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Antibody response against *Litomosoides carinii* and the distribution of bound antibodies on microfilariae from the different internal organs of cotton rats

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Summary

The courses of IgG and IgM antibody levels against adult worm and microfilarial antigen were determined in isogenetic cotton rats infected quantitatively with *Litomosoides carinii*. Against both antigens, IgG as well as IgM, antibody levels exceeded significantly those of noninfected animals, and the IgG levels were generally higher than the IgM levels. The total antibody production was depressed transiently 8 weeks p.i. by the appearance of microfilariae in the peripheral blood. A second transient depression occurred two weeks earlier against microfilarial antigen than it occurred against adult worm antigen. At 3 different times after the infection, the amount of adsorbed antibodies was assessed on adult worms and on microfilariae, which were isolated from blood and by a specially developed method from the internal organs. The percentual distribution of microfilariae in the different organs and blood changed during patency. In spleen, kidney, and lung a continuous increase was observed, whereas in heart and liver the percentage initially increased, and then decreased. The opposite course was seen in blood. Most antibodies adsorbed on the surface of microfilariae and adult worms belonged to the IgM class. Predominantly, the antibodies were detected on organ microfilariae, particularly on those in spleen and kidney, while blood microfilariae had lower amounts of bound antibodies. Furthermore, by complement fixation, no common antigens could be detected on the surface of host cells and the different *L. carinii* stages.

Key word: *Litomosoides carinii*; antibody level; adsorbed antibodies; organ microfilariae.

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Introduction

In filariasis, several mechanisms stimulating as well as suppressing the immune response have been reported. One of the most intensely investigated of them, the antibody level against adult worm antigen, correlates with the number of worms in prepatency and later with that of microfilariae in *Mastomys natalensis* infected with *Litomosoides carinii* (Zahner, 1974). A strong correlation was observed between antibodies against microfilariae and amicrofilaraemia in *L. carinii* infected cotton rats (Jaquet, 1978), and in men infected with *Brugia malayi* (McGreevy et al., 1980). Bound IgG and IgM antibodies were detected on the surface of blood microfilariae of *Dirofilaria immitis,* but not on *Brugia pahangi,* both in dogs (Hammerberg et al., 1984). A portion of circulating antibodies is adsorbed on microfilariae in the lung, where a bulk of them accumulates (Wenk and Wegerhof, 1982) and are destroyed subsequently (Weil et al., 1982). It has been suggested, that a minority of the microfilarial population circulates in the peripheral blood, whereas most remain stationary in the capillary systems of the lung, liver and spleen (Wenk, 1986). Probably both populations differ in regard to the amount of adsorbed antibodies.

In this study, the distribution of microfilariae in internal organs, and the amounts of antibodies adsorbed on their surface were assessed and compared to those of blood microfilariae at 3 different times during patency. Furthermore, the courses of the IgG and IgM antibody levels against adult worm as well as against microfilarial antigen were determined.

Materials and Methods

Isogenetic male cotton rats (CR) were maintained and quantitatively infected with 30 third-stage larvae of *Litomosoides carinii* as described by Müller-Kehrmann and Wenk (1986). The parasitaemia was determined microscopically by a dilution-method in a counting chamber for blood cells (Raether and Meyerhöfer, 1967). For serum preparation, blood was allowed to clot 2 h at 4°C, and the sera stored frozen at −70°C until used.

Production of anti-CR serum: Blood from normal cotton rats was separated with Ficoll-Paque as recommended by the manufacturer (Pharmacia, Sweden). 10^8 of the separated mononuclear leucocytes and erythrocytes each were together suspended in one ml phosphate buffered saline (PBS) and subcutaneously injected in one guinea pig (*Cavia* sp.; Savo-Ivanovas, Kisslegg). After four and six weeks, this procedure was repeated. A further two weeks later, blood was taken by heart puncture, and serum prepared. In an agglutination test, using blood cells of cotton rats and the guinea pig anti-CR serum, agglutination could be observed up to a dilution of 1:30,000 (data not shown).

