Litomosoides carinii仔虫の免疫による感染防御

誌名	Japanese journal of veterinary science
ISSN	00215295
著者	野上, 貞雄 林, 良博 村田, 道里
巻/号	50巻5号
掲載ページ	p. 1035-1039
発行年月	1988年10月

Stage-specific Protective Immunity to Microfilariae of *Litomosoides carinii* in *Mastomys natalensis*

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(Received 29 February 1988/Accepted 2 July 1988)

ABSTRACT. Experiments were performed to know whether immunity to microfilariae (mf) is effective to the protection against infection with infective larvae (L_3) of *Litomosoides carinii* in *Mastomys natalensis*. Animals were immunized subcutaneously with 5×10^5 live mf at two week intervals for six times. Mf in peripheral blood were detected on the following day of subcutaneous inoculation, and mf density increased gradually during five weeks. The mf density in the blood began to decline from five weeks and four of 8 animals became amicrofilaremic after six weeks. Immunofluorescence revealed that these immunized individuals developed antibodies not only against mf but also against both L_3 and adult worms. Beginning at 11 weeks after initiation of immunization, animals received the challenge infection with L_3, and the protective effect induced by mf immunization was examined by the worm recovery three and 14 weeks after challenge infection. There was no suppressive effect of immunization with live mf to the challenge infection as determined by recoveries of developing and adult worms. These results indicated that protective immunity induced by mf immunization was stage-specific and not effective against L_3 to adult stage worms of *L. carinii* in *M. natalensis*.—KEY WORDS: *Litomosoides carinii, Mastomys natalensis,* microfilaremia, protective immunity.

Jpn. J. Vet. Sci. 50(5): 1035–1039, 1988

Protective immunity is an area under active investigation in filaria researches. Existence or absence of protective immunity has been reported by various investigators with different filaria-animal models [1, 3, 5, 7, 9, 10, 13, 20]. However, except for the case of vaccination with irradiated infected larvae (L_3) [2, 6, 19], it is still unclear whether the host animals sensitized by immunization or natural infections develop the protective immunity to infective larvae. On the other hand, it is well known that spontaneous amicrofilaremia occurs naturally in some filaria infections and that an anti-microfilarial immunity is mainly due to antibodies [5, 17, 18]. Thus, it is of interest to study whether the immunity to mf leads to the protection against different filarial stages; *i.e.*, L_3 and adult worms. In the present study, protective immunity against transplanted mf was established and protection against infection with L_3 or subsequent developing stages were investigated using *Litomosoides carinii* in *Mastomys natalensis*.

MATERIALS AND METHODS

Animals: *Mastomys natalensis* and the cotton rat, *Sigmodon hispidus*, used in this experiment were closed bred and maintained in this laboratory.

Microfilariae: Microfilariae (mf) of *L. carinii* were obtained from the blood of male cotton rats at 15 weeks postinfection. Mf were collected from heparinized blood by a centrifugation method [8] using Lymphoprep® (S. G. 1.072) as the density gradient solution.

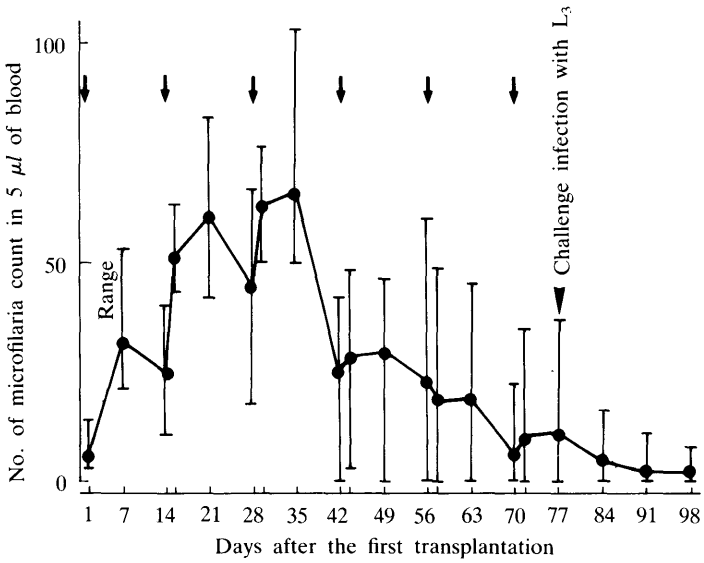

Fig. 1. Density of transplanted microfilariae (mf) of *Litomosoides carinii* in the peripheral blood of *Mastomys natalensis*. Arrows indicate transplantation of 5×10^5 mf.

