TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, VOL. 75, NO. 1, 1981

Dipetalonema viteae (Filarioidea): development of the infective larvae in micropore chambers implanted into normal, infected and immunized jirds*

M. TANNER AND N. WEISS
Swiss Tropical Institute, CH-4051 Basle, Switzerland

Using a micropore chamber technique, larval development of *Dipetalonema viteae* has been followed in jirds (the natural host), hamsters and mice (GASS *et al.*, 1979). Larval development was inhibited in the mouse, an insusceptible host, when this was previously sensitized with live third-stage larvae (L3) (GASS *et al.*, 1979). L3 no longer moulted to the fourth stage (L4). The inhibition of larval development correlated with the appearance of antibodies specific to the cuticle of L3. As in the sera of jirds infected with *D. viteae*, antibodies specific to the cuticle of L3 and L4 can also be detected (WEISS & TANNER, 1981); larval development was also examined in micropore chambers implanted into normal, infected and immunized jirds.

Material & Methods

The filarial parasite, *D. viteae*, was cyclically kept in the jird, *Meirones unguiculatus*, and the tick, *Ornithodorus moubata*. L3 were isolated under sterile conditions exactly as described recently (GASS *et al.*, 1979). Micropore chambers with 5·0 and 0·4 μm pore size membranes (Nucleopore Corp.) were assembled, loaded with 30 L3 and implanted subcutaneously into jirds as already described (GASS *et al.*, 1979). Normally a jird received one 5·0 μm chamber or, in some experiments, a 5·0 and a 0·4 μm chamber simultaneously. Chambers were implanted into jirds two weeks after a previous infection with live L3 (1 × 125 or 5 × 25) or after the immunization with 1 × 125 dead (3 × freeze-thawed) or 1 × 125 irradiated L3 (34 krad, 60 Co). Age-matched uninfected jirds served as controls. Two weeks after the implantation, the chambers were removed, the recovered worms were measured (GASS *et al.*, 1979) and the mortality (number of implanted L3 minus number of recovered motile worms as percentage) was assessed. All the data were recorded separately for each chamber and then pooled for evaluation as described in GASS *et al.* (1979). Antibodies to the cuticle of L3 and L4 were detected by the indirect immunofluorescent antibody test (IFAT) as described in WEISS & TANNER (1981).

Results & Discussion

Table I summarizes the results of four independent experiments. L3 developed to L4 inside 5 μm chambers in uninfected controls within two weeks. The median length varied between 2·3 and 3·6 mm in the four experiments. These results as well as the mortality data (with the exception of Expt. III) are consistent with earlier results on larval development within micropore chambers implanted into jirds (GASS *et al.*, 1979). A significant larval growth inhibition was observed within 5·0 μm chambers implanted into jirds which had been previously infected with live L3 (1 × 125 or 5 × 25, Expts. I-IV). The median lengths were 1·3 to 1·9 mm which means that most larvae did not complete their moult to L4. In addition, larval mortality was, with one exception (Expt. III), significantly higher. Immunization of jirds with irradiated L3 also resulted in a significant larval growth inhibition (Expt. III). In this respect it is noteworthy that 34 krad-irradiated L3 can complete their moult to L4 in micropore chambers (Fig. 1). Inoculated into hamsters, irradiated L3 never reached the fertile adult stage. Higher irradiation doses (51 krad) reduce the percentage of L3 moulting to L4 (Fig. 1). The mortality among 34 and 51 krad irradiated L3 within micropore chambers was only slightly higher than that of the non-irradiated controls. This is consistent with similar experiments described for *Dirofilaria immitis* (see WONG *et al.*, 1974), for *Brugia* spp. (see RAMACHANDRAN, 1970; OOTHUMAN *et al.*, 1978, 1979) and for *Litomosoides carinii* (see RAO *et al.*, 1977). The immunization of jirds with dead larvae did not provoke any larval growth inhibition (Expt. II).

Inhibited larval development correlated with the detection of antibodies specific to the cuticle of L3 and L4, i.e. the infection of jirds with live L3 and the immunization with irradiated L3 provoke the production of anticuticular antibodies. The immunization with dead L3, however, only stimulated antibodies against somatic antigens (cf also WEISS & TANNER, 1981).