Adult worms and uterus microfilariae were isolated as described by Wegerhof and Wenk (1979). Microfilariae (Mf) from the circulating blood were isolated by the phytohaemagglutinin-method, and in vitro-derived microfilariae were obtained from adult females kept in culture (Wenk et al., 1978). Third-stage larvae were obtained according to Mössinger and Wenk (1986).

Isolation of microfilariae from internal organs: Cotton rats were narcotized with an ether-air mixture and exsanguinated. Heart, lungs, liver, spleen and kidneys were removed, washed three times in medium TC 199 (Serva, Heidelberg), cut into small pieces, and incubated for 30 min in 2 ml TC 199 at ambient temperature. Subsequently the medium containing the emigrated microfilariae was separated with a fine metal sieve, and the organ pieces were incubated a second time for 15 min.

The obtained cell-Mf suspensions and the blood were mixed with 0.5 ml anti-CR serum each, and treated as described for the phytohaemagglutinin method (Wegerhof and Wenk, 1979). In a further step, the microfilariae were separated from remaining CR-cells by Percoll (Pharmacia, Sweden) sucrose gradient centrifugation (Chandrashekar et al., 1984). Approximately half of the total of organ microfilariae, determined by the method of Söffner and Wenk (1985), could be isolated, and the resulting microfilariae were nearly cell-free (1–2 cells per 1000 Mf; data not shown).

Preparation of parasite antigen: Adult worms isolated and washed in PBS were sectioned with scissors and ground in a glass tissue homogenizer. After freezing (–70° C) and thawing three times, the antigen suspension was sonicated for 30 sec at 400 Watts (Branson, USA) and filtered using a 0.22 μl filter (Millipore, USA). Microfilarial antigen was prepared in a similar manner.

Complement fixation: Parasite antigen (1 male or female worm, 100 third-stage larvae and 5000 blood, in vitro or uterus microfilariae, respectively) were mixed with 100 μl anti-CR serum and incubated in one ml merbital buffered saline (MBS) for 1 h at 37° C. After washing the antigen twice in MBS, 25 μl guinea pig complement (Behringwerke, Marburg) was added and incubated 30 min at 37° C. Sheep red blood cells (10^8), coated with Amboceptor as instructed by the manufacturer (Behringwerke, Marburg) were added and the mixture incubated for a further 30 min at 37° C. Subsequently, the cells were centrifuged, and the supernatant measured photometrically at 541 nm. Each infection stage was tested five-fold.

ELISA (Enzyme-Linked-Immunosorbent-Assay) was carried out following the method of Voller et al. (1976). The microtitration plates were coated with 3 μg/ml parasite protein antigen, determined by the BioRad (USA) protein assay. CR sera were diluted 1:50, and the conjugates, alkaline phosphatase labelled anti-rat IgM or IgG (Qualex, USA), were diluted 1:200. Supplementary reagents were purchased from Behringwerke (Marburg), and the absorbance was measured in a microtitration plate reader (Multiscan; Flow, USA) at 405 nm.

For estimation the amount of antibodies bound onto adult worms and microfilariae, the millititer vacuum system with 0.45 μm millititer-plates (Millipore, USA) was used. In the wells, one adult worm or 1000 microfilariae were incubated with 100 μl conjugate diluted 1:200 for 40 min at 37° C. After washing the plates twice with PBS-Tween 20 by aspiration of the wash fluid with vacuum, 100 μl substrate was added and incubated at 20° C for 40 min. For measuring the optical density (OD), the reaction fluid was transferred into a normal flat bottom microtitration plate. Reactions with an OD over 2.000 were diluted 1:2 and measured a second time.

Agglutinations of blood microfilariae were carried out in flat bottom microtitration plates. In each well, 5000 pooled microfilariae isolated 45 weeks p.i. were incubated in a total volume of 200 μl PBS diluted antiserum for 1 h at 37° C. The anti-IgG and anti-IgM sera were used in serial dilutions of 1:2, 1:3 and 1:5, respectively.

All determinations were doubled, and the data expressed as arithmetic means. Statistical analyses were performed with the U-test of Wilcoxon and the H-test of Kruskal and Wallis (Sachs, 1969).