Infective larvae: Infective larvae (L_3) were collected from skin of animals 12 hrs after exposure with heavily infected mites, *Ornithonyssus bacoti*, according to the method described by McCall [11].

Transplantation of mf and infection with L_3: Live mf (5×10^5) were injected subcutaneously into five week-old male *M. natalensis* once every other week for six times. Eleven weeks after the first mf inoculation, animals were inoculated subcutaneously with 50 L_3 into the inguinal region using a capillary pipette. Non immunized age-matched male *M. natalensis* received the same challenge infection as control. Both immune and non-immune group consisted of eight *M. natalensis*.

Observation of microfilaremia in peripheral blood: The number of mf in peripheral blood was counted by a quantitative blood smear method described previously [12]. Significance of difference in mean mf densities in the immunized group as compared to control group was determined by *t*-test.

Serology: Detection of antibodies in immunized animals against different stages of *L. carinii* was studied by indirect immunofluorescence (IF) using FITC conjugated anti-mouse IgG (L+H) rabbit antibody as labeled antibody.

RESULTS

Changes of mf density in the peripheral blood: The number of mf in peripheral blood was counted. Subcutaneously transplanted mf were manifested in the peripheral blood within 24 hrs.

As shown in Fig. 1, mf density increased after each of the first three transplantations, yet some amicrofilaremic individuals occurred as early as 6 weeks after the first immunization. In such individuals, mf were eliminated rapidly even during three times repeated transplantations with mf. No age effect of host on microfilarial elimination was observed in an experiment in which age-matched naive *M. natalensis* received 5×10^5 live mf subcutaneously and mf was detected within one day and continued more

Table 1. Recovery of worms of *Litomosoides carinii* from *Mastomys natalensis* after challenge infection

	3 weeks after infection				14 weeks after infection		
	No. of animal	No. of worms recovered			No. of animal	No. of worms recovered	
		live mean±SD	(%)[a]	dead mean (%)		mean±SD	(%)
Immunized	3	25.7±10.6	(51.4)	4.7(9.4)	5	33.4± 7.7	(66.8)
Control	3	29.7±14.6	(59.4)	3.0(6.0)	5	30.4±10.1	(60.8)

a) Percentage of the number of recovered worms to that of L_3 inoculated.

than one week (data not shown).

Protection against challenge infection with L_3: Protective immunities against challenge L_3 and subsequent developing stages were examined by worm recovery ratio three and 14 weeks after challenge infection. As shown in Table 1, worm recoveries were 51% in the immunized group and 59% in the control group three weeks after infection. Recoveries were 67% in the immunized group and 61% in the control group after 14 weeks. These results indicated that there was no protective effect against L_3 and subsequent developing stages.

Microfilaremia after challenge infection: Mf were detected in the immunized group 7 weeks after challenge infection and high mf density was observed in both groups after 14 weeks. However, enhancement of microfilaremia was observed in the immunized group in only 2 weeks, *i. e.*, 10 and 11 weeks after infection (Table 2).

There was no difference between the two groups with regard to ratios of mf density to female worms after 14 weeks as shown in Table 3.

Antibodies against different stages of L. carinii: Antibodies examined by IF were clearly detected not only against mf but also against L_3 and adult worms in animals immunized with live mf (Table 4). Positive reactions were observed on various sites of *L. carinii* except for the cuticle of both infective larvae and adult worms.

Table 2. *Litomosoides carinii* microfilaremia (mf/5μl) in immunized and control groups of *Mastomys natalensis*

Weeks after infection	No. of microfilariae (mean±SD) Immunized	Control
7	0.3± 0.5	0.0± 0.0
8	39.8± 23.4	22.0± 28.7
9	316.8±146.7	117.2±117.5
10[b]	787.5±191.5	274.0±200.8
11[a]	1198.0± 99.4	514.0±367.8
12	1037.5±163.9	633.2±384.7
13	1252.0±367.3	796.6±384.0
14	1654.8±390.7	1526.0±416.6

Significance level: a), $p<0.05$; b), $p<0.01$.

DISCUSSION

In the present study, experiments were performed to know whether immunity to mf is effective to the protection against L_3.

