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PATTERN OF IMMUNE RESPONSE IN RECIPIENTS AFTER TRANSFER OF MESENTERIC LYMPH NODE CELLS SENSITIZED WITH LOW AND HIGH DOSES OF ANCYLOSTOMA CANINUM*

V. V. VARDHANI and G. N. JOHRI

Helminthology-Immunology Laboratory, School of Studies in Zoology, Vikram University, Ujjain

Abstract. Cellular immune response was detected in female Swiss albino mice with mesenteric lymph node cells during *Ancylostoma caninum* infection. Sensitized lymphocytes responded vigorously to *A. caninum* antigens and it was found that the cells (singly or repeatedly) sensitized with either low or high doses could confer adoptive protective immunity in recipients later challenged with a single dose of 500 *A. caninum* larvae.

In previous communications on the mechanism of adoptive immunity, it has been shown that sensitized mesenteric lymph node and peritoneal exudate cells play a definitive role in the rejection of a challenge dose of *Ancylostoma caninum* larvae by the mouse immune system (Vardhani and Johri 1977, 1978a, b). The precise picture about the involvement of such immune cells in the rejection mechanism is now becoming clearer. It has now been well established that a relation exists between changes in intestinal mast cells, histamine levels and worm expulsion during *Nippostrongylus brasiliensis* infection in rats (Wells 1962) and *A. caninum* infection in mice (Vardhani and Johri 1978c). The results of the present studies further confirm the role of sensitized mesenteric lymph node cells (MLNC) in bringing about specific effects in the immune system of recipients indicative of the specificity of functioning of the lymphoid cells.

MATERIAL AND METHODS

Infective *Ancylostoma caninum* larvae and mesenteric lymph node cells were obtained according to the methods reported previously (Vardhani and Johri 1977). Isogeneic Swiss albino mice (both donors and recipients) used in this study were females at the age of 6—8 weeks (20 to 23 g wt.). They were fed on Gold Mohur mice feed by Hind Lever Co., Bombay and clean drinking water and libitum. Each donor group consisted of 15 mice and 36 mice were included in each recipient group. Singly sensitized (from groups A, 500; B, 2 000), repeatedly sensitized (from groups C, 125 + 125 + +250; D, 500 + 500 + 1 000) and normal (uninfected, from group E) mesenteric lymph node cells were collected and approximately 15×10^4 cells were transferred intraperitoneally into 5 groups (a, b, c, d and e) of recipient mice respectively within 3 hours after their collection. Each mouse was challenged with a single dose of 500 infective larvae at 21 days after cell transfer and necropsied at 4 hourly intervals by Baermann's technique through pepsin digestion process. At each necropsy, the larvae were collected and counted from various organs and body muscles.

Schedule of infection to donors and injection to 5 groups of recipients (four experimental and one control) mice is shown below:

*) Part V of the series Delayed (cellular) hypersensitivity in mice during experimental ancylostomiasis.

Days of infection	Singly infected		Repeatedly infected		Control E
	A	B	C	D	
0	500	2 000	125	500	—
7	—	—	125	500	—
14	—	—	250	1 000	—
21	Cell collection and transfer to 5 groups (a, b, c, d and e) of recipients				
42	Challenge infection of 500 larvae at 21 days after cell transfer				

RESULTS

The total percentage of larvae recovered from adoptively immunized and control groups of mice is summarized in Table 1. Maximum expulsion of challenged larvae took place within the first 4 hours in all the experimental recipients leaving behind a very small percentage of larvae (31.2 % in group a, 23.6 % in b, 36.0 % in c and 27.2 % in d). Larval counts declined sharply thereafter. The experimental group of animals was devoid of larval burden by 120 hours in contrast to controls where the level of burden stood at 19.0 % at the same time. The rate of expulsion and/or destruction of challenged larvae in the experimental groups was maximum from 4—12 hours after challenge (9.2 % and 7.2 % in group a, 6.6 % and 4.4 % in b, 4.6 % and 3.4 % in c and 4.2 % and 8.2 % in d between 4 to 8 and 8 to 12 hours respectively). The gastrointestinal tract from where the migration and expulsion of larvae start as early as 4 hours after challenge became devoid of larval burden at 24 hours from its maximum at 20 hours (3.6 % in group a, 6.6 % in b, 8.4 % in c and 5.8 % in d) in case of recipient group of animals, whereas in controls this occurred fairly delayed from 120 (2.6 %) to 144 (nil) hours. The larvae migrated into muscles within 4 hours in group a (0.6 %), 12 hours in b (0.6 %), 16 hours in both c (5.6 %) and d (4.0 %) and 48 hours in e (16.4 %).

Table 1. Total percentage of *Ancylostoma caninum* larvae from different organs of experimental and control mice at each necropsy*). (Values are expressed in mean derived from three animals.)

Hours of necropsy	Experimental recipient groups				Control recipient group
	a	b	c	d	e
4	31.2	23.6	36.0	27.2	86.2
8	22.0	17.0	32.6	23.0	80.0
12	15.2	12.6	29.2	14.8	70.0
16	14.2	12.2	22.8	13.8	64.0
20	11.6	8.8	14.8	9.2	59.4
24	6.8	7.4	11.6	8.4	57.2
36	6.0	3.4	9.2	4.2	47.4
48	3.6	2.8	6.6	3.8	41.2
72	2.8	2.0	2.8	3.6	33.4
96	1.0	1.2	0.6	2.8	26.8
120	—	—	—	—	19.0
144	—	—	—	—	16.2

*) — separate data from different organs and body musculature are not given separately.