Results

Complement fixation, carried out at the beginning of the experiments, revealed no common antigens on the surfaces of the host's blood cells and the different *L. carinii* stages.

For determination of the antibody levels against adult worm and microfilariae antigen, sera of 5 infected and 5 normal cotton rats were taken, 4, 6, 8, 10, 12, 14, 18, 25 and 45 weeks p.i. and each serum was tested by means of ELISA.

Against adult worm antigen, the IgG antibody level was significantly higher, 4 weeks p.i. (U-Test, 2 a = 0.05) in infected than in control animals

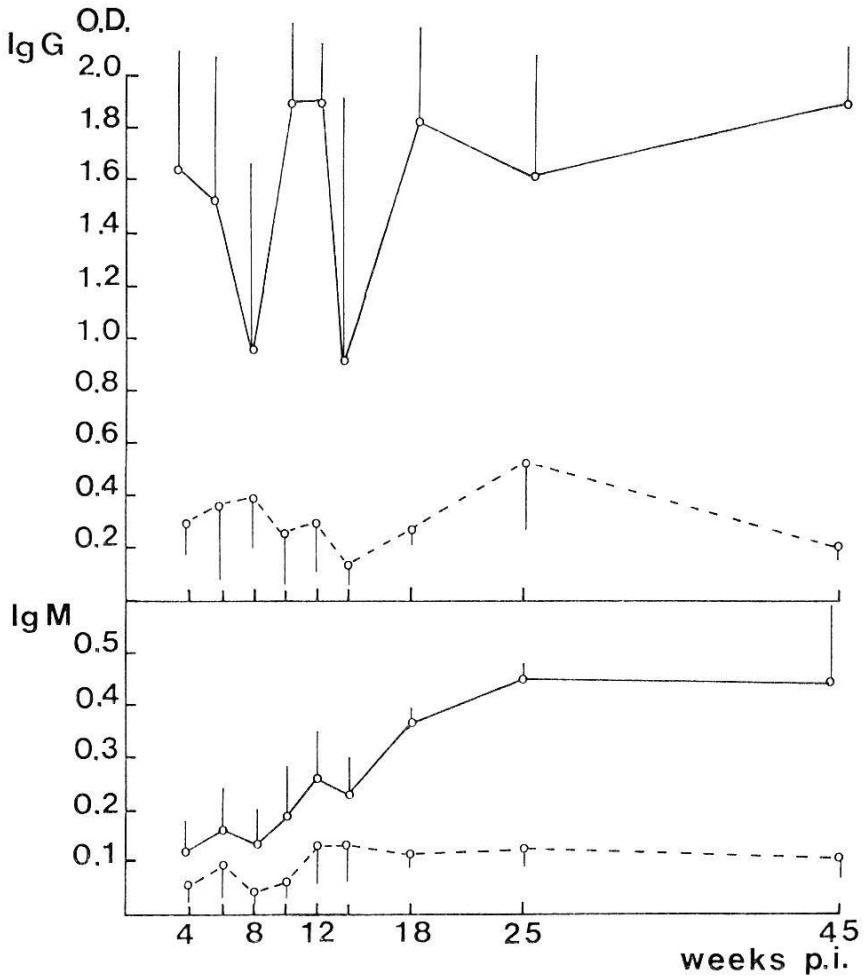

Fig. 1. Antibody-levels (IgG, IgM) against adult worm antigen of *L. carinii* in 5 normal (--○--) and 5 with 30 third-stage larvae infected (—○—) male cotton rats, determined by ELISA at different times post infection (p.i.). Data are reported as mean + or – SD of optical density (OD).

(Fig. 1). No statistical differences could be observed, 8 and 14 weeks p.i., when the antibody levels of the infected CR were transiently depressed. The IgM levels were also lower at these times, but after 18 weeks p.i. the levels exceeded those of the normal animals significantly (U-Test, 2 $\alpha = 0.05$). Using the H-Test, the antibody levels of infected CR were significantly higher (IgG: 2 $\alpha = 0.01$; IgM: 2 $\alpha = 0.05$).

