

Development of *Brugia pahangi* infections and lymphatic lesions in male offspring of female jirds with homologous infections

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Abstract

The development of *Brugia pahangi* infections and associated gross lymphatic lesions were compared in male offspring from *B. pahangi*-infected and uninfected female jirds. The numbers of adult worms recovered did not differ between groups but a greater number of offspring of infected females (92%) developed circulating microfilaraemias than did individuals from uninfected females (50%). Intralymphatic granulomatous thrombi, lymphatic dilatations and lymphatic lesion scores were significantly reduced in offspring from infected mothers.

Introduction

Studies of filariasis patients in areas where lymphatic filariasis is endemic have shown that a wide variation exists in the immunological and pathological responses of individuals. In addition, the development of infections and subsequent filarial disease in transmigrants into areas with endemic filariasis differs from that in the native population. One hypothesis that is consistently cited as an explanation of these phenomena is that *in utero* exposure to filariae, filarial antigens, and/or filarial induced immune effectors may be responsible for the induction of an altered immune response to subsequent filarial infections (OTTESEN, 1980; PIESSENS & MACKENZIE, 1983; OTTESEN, 1984).

Direct evidence exists that fetuses come in contact with filariae and that offspring from infected females show altered responses to subsequent infections. A variety of microfilarial species have been demonstrated to cross the placenta and invade the foetus in a number of different hosts, including; *Dipetalonema viteae* in rats (HAQUE & CAPRON, 1982) and jirds (GEIGY *et al.*, 1967), *Dirofilaria immitis* in dogs (MANTOVANI & JACKSON, 1966), *Onchocerca volvulus* (BRINKMAN *et al.*, 1976) and *Wuchereria bancrofti* in man (BLOOMFIELD *et al.*, 1978). Antibodies to filariae have also been demonstrated in cord blood of fetuses from infected mothers (DISSANAYAKE *et al.*, 1980). The antifilarial foetal IgM antibodies found by Dissanayake *et al.* were suggested to be reproduced by the foetus following natural *in utero* exposure to filarial antigens. *In utero* exposure to microfilariae of *D. viteae* was demonstrated by HAQUE & CAPRON (1982) to alter the immune response in Fischer rats. In those studies, a reversible T-cell dependent unresponsive state developed, and rats born of microfilaraemic mothers developed infections when challenged with *D. viteae* third-stage larvae while age-matched normal Fischer rats were refractory to the challenge. Female offspring from female jirds with *Brugia malayi* infections have been shown to develop more readily microfilaraemias following subcutaneous challenge with homologous infective larvae than females from uninfected mothers (SCHRATER *et al.*, 1983).

Studies on lymphatic lesions in *B. pahangi*-infected jirds have shown that the granulomatous reaction to filariae is altered by the immune response and that the lesion severity can be enumerated in this model (KLEI

et al., 1981, 1982). The purpose of the present paper is to report initial observations on the development of *B. pahangi* infections and associated lymphatic lesions in male offspring from *B. pahangi* infected female jirds.

Materials and Methods

12 pairs of inbred jirds (*Meriones unguiculatus*, Tumblebrook Farms West Brookfield, Mass., USA) were used as initial breeding pairs. Females in six pairs were inoculated subcutaneously with 100 *B. pahangi* infective larvae (L3) and intraperitoneally with 200 L3. Offspring used were limited to age matched males from infected and uninfected mothers. As a result, all offspring used were from three infected and three uninfected females and were born between 90 and 270 days post inoculation (DPI). When three to eight weeks old, age-matched offspring from infected and uninfected female jirds were inoculated subcutaneously with 100 *B. pahangi* L3. Offspring were bled at weekly intervals from the periorbital sinus beginning 56 DPI. Necropsies of offspring were performed at 110 to 120 DPI. A total of 12 male offspring were examined in each group.

Parasite rearing, microfilaraemia determinations, necropsy procedures, and lymphatic lesion quantitation methods have previously been described (KLEI *et al.*, 1982). Briefly, lymphatic lesion severity was judged by the degree of spermatic cord and lumbar lymphatic vessel dilatation, the degree of enlargement and number of subcapsular granulomata in renal and lumbar lymph nodes, and the number of intralymphatic granulomatous thrombi (ILT) present in spermatic cord and lumbar lymphatics. These observations were used to determine the lymphatic lesion score ranging from 0 to 4. A score of 0 represented no lesions and a score of 4 represented maximum lesion severity. The degree of spermatic cord lymphatic vessel dilatation was quantitated from photographic enlargements as previously described (KLEI *et al.*, 1982).

Brugia pahangi antigen and immunological methods used to assess immunological reactivity of female and offspring to *B. pahangi* were as described previously (KLEI *et al.*, 1982). Antibody titres were measured by indirect haemagglutination. Titres are expressed as the Log₂ of the last serum dilution showing complete agglutination. Foot pad hypersensitivity was measured at 30 min and at 24 hours following inoculation of 10 µg of antigen in 0.05 ml into one hind footpad and subtracting the swelling in the other foot which received 0.05 ml of sterile saline.

Differences in values obtained were compared statistically with a two tailed paired Student's-t test.

