

Immunogenicity of the surface of filarial larvae (*Dipetalonema viteae*)*

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Considering the long survival of filarial worms in their hosts one might suggest that an antigenically inert cuticle protects the worm from immune responses of the host. In certain situations, however, an immune response against the surface of filarial larvae has been demonstrated: anticuticular antibodies against microfilariae (mf) have been found in various amicrofilaraemic hosts, i.e. man (WONG & GUEST, 1969), dog (WONG, 1964), cat (PONNUDURAI *et al.*, 1974) and hamsters (WEISS, 1978). Recent experiments have shown that such antibodies are a prerequisite for the cell-mediated destruction of mf in the hamster (WEISS & TANNER, 1979). Antibodies have also been demonstrated against the surface of infective larvae (L3) in Brugian filariasis (WONG & GUEST, 1969) and in dogs immunized with irradiated *Dirofilaria immitis* L3 (WONG *et al.*, 1974).

A preliminary indication of the stage- and species-specificity of anticuticular antibodies was obtained in an immune adherence assay with sera from rabbits immunized with *Breinhia sergenti* L3: there was reactivity only against L3, not against mf or adult worms of the same species and not against *B. pahangi* or *B. malayi* L3 (NELSON *et al.*, 1971).

The surface immunogenicity of *Dipetalonema viteae* stages was analysed in various hosts by the indirect immunofluorescence antibody test (IFAT).

Materials and Methods

The following experimental animals were used:

(i) randomly bred jirds *Meriones unguiculatus*, (ii) randomly bred male hamsters *Mesocricetus auratus* (strain LAKZ), (iii) inbred male mice (strains: C57B1/6J and DBA/2J), (iv) randomly bred white rats (strain SIV) and (v) randomly bred New Zealand white rabbits.

The isolation of third-stage larvae (L3) from soft ticks (*Ornithodoros moubata*) and the micropore chamber method to produce fourth-stage larvae (L4) have already been described in detail (GASS *et al.*, 1979). L4 were recovered from chambers in jirds in which they had previously developed for two weeks.

Microfilariae (mf) were obtained from *in vitro* maintained female worms (WEISS & TANNER, 1979).

Cuticular fluorescence on adult female worms was demonstrated on frozen sections (WEISS, 1978); for all larval stages IFAT was done in suspension. Larvae were carefully washed in phosphate buffered saline (PBS, pH 7.2), fixed in 2% formalin (in PBS) and stored at 4°C. IFAT on mf has been described elsewhere (WEISS, 1978). IFAT on intact L₃ (30 to 50 per test) and on L₄ (5 to 7 per test) was carried out in small watch glasses. Before the test and after each incubation step (37°C, 30 min) larvae were washed three times with PBS by

sucking off the liquid over the larvae under microscopical control and adding about 1.5 ml of PBS. After sedimenting the larvae this procedure was repeated.

The minimal dilutions for normal serum of each species were carefully established to avoid any non-specific fluorescence. The following FITC-conjugated IgG fractions of rabbit antisera (Miles-Yeda Ltd., Israel) at a dilution of 1 in 40 were used: anti-hamster IgG for jirds and hamsters, anti-mouse IgG and anti-rat IgG. A goat anti-rabbit IgG antiserum was used at 1 in 80. Fluorescent readings were made using a Zeiss-microscope equipped with a Ploem illumination system and the interference filters for FITC.

Results and Discussion

Host-dependent immunogenicity of filarial larvae

Table I summarizes the humoral immune response of susceptible and insusceptible hosts against the surface of different worm stages after the inoculation of living third-stage larvae (L3). During the prepatent period anticuticular antibodies against L3 and L4 were detected in the jird. Hamster sera were always negative when tested against L3 either during primary (150 L3), secondary (2 × 150 L3) or trickle infections (12 × 25 L3). During prepatency, hamster sera reacted against L4 and, in contrast to all other hosts, also against microfilariae (mf) at the end of the transient microfilariemia (WEISS, 1978). C57B1 mice, their nu/nu counterparts and DBA-mice developed antibodies against the cuticle of L3 after two inoculations of 25 L3 two weeks apart. These sera also reacted with the postanal region of L4. However, no such antibodies were found in the sera of rats after the inoculation of 2 × 50 resp. 500 L3. A rabbit immunized with a total number of 2000 L3 (three inoculations during a period of three weeks) raised antibodies against the cuticle of L3 and L4. This serum and sera from infected jirds reacted with the cuticle of adult worms.

