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Protective Immunity to *Hymenolepis nana* in Mice II. Actively, Dividing Mesenteric Lymph Node Cells are Responsible for Transfer of Immunity to *Hymenolepis nana* in Nude Mice

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Abstract

We examined ^{3}H-Thymidine incorporation of mesenteric lymph node cells (MLNC), prepared from mice infected with *Hymenolepis nana*, and transfer of protective immunity to nude mice by the injection of MLNC fractionated with Percoll density gradient.

^{3}H-Thymidine incorporation of MLNC prepared from mice infected with 1,000 eggs was higher than that of non-infected control. Treatment with anti-Thy-1.2 monoclonal antibody plus complement reduced incorporation of ^{3}H-Thymidine of MLNC.

The blast-enriched population of MLNC, prepared from mice infected for 4 days, transferred a degree of immunity comparable to that induced by transfer of unfractionated populations. However, such a effect on the rejection was not observed in the recipient nude mice receiving other cell populations.

Key words: *Hymenolepis nana*, nude mouse, protective immunity, adoptive transfer, lymphoblast

Introduction

In direct infection of *Hymenolepis nana,* a rapid and long lasting resistance to reinfection develops in infected mice and the challenging dose is completely blocked (Hearin, 1941). However, repeated infections are established when the congenitally athymic nude mice given a single oral inoculation with *H. nana* eggs (Reed *et al.,* 1977). It has been also shown that the protection is abolished by neonatal thymectomy (Okamoto, 1968) and by administration of anti-thymocyte serum (Okamoto and Koizumi, 1972). In order to analyse this type of immunity, several transfer experiments have been reported using immune spleen cells (Friedberg *et al.,* 1967) and peritoneal exudate cells (Gupta *et al.,* 1980). However, the degree of immunity produced by injection with these cells was not of the order elicited by a primary infection. On the other hand, Asano *et al.* (1986) reported that transfer of mesenteric lymph node cells (MLNC) collected from donor mice on day 4 of infection (4-day-MLNC) produced a complete rejection of cysticercoids infection, but the complete rejection was not observed in the recipient nude mice received MLNC collected from donor mice on day 21 of infection (21-day-MLNC). However, the reasons are not yet fully understood why short-term (4 days) sensitized MLNC are competent for adoptive transfer of complete rejection and long-term (21 days) sensitized MLNC do not provide complete rejection.

The purpose of present study was to monitor blastic activity of MLNC using ^{3}H-Thymidine and to examine the correlation between blastic activity of MLNC and ability of MLNC to transfer immunity.

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Materials and Methods

Mouse: Male normal BALB/c mice (Charles

River Japan) were used as donors at about 5 weeks of age. Male athymic nude mice (CLEA Japan), progeny of BALB/c strain, were used as recipient at 4 weeks of age.

Parasite: The methods for preparing egg suspension of *H. nana* and for examining cysticercoids were as described previously (Okamoto, 1968). The number of eggs used in this study was 1,000 eggs per mouse.

Cell suspension: Suspension of MLNC in RPMI-1640 medium (Boehringer Mannheim) supplemented with 2% heat-inactivated FCS (GIBCO, Lot No. 26P9061), 100 U/ml penicillin and 100 μg/ml streptomycin (RPMI-FCS) were prepared from donor mice by the method described previously (Asano *et al.*, 1986). The number of cells presented in the suspension was counted in a haemocytometer. The viability of cells was assessed by a trypan blue dye exclusion test.

Antibody: Since it has been shown that Thy-1.2 antigen is presented on T cells of BALB/c mouse, monoclonal antibody to Thy-1.2 (Miles Yeda, Lot No. 6) was used to specifically inhibit the immunological properties of T cells. The amount of antibody for 50% killing of thymocytes was 1.0 μg/5 x 10^5 cells.

Complement: Guinea pig serum was used as source of complement. It was absorbed with Noble Agar (Difco) in order to remove natural cytotoxic antibody to mouse T cells (Cohen and Schleginger, 1970).

Complement-mediated cytolysis: Suspension of 2 x 10^7 cells (0.1 ml), 100 μg antibody and 1 ml of guinea pig serum diluted 1:3 were placed in a test tube and mixed with a pasteur pipet. After incubation at 37°C for 45 min, the cells were washed three times with RPMI-FCS and the viable cell concentrations were adjusted to 4 x 10^6 cells in 1 ml of RPMI-FCS.