Against microfilarial antigen, the IgG as well as the IgM antibody level increased in infected CR from 4 to 6 weeks p.i., and at the onset of patency (8 weeks p.i.) the levels of both antibody classes were depressed (Fig. 2). A second reduction appeared 12 weeks p.i., which was 2 weeks earlier than with adult antigen. Using the U-Test (2 $\alpha = 0.05$) the IgG levels of infected animals were significantly higher beginning at 10 weeks p.i. while the IgM levels significantly differed from 18 weeks p.i. The course of the IgM (2 $\alpha = 0.05$) and IgG (2 $\alpha = 0.01$) curves differed significantly from those of normal CR in the H-test.

Against both antigens, the IgG levels were usually higher than the IgM levels. Furthermore, the antibody levels against adult worms generally exceeded

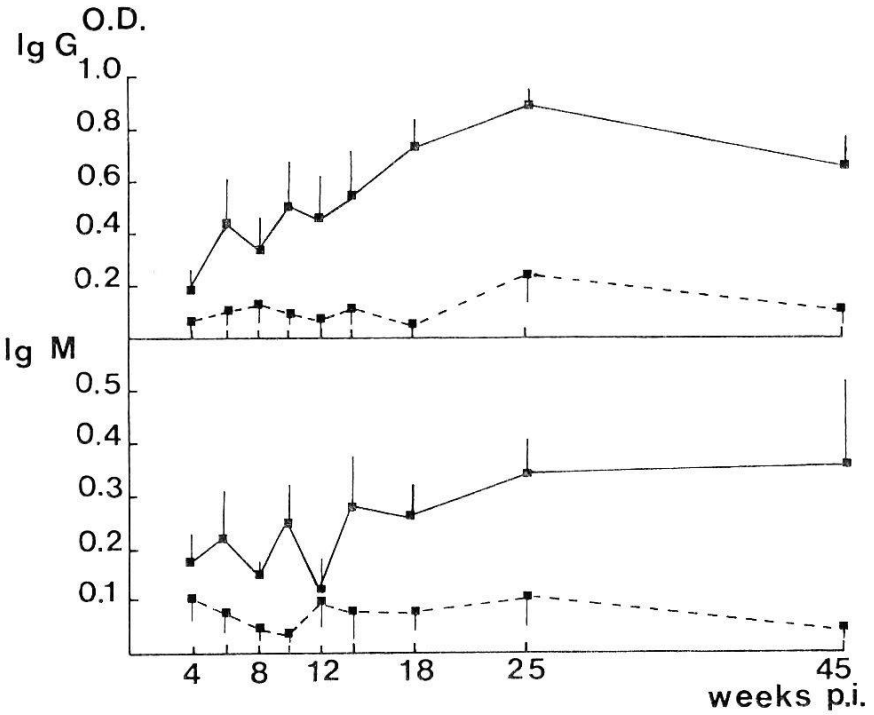

Fig. 2. IgG and IgM level against antigen of *L. carinii* microfilariae in 5 normal (--■--) and 5 with 30 third-stage larvae infected (—■—) male cotton rats, determined by ELISA at different times post infection (p.i.). Data are reported as mean + or – SD of optical density (OD) .

those against the microfilariae. At the end of patency, 45 weeks p.i., both IgG and IgM antibody levels against adult worm antigen increased. Against microfilarial antigen only the IgM levels increased, while the IgG level decreased.

Microfilariae of the internal organs were isolated from each of three male CR, which were infected 12, 25 and 45 weeks before. The distribution of the microfilariae in the organs changed during patency and also varied strongly between the animals (Table 1). In all organs the highest number of microfilariae were detected 25 weeks p.i., while the number in the peripheral blood decreased from 12 to 45 weeks p.i. The percentage of microfilariae in blood as a proportion of total microfilariae diminished continuously. In contrast, the portion in the lung, the organ with the second greatest number of microfilariae, increased from 12 to 45 weeks p.i. A similar change was observed in the spleen and the kidney, while the percentage in the liver and the heart increased from 12 to 25 weeks p.i. and then decreased. The total of the isolated microfilariae increased from 12 to 25 weeks p.i. and decreased by half at 45 weeks p.i.

