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USE OF THE *LITOMOSOIDES SIGMODONTIS* – MOUSE MODEL IN DEVELOPMENT OF AN *ONCHOCERCA* VACCINE. II – *L. SIGMODONTIS* IN THE BALB/c MOUSE : VACCINATION EXPERIMENTS ; PRELIMINARY IMMUNOLOGICAL STUDIES

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Early experiments demonstrated genetic restriction in the pattern of infection with *L. sigmodontis* in various inbred strains of mice (Petit *et al.*, 1992). BALB/c mice were the most susceptible and this strain has been used for vaccination experiments.

FATE OF *L. SIGMODONTIS* IN BALB/C MICE ; VACCINATION

Two months after inoculation with normal 25 L3, all mice inoculated harboured adult filariae with a mean of 4.6 worms (range from 1.5 to 10.6 based on 9 experiments with 7 to 15 mice per experiment), and 45 % of mice presented with a microfilaremia (range between 25 and 86 % for individual experiment). Similar results were obtained when mice were either 1- or 2- month old at time of inoculation.

Different protocols of vaccination with irradiated infective larvae were investigated. Best results were obtained when mice were inoculated at weekly intervals with three doses of 25 L3 irradiated with 60 Krads : this regimen resulted in a 83 % reduction in worm burden.

Other vaccination experiments were performed using extracts of adult *L. sigmodontis* , adult *O. volvulus* and 4 *O. volvulus* recombinant antigens (as described in Part I). The results are presented in Table I. Vaccination with recombinant antigen O.v. 3.11 had a semi-sterilizing effect. Thus 25 % of vaccinated mice presented with a microfilaremia compared to 75 % of the control group (almost significant), the mean microfilaremia was 0.5 mf/10 mm³ compared to 2.7 mf/10 mm³ of the control group, and the density of uterine microfilariae was significantly lower in the vaccinated group (Table II).

In contrast, immunisation with a Triton insoluble fraction of *L. sigmodontis* resulted in a "facilitating effect" : increase in the mean microfilaraemia (statistically significant), increase in adult worms, a higher density of uterine microfilariae and all adult male had normal spicule morphology.

ANTIGEN RECOGNITION

Differences were observed in the recognition patterns of *L. sigmodontis* antigens by infection sera collected from the different mouse strains (Diagne, 1990). As summarised in Table III, recognition of a number of antigens appears to be associated with particular background genes ; however an association with H-2 was not observed.

Further differences were observed in the recognition pattern of *L. sigmodontis* antigens by different antibody isotypes within the BALB/c mice normally infected or vaccinated with homologous irradiated L3 (Table IV).

Western blotting and immunoprecipitation experiments performed with radiolabelled *L. sigmodontis* antigens and human *O. volvulus* infection sera and serum raised in rabbits against *O. volvulus* antigens demonstrated considerable immunological cross-reactivity between the two species. This cross-reactivity extended to the *O. volvulus* recombinant antigens (Table V). For example, rabbit anti-*O volvulus* GST recognises a 25,000 Mr antigen of *L. sigmodontis* in Western blotting experiments performed on whole worm homogenates. Immunoprecipitation experiments demonstrated the release of this antigen from *L. sigmodontis* maintained *in vitro* for periods up to 48 hours.

In ELISA, sera collected from irradiated L3 vaccinated mice demonstrated a strong antibody response against recombinant *O. volvulus* HSP70 (Figure 1), although this antigen failed to elicit a protective response.

FILARIA		PERCENTAGE REDUCTION				
	Antigens	F	Mf	%Mf	L	%Sp N
L. sigmodontis	Soluble	34	50	42	- 0.4	- 26
	Insoluble	9	- 468	12.5	- 3.6	- 33
	Soluble	40	98	50	11	- 25
O. volvulus	Homogenate	16	- 81	33	8.3	- 22
	O.v 1.9	5	70	12.5	- 1	- 33
	O.v 2.5	- 47	- 121	- 50	- 4	- 25
	O.v 3.11	- 3.2	81	67	5	- 14
	O.v 7.9	30	4	0		

Table I. – *Litomosoides sigmodontis* in BALB/c mice : immunisation experiments.
Results are expressed by the percentage reduction of the means of the control group for adult filariae (F), microfilariae/10 mm3 (Mf), mice with blood microfilariae (% Mf), length of female worms (L), male worms with normal spicules (% Sp N). Negative numbers mean facilitating effect. O.v. : recombinant antigens of *O. volvulus* (O.v. 7.9 = *O. volv.* HSP 70).

Group	FEMALE FILARIA					MALE FILARIA		
	L ±s	W ±s	%DE (n/N)	%Mf ut (n/N)	%F spz (n/N)	L ±s	W ±s	Sp. an
O. v 3.11	68 ±10	176 ±18	34.7 (8/23)	26.1 (6/23)	13 (3/23)	19 ±2.2	97 ±7.7	11%
Control	72 ±7	191 ±14	78.9 (15/19)	57.9 (11/19)	36.8 (7/19)	19.2 ±2.7	102 ±6.2	22%

Table II. – Effect of O.v. 3.11 on fertility and growth of *Litomosoides sigmodontis* in BALB/c mice.
L : body length of filariae, in mm. W : body width, in µm. % DE : percentage of female filariae with divided eggs ; % Mf ut : percentage of female filariae with uterine microfilariae ; F spz : percentage of female with spermatozoa in seminal receptacle ; Sp.an. : percentage of studied male filariae with abnormal or no spicules ; N : number of filariae studied ; n : number of female filariae with divided eggs or uterine microfilariae or spermatozoa.

