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***Dipetalonema viteae* (Filarioidea):
evidence for a serum-dependent cytotoxicity
against developing third and fourth stage larvae in vitro¹**

Short communication

M. TANNER, N. WEISS

Several studies have shown that developing filarial larvae can be overcome by the host's immune response after a previous sensitization with irradiated infective larvae (L3). Partial immunity, deduced from decreased adult worm recoveries, has been detected for *Dirofilaria immitis* in dogs (Wong et al., 1974) for *Litomosoides carinii* in rats (Rao et al., 1977 and 1980), and for *Brugia pahangi* in cats (Oothuman et al., 1979). For *Dipetalonema viteae*, the immunization of jirds (*Meriones unguiculatus*) with 34-krad irradiated L3 lead to a significant lower peak microfilaraemia and significant lower adult worm load compared to that of controls (Table 1, results and experimental design). In contrast, the immunization of hamsters with 150 irradiated *D. viteae* L3 was unsuccessful: all challenged hamsters (75 L3) became patent and neither mean microfilarial peak counts nor adult worm recovery differed from that of controls (data not shown). From micropore chamber experiments in jirds we already know that larval development is inhibited and the moult to the fourth stage (L4) is blocked in immunized animals (Tanner and Weiss, 1981). Adherence of host cells could only occasionally be observed on larvae recovered from implanted chambers. However, we could not demonstrate in vivo that an exclusive humoral effector mechanism was responsible for the larval growth inhibition and subsequent larval death since larval growth was also retarded inside 0.4 μ m chambers within uninfected controls when compared with the corresponding 5.0 μ m chambers (Tanner and Weiss, 1981).

For a further analysis of potential immune mechanism against early mammalian stages of *D. viteae* we made use of the newly developed in vitro system (cf. Tanner, 1981, this volume). We first compared the development of 5 days in vivo triggered L3 in BHK-21 medium supplemented with 10% tryptose-phosphate and either 10% normal or immune jird serum at 36.5° C in an atmo-

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Table 1. *Dipetalonema viteae*: microfilaraemia and adult worm recovery in jirds immunized with 125 34-krad irradiated L3 following challenge infection with 125 live L3 compared to the corresponding controls

Group	I	II	III
34-krad irradiated L3	125	125	—
Live L3 ^a	—	125	125
Mf + /total ^b	0/20 A	8/17 B	10/13 C
Peak mf count (mf/20 mm ³)			
median \pm S.E.	0	12 \pm 3 D	250 \pm 111 E
Number of adult worms ^c			
median (95% confidence limit)	0	1 (0–2) F	10 (3–15) G

^a Injected subcutaneously 3 weeks post immunization

^b Number of microfilaraemic jirds/total number of infected jirds 15 weeks post immunization or 12 weeks post infection, respectively

^c Autopsy at week 15 post immunization

Chi-square-test: $P < 0.001$: A–B, A–C; $P < 0.05$: B–C

U-(rank)-test: $P < 0.005$: D–E, F–G

mf = microfilariae, L3 = third stage larvae

sphere of 90% N₂, 5% O₂ and 5% CO₂. The immune serum was collected from jirds which inhibited larval growth within micropore chambers (Tanner and Weiss, 1981). This serum pool contained antibodies to the cuticle of L3 (titre: 1 in 80) and L4 (1 in 40) as measured by an indirect fluorescent antibody test (IFAT) on intact larvae (Weiss and Tanner, 1981). From day 2 of culture onwards the larvae in medium supplemented with immune serum started to die and all larvae were dead by day 6 (Fig. 1). Larvae neither grew nor completed their moult to L4. Dying larvae lost their motility and were obviously damaged (puffing up) at their anterior end. In control cultures (containing 10% normal jird serum), 33% of the larvae moulted and grew on to a maximal length of 3.4 mm at day 6. At that time, the medium length was 2.2 mm compared to 1.8 mm at the beginning of the culture. During the 6 days culture larval mortality reached only 17% (Fig. 1). The effect of the immune serum was even more pronounced in another experiment: all larvae were dead at day 2 of culture, but none in the control cultures. If the immune serum was inactivated (30 min, 56° C) the larvicidal activity was no longer observed.

The immune serum killed round-headed L3⁺ (the transformation of the anterior end is the first obvious sign of an infective larva having adapted to the vertebrate host (cf. Tanner, 1981), and L4, but not vector-derived L3. The transformation of point-headed L3 to L3⁺ was not affected by the immune serum. However, no difference in the intensity of antibody binding to the cuticles of L3 or L3⁺ could be detected by IFAT (Weiss and Tanner, 1981).