No larvae migrated into lungs in all the four experimental groups throughout the experimental period and a maximum migration of 6.2 % by 20 hours after challenge to lungs took place in case of control animals. Larval migration into liver, however, reached a maximum of 5.2 % by 24 hours in case of group b; in controls it was 20.0 % by 12 hours.

DISCUSSION

The elimination of measurable worm burden as early as 4 hours after challenge in experimental animals proves that both singly and repeatedly sensitized MLNC could produce detectable adoptive immunity fairly early in recipients. Nevertheless, groups with singly sensitized cells provided greater immune response compared to those counterpart groups with repeatedly sensitized cells, the donors of which had received the same number of larvae but in repeated doses. Within the gastrointestinal tract itself, the larval burden was higher in groups a (30.0 %) and d (27.2 %) than in groups b (23.6 %) and c (23.6 %) at 4 hours after challenge with some of the larvae having migrated into liver and muscles (especially in groups a and c); it is significant that the larvae had been forced to move away from their normal site within an extremely short interval. In fact, it seems that these larvae did not stay in the intestine and were most probably also subjected to an unleashing attack by specific antibodies and/or cellular factors essential to drive them out of the gut and/or resort them to forced migration. Findings somewhat similar to these have also been reported by Ogilvie and Jones (1971) with *N. brasiliensis* in rats.

An important finding in the present investigation is that severe restrictions are placed on the migration of larvae; very few could migrate and reach the liver and none penetrated into the heart or lungs. The larvae boring through the intestinal wall and emerging into the peritoneal cavity find another barrier, that of sensitized peritoneal fluid and exudate cells which have increased enormously preventing them from boring the liver capsule (their probable route of entry into the organ). Thus, in the normal course, control animals exhibit a sizeable percentage of larvae in the liver and lungs (Banerjee et al. 1970) and this migration is essential for their survival (Larsh 1975). It seems most unlikely that the larvae which failed to reach liver would, after getting damaged in the peritoneal cavity, pierce through the diaphragm to reach the pleural cavity and the lungs, resulting in absolute prevention into lungs either by way of liver or through the diaphragm in experimental animals. Also, there are unrestricted channels to reach their place of choice. This is also indicative of the fact that the larvae are incapable of lysing the host tissue for penetration and it is possible that the enzymes required for this might have been destroyed during the immunological injury to them. Such findings are also supported by Lewert and Lee (1954) who found penetrating enzymes in *A. caninum* larvae. Lewert (1958) also found penetrating enzymes at least in actively penetrating stages of the parasite.

The earlier migration and rapid concentration of larvae into muscles (within 4 hours in group a, 12 hours in b and 16 in c and d) are the cumulative effects of immunological reactions. Nonsurvival of larvae in the muscles of experimental animals by 96 hours in groups a, b, c and d explains that the transferred cells and the immune system of recipients further effected the already damaged worms previously in gastrointestinal tract. This conclusion seems valid because of the complete rejection of larvae from the muscles. Our findings are also supported by the observations made by Ogilvie and Hockley (1968) and Dineen and Kelly (1973) who stated that transplanted worms (damaged) were more susceptible than the normal ones

Our results also show that there is a correlation between the size of infection in donors and the degree of sensitivity induced, and that the protective immunity can

be transferred to *A. caninum* with MLNC taken from singly and repeatedly infected mice while actively responding to tissue phase. Larsh et al. (1964) failed to produce significant response in three groups of recipients that repeatedly received cells from donor mice also repeatedly infected with *Trichinella spiralis*.

We would thus suggest that three important functional aspects play significant roles in the host's immunological capacity in this model; one concerns the mechanisms involved in the development of the immunopathology, the second the establishment of protective immunity and the third the destruction of the parasite within the host; these are the mechanisms due to which the worms cannot escape detection or damage by the host's immune system.

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ИМУННЫЙ ОТВЕТ РЕЦИПИЕНТОВ ПОСЛЕ ПЕРЕНОСА МЕЗЕНТЕРИАЛЬНЫХ КЛЕТОК ЛИМФАТИЧЕСКОГО УЗЛА, СЕНСИБИЛИЗИРОВАННЫХ НИЗКИМИ И ВЫСОКИМИ ДОЗАМИ *ANCYLOSTOMA CANINUM*

В. В. Вардани и Г. Н. Джори

Резюме. Изучали клеточный имунный ответ у самок мышей, экспериментально зараженных *Ancylostoma caninum*. Сенсибилизованные лимфоциты давали высокий уровень иммунитета против *A. caninum* антигенов. Было обнаружено, что клетки, однократно или повторно сенсибилизированные низкими или высокими дозами, могут вызывать восприимчивый защитный иммунитет у реципиентов при заражении дозой 500 личинок *A. caninum*.

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V. V. V., Helminthology-Immunology Laboratory, School of Studies in Zoology, Vikram University, Ujjain (M. P.) 456010, India