Density gradient fractionation: Percoll (Pharmacia, Lot No. MF 02356) was made isotonic for use with living cells by adding 1 volume of 10 times concentrated Hank's Balanced Salt Solution (HBSS; GIBCO) to 9 volume of commercial preparation. For subsequent use the Percoll solution was diluted at either 60, 50 or 20% in isotonic HBSS. The diluted Percoll solution was carefully layered one after another in 12 ml plastic centrifugation tube (Nalgen). MLNC suspensions as prepared above were resuspended to 100 x 10^6 cells in 1 ml of HBSS. Aliquots of 100 x 10^6 cells were layered on top of the gradient and centrifuged at 3,000 rpm for 15 min at room temperature (Kurnic *et al.*, 1979). After centrifugation, the cells were harvested with a pasteur pipet at the interface between two different densities and bottom of the tube. The cells were washed 5 times with RPMI-FCS. The cells were then counted and resuspended in fresh RPMI-FCS at the appropriate cell concentration suitable for the subsequent experiments.

Incorporation of ^{3}H-Thymidine and determination of radioactivity: The concentration of the viable cell suspension was 4 x 10^6 in 1 ml of RPMI-FCS. Aliquots of the cell suspension (8 x 10^5 cells) were placed into each well of a sterile U-bottomed microculture plate (1-63320, Nunclon) and 2 μCi of ^{3}H-Thymidine (specific activity, 20.0 Ci/mM; New England Nuclear) was added to each well. The plate was maintained for 2 hrs in a humidified 5% CO_2 atmosphere at 37°C. The cells were harvested onto glass fiber filter discs with a semiautomatic cell harvester (Abe Kagaku). The discs were washed 3 times with distilled water and each disc was placed in a 25 x 60 mm scintillation vial (Wheton). They were allowed to stand in an incubator at 60°C to dry the discs. After 45 min, 5 ml of scintillation fluid [Toluen base containing PPO (3 g/l), POPOP (0.1 g/l)] was introduced into the vial. Radioactivity was measured in a Packard Tricarb liquid scintillation spectrometer (Packard). The results were expressed as the mean counts per minute (cpm) for triplicate cultures with the standard deviation.

Cell transfer and assay of protective immunity: Recipient nude mice were intravenously injected three times with MLNC on days -6, -4 and -2 relative to the challenge. The cell dosage transferred was 3 x 10^7 cells/injection in one experiment and 5 x 10^7 cells/injection in

another. All the recipient nude mice were challenged with 1,000 eggs 2 days after the last injection. All the mice were killed 4 days later and intestinal cysticercoids were counted by the method previously described (Okamoto, 1968).

Results

Experiment I

In this experiment, changes of cellularity in the mesenteric lymph nodes in terms of ^{3}H-Thymidine incorporation were monitored. Donor mice (6 mice/group) were infected with eggs either 4 or 21 days prior to cell harvest. Control mice received no infection. Such experiments were repeated twice. The results are shown in Table 1.

The mean response of cpm was 13,543 in MLNC from non-infected mice, 41,650 from mice infected for 4 days and 24,661 from mice infected for 21 days. ^{3}H-Thymidine incorporation of MLNC was completely eliminated, when they were treated with anit-Thy-1.2 monoclonal antibody plus complement.

Experiment II

In this experiment, MLNC prepared from infected mice were fractionated by Percoll density gradient centrifugation. Fractionated cells were tested for their ability to transfer immunity. Four fractions were obtained by density gradient centrifugation; cells harvested on the top of 20% Percoll (Fraction I), at the interface between 20% and 50% Percoll (Fraction II), at the interface between 50% and 60% Percoll (Fraction III) and the bottom of tube (Fraction IV). The maximum ^{3}H-Thymidine incorporation of fractionated cells was observed in Fraction II and the minimum in Fraction IV. A comparison was then made of ability of fractionated cells to transfer immunity by injecting cells with unfractionated, Fraction II and Fraction IV. To accomplish this, recipient nude mice were divided into 7

Table 1 ^{3}H-Thymidine incorporation of mesenteric lymph node cells obtained from BALB/c mice infected with 1,000 *Hymenolepis nana* eggs.

Days after infection	^{3}H-Thymidine incorp. (cpm/8×10^{5} cells)	
	non-treated	treated with anti-Thy-1.2+complement
—	13543±1374	2340±471
4	41650±5660	1791±446
21	24661±3994	3381±491

Table 2 Transfer of protective immunity against *Hymenolepis nana* in nude mice by the injection of density gradient (Percoll) separated immune mesenteric lymph node cells.