Results

Three of the *B. pahangi*-infected female jirds produced offspring. Although all of the uninfected

females reproduced, offspring were only used from three. Patent infections were developed in all of the infected females from which offspring were obtained and adult worms and granulomatous lesions were observed in the peritoneal cavity of these animals at necropsy 360 DPI. A comparison of the anti-*B. pahangi* antibody titres and foot pad swelling responses of these females 90 DPI is summarized in Table I.

Parasitological data recovered from offspring from infected and uninfected mothers is shown in Table II. Significant differences between the numbers and locations of adult worms found was not seen. However, fewer patent infections developed by 120 DPI in offspring from uninfected females than in those from infected mothers.

The quantitation of gross lymphatic lesions in offspring from infected and uninfected females is

summarized in Table III. Antibody titres and foot pad swelling responses of offspring from infected and uninfected female jirds measured immediately before necropsy did not differ.

Discussion

The percentage recovery of adult worms and their locations within the tissues of individuals did not vary between offspring from infected and uninfected females. Thus, infections of mothers did not enhance the susceptibility or decrease the natural resistance to initial infections in their offspring. This is in contrast to the increased susceptibility to *D. viteae* observed in Fischer rats born to females with surgically induced infections (HAQUE & CAPRON, 1983). Also it is important to note that *B. pahangi* microfilariae were not observed in circulation of jirds born to microfilaraemic females in the present experiments as has been

Table 1—Summary of immune response to *Brugia pahangi* antigen of female jirds used as parents for offspring at 90 days post inoculation

Treatment	IHA Titre*		Foot Pad swelling† Response	
	Mean	Range	30 min	24 hr
Infected	12	12-13	0.8	0.6
Uninfected	2	1-2	0.3	0.1

*Titres are expressed as the Log^2 of the dilution showing complete agglutination.

†Mean foot pad thickness in mm of antigen injected foot minus the contralateral saline injected foot.

Table II—Summary of parasitological data from male offspring necropsied 110 to 120 days following inoculation

Origin	Worms recovered	Worms in lymphatics	Worms per SPCD	Microfilariae† at necropsy	Mean day of patency	% Patent
Uninfected mothers	12.9 ± 6.7	8.3 ± 3.7	3.9 ± 2.1	10.6 ± 16.7	97	50
Infected mothers	10.6 ± 6.5	6.3 ± 4.0	6.3 ± 3.2	10.2 ± 12.4	93	92

SPCD—spermatic cord lymphatic vessels.

Mean ± S.D.

†Microfilariae per 20 µl of blood.

Table III—Summary of mean quantitative gross lymphatic lesions from infected offspring

Origin	Lesion score	Lesion score per worm	ILT Per SPCD	ILT per worm in SPCD	% Increase† in SPCD lymphatic	% Increase in renal L.N.
Uninfected mothers	2.1*	0.26	2.5*	0.62*	458*	185
Infected mothers	1.3	0.20	0.5	0.1	316	195

ILT—intralymphatic thrombi

SPCD—spermatic cord lymphatic vessels

†% increase of infected spermatic cord vessel as compared to uninfected standard.

* Values are significantly different ($P < 0.05$).

described in several other filarial-host systems (*loc. cit.*). However, a higher percentage of offspring from infected females became microfilaraemic by 110 DPI than did those from uninfected females. This difference corresponds in some degree with observations of SCHRATER *et al.* (1983) on offspring born to *B. malayi*-infected female jirds.

The degree of lymphatic lesion development as indicated by the numbers of intralymphatic granulomatous thrombi, the degree of lymphatic dilatation, and lymphatic lesions scores, were reduced in offspring from infected females as compared to offspring from uninfected females. These observations are similar to the reduction in gross lymphatic lesions which develop following subcutaneous inoculation of *B. pahangi* L3 into jirds with existing intraperitoneal infections (KLEI *et al.*, 1982) and support the hypothesis advanced by others dealing with human populations (OTTESEN, 1980; PIESSENS & MACKENZIE, 1983; OTTESEN, 1984) that *in utero* exposure to filariae induces a tolerance to subsequent filarial infections and a reduction in resulting disease. Immunological tolerance has been hypothesized to be responsible for the reduced granulomatous response to *Schistosoma mansoni* ova that was seen in mice born to *S. mansoni*-infected mothers (LEWERT & MANDLOWITZ, 1969). This response was later shown to be dependent on the concentration of antigen to which foetal mice were exposed (HANG *et al.*, 1974). Recent studies on the *B. pahangi*-jird model have demonstrated that antigen specific and non-specific regulatory mechanisms of lymphocyte responses occur in association with the onset of microfilaraemia and that specific suppression is not effected by cyclophosphamide treatment (KATZ & LAMMIE, 1984). Suppression in chronic *B. pahangi* infections in jirds has also been associated with serum factors that are not immunoglobulin or immune complex in nature (LAMMIE *et al.*, 1984). Although the mechanism of the suppression of lymphatic lesion development observed in the present study is unknown, the induction of immune suppressor mechanisms described by Katz & Lammie may be involved. Data obtained indicate that the *B. pahangi*-jird model may be useful in the further characterization of altered responsiveness of offspring born to mothers with filarial infections.

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