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Strain MHC		B10 b	BALB/B b	BALB/c d	DBA/2 d	C3H k
Size	230	+	+	+	+	+
	175	+	+	+	+	+
	125	+	+	-	+	-
	115	+	+	+	+	+
	72	+	+	-	+	+
	61	?	+	?	+	-
	53	+	-	-	+	-
	49	+	+	?	+	+
	40	+	+	+	+	+
	37	?	+	+	?	+
	36	+	+	+	+	+
	32	-	-	-	+	+
	30	-	+	+	+	+
	23	+	-	-	+	+
	21	-	-	-	+	-
	19	-	-	-	+	+
	17	+	+	+	+	+
	16	-	+	+	+	-
	11	-	+	+	-	-

Table III. – Pattern of antigen recognition of total IgG antibodies collected from different mouse strains infected with *L. sigmodontis*.
The responses were assessed by immunoprecipitation of radiolabelled antigens separated by SDS gel electrophoresis and detected by fluorography. (+) = positive ; (-) = negative ; (?) = uncertain. Susceptible mouse strains : BALB/c and BALB/B ; resistant strain : B.10 ; intermediary strains : DBA/2, C3H (Petit *et al.*, 1992). Molecular weights x 10^{-3} are indicated.

:	N	V	N	V	N	V	N	V
Size	IgG1	IgG1	IgG2a	IgG2a	IgG2b	IgG2b	IgG3	IgG3
230	+	+	+	+	+	+	+	+
82	-	-	+	-	+	-	+	-
64	?	+	?	+	+	+	+	+
53	-	-	-	-	-	-	-	-
40	?	?	?	?	?	?	?	?
32	+	-	-	-	-	-	-	-
30	+	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-
17	+	+	?	+	+	+	+	+
16	-	-	+	+	+	+	?	+
11	+	+	?	-	?	-	-	-

Table IV. – Pattern of antigen recognition by individual IgG isotypes of BALB/c mice with a chronic infection of *L. sigmodontis* : comparison between mice vaccinated with homologous irradiated L3 larvae (V) and control mice normally infected (N).
The responses were assessed by immunoprecipitation of radiolabelled antigens using isotype specific monoclonal antibodies immobilised on Sepharose. Precipitated antigens were separated by SDS gel electrophoresis and detected using fluorography. Molecular weights x 10^{-3} are indicated.

Size	Adult	2ME	GBP	GST	1.9	2.5	3.11	HSP70
200	+	-	-	-	-	-	-	-
120	+	+	-	-	-	-	-	-
100	+	-	-	-	-	-	-	-
72	+	-	-	-	-	-	-	-
70	+	-	-	-	-	-	-	+
50	+	-	-	-	-	+	-	-
42	+	-	-	-	-	-	+	-
31	-	+	-	-	-	-	-	-
25	+	+	+	+	-	-	-	-
19	-	-	-	-	+	-	-	-
18	-	+	-	-	-	-	-	-

Table V. – Cross reacting antigens of *L. sigmodontis* and *O. volvulus* as detected by immunoprecipitation of radiolabelled antigens using a variety of rabbit antisera.
Precipitated antigens were separated by SDS gel electrophoresis and detected using fluorography. Molecular weights x 10^{-3} are indicated. Adult = rabbit anti-adult female *O. volvulus* ; 2ME = rabbit anti-2-b-mercaptoethanol soluble surface antigens of *O. volvulus* ; GBP = rabbit anti-*O. volvulus* glutathione binding protein ; GST : rabbit anti *O. volvulus* glutathione-S-transferase ; 1.9 = rabbit anti-*O. volvulus* recombinant antigen O.v1.9 ; 2.5 = rabbit anti-*O. volvulus* recombinant antigen O.v2.5 ; 3.11 = rabbit anti-*O. volvulus* recombinant antigen O.v3.11 ; HSP = rabbit anti-*O. volvulus* recombinant heat shock protein 70.

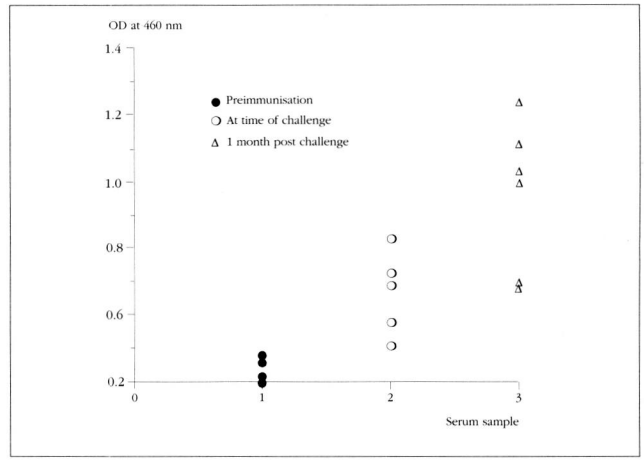

Figure 1. – ELISA Anti-*O. volvulus* HSP70 responses in mice vaccinated with 25 irradiated L3 of *Litomosoides sigmodontis*.
The "preimmunisation" control sera (1) were collected immediately before inoculation of the irradiated L3 larvae. A second serum sample (2) was taken at the time of challenge with normal L3 larvae and a third sample (3) was collected 1 month after the challenge.

These preliminary data demonstrate the considerable potential of the *L. sigmodontis* - mouse model for investigation of protective immunity against filarial infections.

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