Group	Interval between oral infection and collection of cells from donor	Donor cells			No. of mice examined	No. of cysticercoids in individual mice
		Type	^{3}H-Thymidine incorp. (cpm/8 $\times10^{5}$ cells)	Total no. of cells injected ($\times10^{7}$)		
1	—	—	—	None	4	171 243 305 360
2	4	Unfractionated	41650±5660	15	6	0 0 0 0 0 1
3	4	Fraction II	58526±1662	15	7	0 0 0 0 0 1 3
4	4	Fraction II	—	9	5	0 0 0 0 1
5	4	Fraction IV	4699±1123	15	7	161 169 204 219 220 307 311
6	21	Unfractionated	24661±3994	15	3	127 135 135
7	21	Fraction II	53065±5746	15	5	27 29 48 52 64

groups. Mice of Group 1 served as non-injected control. Group 2 received 15×10^7 unfractionated 4-day-MLNC, others (Groups 3 and 5) fractionated 4-day-MLNC. Mice of Group 4 were injected with 9×10^7 fractionated 4-day-MLNC. Group 6 received 15×10^7 unfractionated 21-day-MLNC and the remaining one group were injected with 15×10^7 fractionated 21-day-MLNC (Group 7). The results are shown in Table 2.

All the recipient nude mice of Group 1 harboured cysticercoid ranging from 171 to 360 per mouse. Adoptive immunity was successful in 5 out of 6 mice of Group 2, in which mice received unfractionated MLNC prepared from donor mice immunized 4 days before cell collection. The remaining one mouse of this group harboured only one cysticercoid. Similar results were obtained in Groups 3 and 4, in which mice received 4-day-MLNC in Fraction II. However, mice received 4-day-MLNC in Fraction IV were all positive for cysticercoids and harboured cysticercoid ranging from 161 to 311 per mouse. Little or no effect was observed on cysticercoid rejection in mice that received 21-day-MLNC (Group 6), but the number of cysticercoid for Group 7, which received 21-day-MLNC in Fraction II, was significantly lower than that of Group 6.

Discussion

In *H. nana* – mouse system, mice received a primary infection with eggs are able to reject completely cysticercoids from challenge infection for their life time. In spite of this long lasting protective immunity to reinfection, adoptive transfer of immunity is possible only with cells prepared from donor mice within a limited period after egg inoculation. This fact may be interpreted to indicate that the cells responsible for transferring immunity are short-lived and survived for a limited period in donor mice. Although Palmas *et al.* (1984) reported that the protective immunity was adoptively transferable to naive mice with rapidly dividing mesenteric lymph node cells, the criterion for success of the transfer of protective immunity was the reduction in number of adult worms in the intestine of recipients. Since the inoculation with eggs leads to absolute protection to development of the cysticercoid in the intestinal villi, it is necessary that the criterion for success of the transfer immunity is the absence of cysticercoid in the intestinal villi of recipients. Consequently, their report does not make clear the characteristics of cells responsible for transfer immunity.

It is recognized that, when the lymphocytes contact with antigens, the DNA synthetic activity are increased. The degree of activity is assessed by measuring the amount of ^{3}H-Thymidine or other radiolabeled materials incorporated into newly synthetized DNA. The results presented in this paper lead to the suggestion that there is continuing antigenic recognition in the mesenteric lymph nodes of mice infected with *H. nana* and the antigens stimulate only T cells (Table 1).

In the next experiments, we examined the correlation between blastic activity of MLNC and capacity of MLNC to passively transfer immunity. As can be readily observed in Table 2, the two blast-enriched populations isolated from 4-day-MLNC transferred a degree of protective immunity comparable to that transferred by unfractionated populations. However, the blast-depleted population could not transfer protective immunity. Although the complete rejection of cysticercoids was not observed in the recipient mice received the blast-enriched population from 21-day-MLNC, the number of cysticercoids in individual mice was smaller than that in Group VI. As stated above, the mice receiving an oral inoculation of *H. nana* eggs inhibit cysticercoid development in the intestinal villi. Therefore, Short-lived proliferating cells activated during cysticercoid development are responsible for transferring immunity to nude mice.

The population of lymphoblasts prepared from the mesenteric lymph nodes of mice infected with *Trichinella spiralis* that can be labeled after incubation *in vitro* ^{125}I-UdR have

a marked tendency to migrate to the small intestine after injection into normal mice (Rose *et al.*, 1976). However, in *H. nana* – mouse system, the fate of lymphoblasts isolated from MLNC, after the intravenous injection to nude mice, is still unclear. Thus, further experiments are needed to clarify this point.

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