It is well known that anti-microfilarial immunity is mainly due to humoral immunity [4, 17, 18, 20]. Since, live mf are known to be more antigenic than dead ones or mf extracts [1, 15–17], thus we used live mf collected from blood as the immunizing antigen. As protective immunity against various stages of filarids is manifested differently in the individual filaria-animal models [3, 5, 9, 20] we, at first, confirmed the

Table 3. The microfilariae (mf) density and recovery of female worms of *Litomosoides carinii* in *Mastomys natalensis* 14 weeks after challenge infection

	Immunized				Control				
(A) Mf density (mf/5μl)	1424	1752	2161	1282	1622	1640	810	1904	1654
(B) No. of female worms recovered	11	17	23	21	8	21	14	11	20
Ratio (A/B)	129.5	103.1	94.0	61.1	202.8	78.1	57.9	173.1	82.7
Mean±SD		96.9±28.2				118.9±64.6			

Table 4. Detection of antibodies against various antigens of *Litomosoides carinii* by immunofluorescence in *Mastomys natalensis* immunized with live mf

Animal No.	Antigens			
	Microfilaria	Infective larva	Female adult	Male adult
A-2[a)]	++	++	+	++
A-3[a)]	++	+	+	++
A-4	++	++	+	++
B-1	++	+	+++	++
B-2	++	++	++	++
B-3	++	++	++	+++
B-4[a)]	++	++	+++	++
B-5[a)]	++	++	+++	++

a) Amicrofilaraemia at challenge infection with L_3.

ability of mf of *L. carinii* to induce anti-microfilarial immunity in *M. natalensis*. Previously Hass and Wenk [5] have reported anti-microfilarial immunity in *L. carinii* in the cotton rat by intravenous transplantation of live mf derived from uteri of adults. In the present study, similar anti-microfilarial immunity was established and expressed within a period of six weeks after the first immunization, although the sensitization method was different. However, this anti-microfilarial immunity was not protective against L_3, developing worms or adults as determined by challenge infection.

Recently Kazura *et al.* [7] reported protective immunity to L_3 following immunization with an extract antigen of mf in *Brugia malayi*-jird model. Moreover, Denham *et al.* [3] reported anti-microfilarial immunity after several repeated infections with L_3 in *B. pahangi*-cat model. These results have indicated that protective mechanism(s) between L_3 and mf was non-stage-spcific. In the present study, antigenic cross-reactivity between mf and both L_3 and adult worms was observed by indirect IF, but protective immunity to mf was mf-specific and not effective against L_3 to adult stage worms of *L. carinii* in *M. natalensis*.

It has been reported that immunization with mf suppressed mf production by adult female filariid worms [4, 14, 16, 17, 20]. On the other hand, enhancement of infection by a prior sensitization has been reported for *Dipetalonema viteae* in hamsters [4], *B. pahangi* in the jird [9], *B. malayi* in mice [6] and *L. carinii* in cotton rats [15]. Interestingly, enhancement of microfilaremia in the immunized group at the early patent period was observed in this study. This phenomenon in the present study should be investigated by further experiments.

ACKNOWLEDGEMENTS. We acknowledge Prof. Bruce Hammerberg, North Calorina State University for reviewing the manuscript.

This investigation was supported in part by Grant-in Aid (Nos. 61570190 and 62770267) from the Scientific Research Fund of the Ministry of Education, Science and Culture, Japan.

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要　　約

Litomosoides carinii 仔虫の免疫による感染防御：野上貞雄・林　良博・村田道里[1)]・中垣和英[2)]・田中　寛（東京大学医科学研究所寄生虫研究部，[1)]自治医科大学動物センター，[2)]東京農工大学獣医学科獣医内科学教室）——ミクロフィラリア（mf）に対する防御免疫が感染幼虫（L_3）の感染防御に関与するか否かを *Litomosoides carinii* を用い，マストミス（*Mastomys natalensis*）で実験した．生きた50万の mf をマストミス皮下に2週間隔で6回注入し，初回注入から11週後に L_3 の定量感染を行い，3週と14週後の虫体回収率から感染防御の有無を検討した．皮下に注入された mf は1日後に流血中で検出され，移入5週後までは注入毎に mf 密度は増加した．しかし6週以後は mf を注入しても減少し，8例中4例は無 mf 血症になり mf に対する強い防御効果が示された．一方，虫体回収率は，無処置対照群と差がなく防御効果は認められなかった．免疫蛍光法で mf と L_3 に共通抗原の存在が認められたものの，移入 mf に対する防御免疫は stage-specific で，L_3 には有効でないことが示された．