Most of the total detected IgM and IgG antibodies (Mf×OD) on the microfilarial surface, were bound to the microfilariae of the internal organs, except the IgM level 12 weeks p.i. (Tables 2 and 3). The amount of antibodies bound to one microfilaria (OD/Mf) was generally the lowest in blood. Smaller amounts were only observed on heart (IgG) 25 weeks p.i. and on kidney microfilariae (IgM) 45 weeks p.i. The highest amount of IgM antibodies were bound to liver, 12 weeks p.i., and at the other times to spleen microfilariae, in contrast

Table 1. Number and percentual distribution of *L. carinii* microfilariae in the internal organs and the peripheral blood of 3 cotton rats each, infected with 30 third-stage larvae and autopsied 12, 25 and 45 weeks p.i., respectively

	Week 12 p.i.			Week 25 p.i.			Week 45 p.i.		
	Mf × 1000	SD ±	%	Mf × 1000	SD ±	%	Mf × 1000	SD ±	%
Heart	13.7	2.8	1.3	80.3	27.7	6.6	17.0	8.2	3.2
Spleen	7.1	5.7	0.7	24.0	13.1	1.9	11.5	4.3	2.2
Liver	16.0	4.9	1.5	130.0	112.5	10.7	20.6	10.5	3.9
Lungs	113.1	99.6	10.8	373.8	112.9	30.6	220.3	86.1	42.3
Kidney	30.1	11.2	2.8	50.6	8.1	4.1	26.7	2.8	4.9
Blood	862.5	362.2	85.9	563.0	187.4	46.1	225.0	69.7	43.5
Total	1042.5		100.0	1221.7		100.0	521.1		100.0

Table 2. Amounts of IgG and IgM antibodies in optical density (OD) bound to one adult-worm or to 1000 microfilariae isolated from each internal organ and the peripheral blood of 3 cotton rats each, infected with 30 third-stage larvae and autopsied 12, 25 and 45 weeks p.i., respectively. The levels were determined by ELISA using the Millititer-System (Millipore, USA).

	Week 12 p.i.		Week 25 p.i.		Week 45 p.i.	
	OD	SD	OD	SD	OD	SD
IgG						
Worms ♀	0.120	0.108	0.105	0.100	0.145	0.165
♂	0.049	0.027	0.062	0.058	0.082	0.063
Heart	0.243	0.046	0.069	0.060	0.079	0.038
Spleen	0.884	0.297	0.480	0.298	1.041	0.506
Liver	0.734	0.062	0.709	0.239	0.494	0.063
Lungs	0.300	0.288	0.099	0.070	0.429	0.215
Kidney	1.142	0.625	1.437	0.872	1.501	1.005
Blood	0.069	0.032	0.093	0.048	0.096	0.037
IgM						
Worms ♀	0.622	0.234	0.898	0.453	1.089	0.625
♂	0.184	0.318	0.624	0.218	0.872	0.537
Heart	0.915	0.789	0.802	0.230	0.855	0.881
Spleen	0.831	0.574	1.222	0.638	1.549	0.395
Liver	1.065	0.320	0.802	0.532	0.780	0.428
Lungs	0.829	0.412	0.378	0.143	0.445	0.199
Kidney	0.592	0.336	0.367	0.217	0.351	0.197
Blood	0.277	0.088	0.225	0.085	0.360	0.073

Table 3. Calculation of the total amount of antibodies in optical density (OD) bound to all *L. carinii* microfilariae of one cotton rat ($\frac{\text{number of all MF}}{1000}$ × measured OD of 1000 MF; Mf × OD) and the amount bound to only one microfilaria ($\frac{\text{measured OD of 1000 Mf}}{1000}$ OD/Mf) in the internal organs and peripheral blood of 3 cotton rats each, infected with 30 third-stage larvae and autopsied 12, 25 and 45 weeks p.i., respectively

