 had microfilariae in their blood and 10 had microfilariae in their peritoneal cavity. Every mouse that had adult males and females in the lymphatics after i.p. inoculation had microfilariae in its blood but microfilariae were also found in the blood of 4 mice which had adult worms only in the peritoneal cavity. One mouse had only dead microfilariae in its peritoneal cavity and contained no male worms. It is assumed that the male which fathered these microfilariae died after fertilizing one or more of the 13 female worms. Microfilariae were not seen in 3 mice one of which contained no adult worms, one only a female and the other 6 females and a sterile male.

There was a higher percentage recovery of adult worms from the mice infected intraperitoneally (11.1%) than those infected subcutaneously (6.1%) which reflects the situation we find in jirds.

Discussion

Our results with conventional mice showed that the strains of normal mice used are not susceptible to infection with larvae of *B. pahangi* from mosquitoes. That adult worms developed in some mice infected with either third or fourth stage larvae from jirds suggests that larvae from mosquitoes are either more susceptible to the immune response of the mouse or need some trigger to begin their development which the mouse does not supply.

Adult worms implanted into the peritoneal cavity were able to survive in all the strains of mouse but the recoveries were lower than we obtain from jirds. Of the 5 strains of mouse used BALB/c gave highest recoveries after almost every type of infection.

Athymic, nude, mice were susceptible to every type of infection with *B. pahangi*. The hairy littermates of the nude mice were more resistant to infection than any of the ordinary mice which we studied and it was, therefore, more remarkable that the nudes were so susceptible. It is our intention to attempt to infect nude mice with a BALB/c background and thymectomized BALB/c mice.

The fact that athymic mice can be infected whereas their littermates with a thymus cannot suggests that the failure of the parasite to develop in normal mice is due to an immune response and that this response is mediated by T lymphocytes with or without the cooperation of B lymphocytes. This opens up the prospect of a detailed analysis of the immunological response which kills developing worms in mice.

Most of the nude mice infected by intraperitoneal injection developed microfilaraemia even when adult worms were only found in the peritoneal cavity and this is in contrast to the same type of infection in jirds in which microfilaraemia seldom develops after intraperitoneal infection. Microfilariae may find it easier to cross the peritoneum of mice.

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