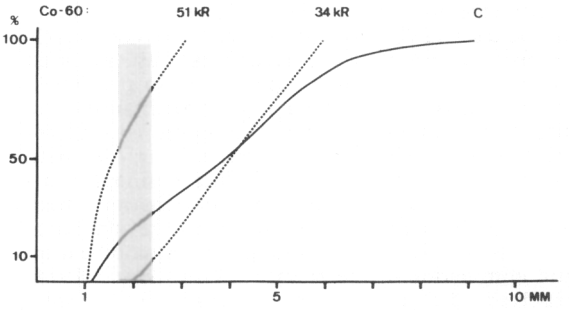

Fig. 1. Cumulative frequency distribution of the length of normal (C) and irradiated larvae from 5·0 μm micropore chambers two weeks after the implantation into uninfected jirds. The shaded area indicates the range of the third moult.

*Supported by the Swiss National Science Foundation grant No. 3.267.78

Table I—Larval development in micropore chambers implanted into normal, infected and immunized jirds

Experiment	Jird	Chamber pre size μm	Na	Mortality %	Number of larvae examined	Length (mm) median	(95% confidence limit)	
I	Controls	5·0	5	55	64	3·60	(3·23—3·78)	A
	1 × 125 L3 live	5·0	7	77***	47	1·49	(1·49—1·55)	B
	5 × 25 L3 live	5·0	4	82***	26	1·37	(1·30—1·43)	C
II	Controls	5·0	4	21	143	2·85	(2·48—3·23)	D
	1 × 125 L3 live	5·0	6	66**	40	1·49	(1·49—1·61)	E
	1 × 125 L3 dead	5·0	6	54**	82	2·54	(1·61—3·23)	F
III	Controls	5·0	8	72	63	3·72	(3·23—4·09)	G
		0·45	6	67	58	2·17	(1·80—2·36)	H
	1 × 125 L3 live	5·0	3	71	26	1·86	(1·30—2·11)	I
		0·45	4	48	56	2·24	(1·80—3·23)	K
	1 × 125 L3 34krad	5·0	5	79	32	1·37	(1·24—1·74)	L
		0·45	7	60	80	1·68	(1·55—1·92)	M
IV	Controls	5·0	6	47	96	2·30	(1·61—2·73)	N
		0·45	7	37	133	1·55	(1·49—1·61)	O
	1 × 125 L3 live	5·0	8	60*	96	1·30	(1·24—1·37)	P
		0·45	7	65**	74	1·37	(1·30—1·37)	Q

[a] Number of chambers examined
χ^2-test (compared to the dor-esponding control)
* : $2P < 10^{-2}$, **: $2p < 10^{-3}$, ***: $2P < 10^{-4}$

U-(rank) test : $2P < 0{\cdot}05$:G-H, I-K, L-M
$2P < 10^{-2}$:A-B, A-C, E-F
$2p < 10^{-4}$: D-E, G-I, G-L, N-O, N-P, O-Q

In order to get information on the mechanisms mediating larval growth inhibition, 0·4 μm chambers were implanted. These chambers prevent host cell immigration. Thus, the effect of antibodies alone can be measured. Experiments III and IV show that larval growth inside 0·4 μm chambers is significantly inhibited also within uninfected controls when compared with the corresponding 5·0 μm chambers. Nevertheless, the moult to L4 could still take place in such 0·4 μm chambers in controls (Expt. III). This was also the case within 0·4 μm chambers implanted into previously infected (125 live L3) or immunized (125 34 krad L3) jirds, while larval development in the corresponding 5·0 μm chambers was significantly inhibited (Expt. III). However, a replicate experiment (IV) was inconsistent. Larval development within 0·4 μm chambers was inhibited in controls to the same proportion as in previously infected jirds. Thus it is not yet clear if antibodies alone are responsible for the observed growth inhibition. Preliminary studies *in vitro* with peritoneal exudate cells—including eosinophil enriched cell populations—in combination with anticuticular antibodies did not promote adherence of cells to L3 and subsequent immobilization and elimination of the larvae. WONG *et al.* (1974) reported the appearance of antibodies specific against L3 in beagle dogs immunized with irradiated L3 of *D. immitis*. There appeared to be some evidence of correlation between L3 antibody levels and the degree of resistance of these immunized dogs.

The significance of our present results in respect to protection following challenge or superinfections is currently being investigated.

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