To test the possibility of an (additional) antibody-dependent cell-mediated effector mechanism, L3 isolated from ticks and 5-day in vivo triggered L3⁺ were

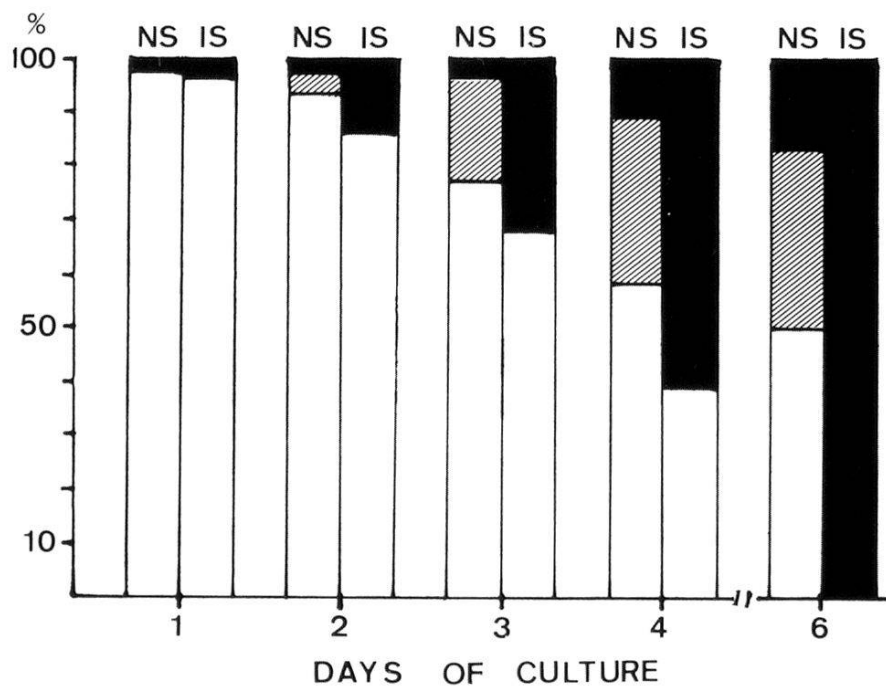


Fig. 1. In vitro cultivation of 5-day in vivo triggered *D. viteae* third stage larvae in the presence of 10% fresh normal jird serum (NS) or 10% fresh immune jird serum (IS). Percentage of dead larvae (black), of moulting L3 plus live L4 (hatched) and of live L3 (white). The daily observations included all larvae in the culture ($n = 75$ in NS, $n = 94$ in IS).

cultured in the presence of 10^6 peritoneal cells (from uninfected jirds sensitized with 2 ml of proteose-peptone for two days) or 10^6 normal jird spleen cells in 24-well tissue culture plates (\varnothing 16 mm Costar Inc., Cambridge, Mass., USA). To 1 ml BHK-21 medium (supplemented with 10% tryptose-phosphate broth and 10% normal or immune jird serum) approximately 50 larvae were added and observed for 3 days. None of the cross-checked combinations lead to the adhesion of spleen or peritoneal cells to L3 or L3⁺, thus confirming our observations from micropore chamber experiments (Tanner and Weiss, 1981).

To summarize our present knowledge on immune mechanism against developing *D. viteae* larvae, we assume that the larvicidal effects seem to be exclusively mediated by heat-labile serum factors. One might speculate that larval killing is only achieved in the presence of specific antibodies and complement. It is important to note that the surface of infective larvae does not directly bind and activate complement as demonstrated with immune adherence tests (Kigoni, 1978). Micropore chamber experiments with mice (Gass et al., 1979) and jirds (Tanner and Weiss, 1981) revealed that larval growth inhibition and mortality was clearly associated with the presence of serum antibodies to the surface of the cuticles of L3 and L4. They were shown to be of the IgM class (Weiss and Tanner, 1981). However, we still have no conclusive evidence for their protective role. The failure to immunize hamsters with irradiated larvae associated with the absence of anticuticular antibodies against L3 in their serum is a further element to postulate that surface antigens are of prime importance as functional antigens. From the fact that only developing larvae (L3⁺ and L4)

but not untriggered (vector-derived) L3 are affected one might speculate that anticuticular antibodies (plus complement) interfere primarily with a cuticle during its growth and moulting phase. In this respect it has to be shown whether the transcuticular uptake of nutrients can be impaired by the anticuticular antibodies. Further attention should be given to the potential role of excretory/secretory antigens in inducing protective immune responses against filarial larvae.

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