	Week 12 p.i.		Week 25 p.i.		Week 45 p.i.	
	Mf × OD ($\times 10^3$)	OD/Mf ($\times 10^{-5}$)	Mf × OD ($\times 10^3$)	OD/Mf ($\times 10^{-5}$)	Mf × OD ($\times 10^3$)	OD/Mf ($\times 10^{-5}$)
IgG						
Heart	3.32	24.3	5.54	6.9	1.34	7.9
Spleen	6.27	88.4	11.52	48.0	11.97	104.1
Liver	11.74	73.3	92.17	70.9	10.17	49.4
Lungs	33.93	30.0	37.00	9.9	94.50	42.9
Kidney	34.92	114.2	72.71	143.7	40.07	150.1
Int. org.	89.63 (60.1%)		218.94 (80.7%)		158.10 (88.3%)	
Blood	59.51 (39.1%)	6.9	52.35 (19.3%)	9.3	21.60 (11.7%)	3.7
	149.14		271.29		179.70	
IgM						
Heart	12.53	91.5	64.40	80.2	15.05	85.5
Spleen	5.90	83.1	29.32	122.2	17.81	154.9
Liver	17.04	106.5	104.26	80.2	16.07	78.0
Lungs	93.75	82.9	141.29	37.8	98.03	44.5
Kidney	17.81	59.2	18.57	36.7	9.37	35.1
Int. org.	147.08 (38.1%)		357.84 (73.9%)		156.33 (65.9%)	
Blood	238.91 (61.9%)	27.7	126.67 (26.1%)	22.5	81.00 (34.1%)	36.0
	385.94		484.51		237.33	

to the highest IgG amounts which were always found on microfilariae from the kidney. In the lung, the low IgG amount bound to one microfilaria was comparable to that of blood microfilariae 25 weeks p.i. The total of IgM antibodies bound to all lung microfilariae was always the highest of the organs.

On the surface of male and female worms the IgM levels were generally higher than those of IgG. Both antibody levels on the males increased continuously from 12 to 45 weeks p.i., whereas on female worms only the IgM level increased. The IgG levels decreased from 12 to 25 weeks p.i. and increased later.

Agglutinations of blood microfilariae isolated 45 weeks p.i. were observed up to a dilution of 1:81 using anti-IgG and up to 1:4096 using anti-IgM.

Discussion

Common surface antigens of the different *L. carinii* stages and on the host's blood cells could not be detected by the complement fixation. However, the microfilariae try to escape the host's immune reactivity by coating their surface with CR albumin (Stäb, 1985).

Against microfilariae, the IgG and IgM antibody levels were higher 4 weeks p.i. than in controls. At this time no microfilariae have been yet produced (Mössinger and Wenk, 1986).

Apparently a portion of the antibodies produced against adult worms crossreact or are identical to those against microfilariae. On the other hand, there must be different antibody specificities. Thus, in normal and particularly in infected CR, the IgG levels against adult worms were much higher than those against microfilariae. Moreover, the second transient lack of the IgG as well as of the IgM antibody responses against adult worms was more pronounced, and emerged two weeks later. This also indicates that two different but specific suppressor mechanisms exist, which were expressed when Müller-Kehrmann and Wenk (1986) observed the beginning of the normal T-cell suppression. Partially different antibodies have also been observed against third-stage larvae and microfilariae (Stäb, 1985).

The first transient depression of the anti-adult worm and anti-microfilariae antibody production was caused by the appearance of the microfilariae in the peripheral blood, i.e. when a bulk of foreign protein is introduced into the host (Müller-Kehrmann and Wenk, 1986). Towards the end of patency, all antibody levels increased with exception of the IgG levels against microfilariae. Increased antibody levels at patency were also observed in other filarioses (McGreevy et al., 1980; Rzepczyk and Bishop, 1984; Weiss, 1978; Jaquet, 1978).