In mice, the antibody response was followed after the implantation of L4 (30 to 40 per animal). Sera from these animals strongly reacted with the cuticle of L3 but only moderately with L4. Microfilariemia was not observed and no adult worms were recovered.

Appropriate controls were made; sera from mice sensitized with tissues and haemocoel fluid from uninfected ticks gave no reaction with L3. In addition, no host material could be detected on L4 using either a direct fluorescence test or sera from mice immunized with total jird serum in IFAT.

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Table I—Humoral immune response against the cuticle of different stages of *Dipetalonema viteae* after the injection of living third-stage larvae into various vertebrate hosts

Host	Anticuticular antibodies			Mf	Course of infection
	L3	L 4	Adult		
Jird	(+)	(+)	+	—	persistent microfilaraemia low adult worm recovery
Hamster	—	(+)	—	+*	transient microfilaraemia high adult worm recovery
Mouse	+	+ PA	—	—	no microfilaraemia no adult worm recovery
Nude mouse	+	+ PA	—	—	
Rat	—	—	—	—	
Rabbit	+	+	+	—	

PA = Post-anal fluorescence, Mf = Microfilariae, () = Only transient, * = Persistent during postpatency

The immunogenicity of mf for mice was tested by the injection of living mf. Interestingly, mice injected with approximately 10^6 mf showed microfilaraemia of long duration: four to seven months in C57B1 and eight to nine months in DBA. In contrast, hamsters tolerate mf for only two months (WEISS, 1970). In addition, after a second injection, mf circulated for more than four months in DBA-mice, whereas most hamsters cleared circulating mf within two weeks (WEISS, 1970). Thus, it seems that mf are less immunogenic in mice than L3, which is just the opposite situation to that in the hamster.

Hamster antibodies against the cuticle of mf were shown to be of 19 S class and were 2-mercaptoethanol sensitive (WEISS & TANNER, 1979). All anticuticular antibodies, whether from mice, jirds or rabbits and whether against L3 or L4, were 2-mercaptoethanol sensitive. In addition, rabbit antibodies against L3 and L4 were found in the first peak fractions of a Sephadex G-200 gel filtration.

Stage-specific surface antigenicity of filarial larvae

No cross-reactivity was evident when testing sera from postpatent hamsters (anti-mf) against L3 and L4 or sera from jirds, mice or rabbits containing anti-L3 and/or L4 antibodies against mf. Thus, an antigenic change occurs from mf to L3 in the tick vector. Fluorescence patterns on mf and L3 were uniform. There is also evidence for an antigenic change from L3 to L4, hamster sera reacting uniformly with the L4 surface did not cross-react with L3. It appears that some L3-antigens might have been retained on L4, as mouse sera containing anticuticular antibodies against L3 did react with the posterior part of L4, especially with the postanal cuticle. The important structural alterations of the cuticle during the development to the adult worm (COLLIN, 1971, and our unpublished results) could well be accompanied by antigenic changes.

Anticuticular antibodies against L3 were never seen in mice when dead L3 (three times freeze-thawed) or when various adult worm antigens (whole homogenized worms or saline extracts) were

used for immunization. Irradiated L3 (34 krad), but not dead L3, provoked anticuticular antibody production in jirds. Since living and irradiated L3 did moult to L4 in jirds (TANNER & WEISS, 1981) and mice (GASS *et al.*, 1979) the moulting process might be crucial for the stimulation of anticuticular antibodies. Micropore chamber experiments are in progress to see if—according to this hypothesis—L3 can complete their moult in the rabbit but not in the rat. It remains to be elucidated why the hamster is unable to produce antibodies against the cuticle of L3 following infections (see above) or the injection of irradiated L3 (150 L3, 30 to 72 krad) of dead L3 (3×150 L3, 3×1000 L3). Experiments are under way to obtain antigens released during the moulting process *in vivo* (from micropore chambers) or *in vitro*. It is worthy of mention that culture fluid in which *Ascaris suum* larvae developed from the third to the fourth stage could induce resistance to challenge infections in the guinea-pig (STROMBERG & SOULSBY, 1977). Immunization experiments with such antigens should show if the observed host differences in the surface immunogenicity of filarial larvae are due to an inability of an adequate antigen processing of the intact cuticle.

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