The total of blood microfilariae diminished from 12 to 45 weeks p.i., while the number in the organs first increased and then decreased. In muscles, testes, brain, and skin practically no microfilariae could be observed (Bayer, pers. comm.). The percentage of microfilariae in heart and liver increased and later decreased, whereas a continuous increase was observed in spleen, kidney, and

especially in lung. This organ functions a sieve, where the bulk of microfilariae is accumulated (Wenk and Wegerhof, 1982). This was also observed by injection of isolated *L. carinii* microfilariae in native CR (Seeger, 1986; Wenk, 1986), as well as of *Dirofilaria immitis* microfilariae in dogs (Weil et al., 1982).

The amount of IgM antibodies bound to the surface of adult worms was higher than that of IgG. This is surprising since in the sera, the IgG levels against adult worm antigen were much higher than those of IgM and normally IgG molecules have a higher avidity. A part of the antibodies against adult worms are probably eliminated in the pleural cavity by absorption to excretion and secretion products of the adult worms as described by Ischii (1970).

Most antibodies on the surface of microfilariae belonged to the IgM class, which was found to be the functional class in *D. immitis* infected dogs (Rzepczyk and Bishop, 1984), in hamsters infected with *Dipetalonema viteae* (Weiss, 1978) as well as in CR infected with *L. carinii* (Stäb, 1985). The highest total of bound IgM antibodies was determined in the lung. In this organ about 7/8 of all produced microfilariae were destroyed (Wenk, 1986). The majority of antibodies of both classes were predominantly bound to organ microfilariae, especially in the spleen (mainly IgM) and in the kidney (IgG), while the lowest amounts were mostly observed on blood microfilariae. Furthermore in the peripheral blood, no agglutinated *L. carinii* microfilariae were found (Stäb, 1985) and also the fewest immobilized ones. In contrast, the most immobilized microfilariae were observed in CR with a low worm burden in the kidney and in those with a high burden in the spleen (Haas and Wenk, 1981). Evidently the bulk of antibodies against microfilariae was adsorbed by organ microfilariae, so that the blood microfilariae were not influenced in their mobility and could be taken up by the vector. Another mechanism to reduce the immune reactivity against microfilariae is apparently the large amount of antibodies on spleen microfilariae. Probably this bulk simulates an excess of antibodies and induces a feedback mechanism; an increase of specific T-suppressor cells against microfilarial antigen. Since there are no adult worms in the spleen, this might explain, why the antibody levels against adult worms were higher. A T-cell suppression in the spleen, beginning about 14 weeks p.i. has been observed in jirds infected with *Brugia pahangi* (Lammie and Katz, 1983) and in with *L. carinii* infected CR (Stäb, 1985).

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Chandrashekar R., Rao U. R., Rajasekariah G. R., Subrahmanyam D.: Separation of viable microfilariae free of blood cells on Percoll gradients. J. Helminth. *58*, 69–70 (1984).

Haas B., Wenk P.: Elimination of microfilariae (*Litomosoides carinii* Filarioidea) in the patent and in the immunized cotton rat. Trans. roy. Soc. trop. Med. Hyg. *75*, 143–144 (1981).

Hammerberg B., Rikihisa Y., King M. W.: Immunoglobulin interactions with surfaces of sheated and unsheated microfilariae. Parasit. Immunol. *6*, 421–434 (1984).

Ishii A.: Antigenicity of excretory and secretory products of cotton rat filaria, *Litomosoides carinii*. Jap. J. exp. Med. *40*, 39–45 (1970).

Jaquet C.: *Litomosoides carinii* (Travassos, 1919) Chandler, 1913 (Filarioidea) in cotton rats (*Sigmodon hispidus*, say et ord, 1825) and jirds (Meriones unguiculatus, Milne-Edwards, 1867): Comparison of the infection in relation to the immune response. Dissertation, University of Neuchâtel 1978.

Lammie P. J., Katz S. P.: Immunoregulation in experimental filariasis. I. In vitro suppression of mitogen-induced blastogenesis by adherent cells from jirds chronically infected with *Brugia pahangi*. J. Immunol. *130*, 1381–1385 (1983).

McGreevy P. B., Ratiwayanto S., Sekar Tuti, McGreevy M. M., Dennis D. T.: *Brugia malayi*: relationship between anti-sheath antibodies and amicrofilaremia in natives living in an endemic area of south Kalimantan, Borneo. Amer. J. trop. Med. Hyg. *29*, 553–562 (1980).

Mössinger J., Wenk P.: Fecundity of *Litomosoides carinii* (Nematoda, Filarioidea) in vivo and in vitro. Z. Parasitenk. *72*, 121–131 (1986).

Müller-Kehrmann H., Wenk P.: T-cell dependent and T-cell independent immune response in normal and in with *Litomosoides carinii* infected cotton rats. Trop. Med. Parasit. *37*, 364–368 (1986).

Raether W., Meyerhöfer W.: Quantitative Untersuchungsmethoden zum Nachweis von Mikrofilarien (*Litomosoides carinii*) mit Hilfe von Zählkammern nach Fuchs-Rosenthal und Jessen sowie Anreicherungsverfahren. Trop. Med. Parasit. *18*, 99–108 (1967).

Rzepczyk C. M., Bishop C. J.: Immunological and ultrastructural aspects of the cell-mediated killing of *Dirofilaria immitis* microfilariae. Parasit. Immunol. *6*, 443–457 (1984).

Sachs L.: Statistische Auswertungsmethoden. 2. Auflage. Springer-Verlag, Berlin 1969.

Seeger V.: Die Verteilung der Mikrofilarien von *Litomosoides carinii* (Nematoda, Filarioidea) in den inneren Organen der Baumwollratte. Staatsexamensarbeit 1986.

Söffner J., Wenk P.: Wirkung von Ivermectin auf die Verteilung der Mikrofilarien in den Organen und auf die Embryogenese bei der Baumwollratten filarie *Litomosoides carinii* (Nematoda: Filarioidea). Mitt. Österr. Ges. Tropenmed. Parasit. *7*, 229–234 (1985).

Stäb F.: Isolierung und Charakterisierung eines funktionellen Immunglobulins und Nachweis einer spezifischen Immunsuppression bei der Nagerfilarie *Litomosoides carinii* (Nematoda, Filarioidea). Dissertation, University of Tübingen 1985.

Voller A., Bartlett A., Bidwell D. E.: Enzyme immunoassays for parasitic diseases. Trans. roy. Soc. trop. Med. Hyg. *70*, 98–106 (1976).

Wegerhof P. H., Wenk P.: Studies on acquired resistance of the cotton rat against microfilarie of *Litomosoides carinii*. I. Effects of single and repeated injections of microfilariae. Z. Parasitenk. *60*, 55–64 (1979).

Weil G. J., Powers K. G., Parbuonl E. L., Line B. R., Furrow R. D., Ottesen E. A.: *Dirofilaria immitis*. VI. Antimicrofilarial immunity in experimental filariasis. Amer. J. trop. Med. Hyg. *84*, 477–485 (1982).

Weiss N.: Studies on *Dipetalonema viteae* (Filarioidea). I. Microfilaraemia in hamsters in relation to worm burden and humoral immune response. Acta trop. (Basel) *35*, 137–150 (1978).

Wenk P.: The function of non circulating microfilariae: *Litomosoides carinii* (Nematoda: Filarioidea). Dtsch. tierärztl. Wschr. *93*, 414–418 (1986).

Wenk P., Wegerhof P. H.: Studies on aquired resistance of cotton rat against microfilariae of *Litomosoides carinii*. II. Injection of microfilariae during prepatency. Z. Parasitenk. *68*, 321–329 (1982).

Wenk P., Illgen B., Seitz H. M.: In vitro Versuche mit *Litomosoides carinii* (Nematoda: Filarioidea). I. Haltung von adulten Weibchen und Mikrofilarien sowie Ausstoss von Mikrofilarien in verschiedenen Kulturmedien. Z. Parasitenk. *55*, 63–72 (1978).

Zahner H.: *Litomosoides carinii*-Infection der *Mastomys natalensis:* serologische Verlaufuntersuchungen mit der Komplementbindungsreaktion und indirekten Hämagglutination. Z. Parasitenk. *43*, 181–196 (1974).