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Transfer of T-cell mediated immunity to *Hymenolepis nana* from mother mice to their neonates

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Abstract. Administration of lymph node cells from *Hymenolepis nana*-infected mice into lactating mothers, or directly suckling neonates successfully transferred immunity to the neonates. The capacity of lymph node cells to transfer immunity was completely abrogated by pretreatment with anti-Thy-1.2 monoclonal antibody and complement.
Key words. Hymenolepis nana; maternal transfer; protection; T-cell mediated immunity.

Immune mediators present in milk protect neonates from microbiological infections at a time when its own defense mechanisms may not be adequately developed. This type of immunity has been also demonstrated for parasitic infections of *Hymenolepis nana* in mice [1], *Taenia taeniaeformis* in mice [2], *Trichinella spiralis* in rats [3, 4] and *Schistosoma mansoni* in rats [5], among others. Although all of these reports showed that humoral factors in milk are important in providing immunity to suckling neonates, the cellular mediators in this immunity have received little attention.

H. nana, the dwarf tape worm of mice, is known to be strongly immunogenic. An oral inoculation with eggs elicits, during worm development in the intestinal villi, strong immune responses which confer protection upon the host by inhibiting larval growth in the intestinal villi: oncospheres from the challenge egg inoculation can invade the intestinal villi but cannot form cysticercoids there [6, 7]. It has been also shown that the immune responses responsible for inhibition of larval growth in the intestine of *H. nana*-infected mice are T-cell dependent. This was substantiated by studies in athymic nude mice [8] and in neonatally thymectomized mice [9, 10]. Furthermore, intravenous injection with mesenteric lymph node cells, especially Lyt-1 positive T cells from immunized donor mice have been shown to be capable of transferring immunity to athymic nude mice [11]. More recently, we reported that intravenous injection of BALB/c mice with monoclonal antibody to L3T4 antigen completely blocked not only development of immunity but also the increase in number of T cells characteristically induced by *H. nana* infection [12].

With regard to humoral antibody, Di Conza [13] indicated that serum from mice that received an oral inoculation of *H. nana* eggs had significant immune activity against subcutaneous larval growth, when the serum was injected into the area where the parasite was developing. Other observations [14, 15] also showed the presence of humoral antibody in serum from *H. nana*-infected mice. Since humoral antibody was detectable in serum only after 2 or more weeks of infection, its development can not account for the fact that the mice acquire immunity very rapidly, within 24 h.

Judging from these reports, the concept may be established that T cells are essential to initiate immunity to *H. nana*. Therefore, the *H. nana*-mouse system seemed to be a particularly good model for investigating maternal transfer of T-cell mediated immunity. We used this system in this study as a model of maternal transmission of immunity and examined whether T-cells responsible for development of immunity were adoptively transferred into suckling neonates via the milk.

Materials and methods

Mouse and parasite. BALB/c mice maintained under specific-pathogen free conditions were purchased from

Charles River Japan Incorporation (Atsugi, Japan) and used throughout this study.

H. nana utilized in this study is routinely maintained in this laboratory for about 20 years. The methods used for preparing egg suspension and for egg inoculation were similar to those used in our previous report[9].

Monoclonal antibody. The monoclonal antibody used in this study was mouse Ig G2b antibody against the Thy-1.2 antigen. The minimum amount of antibody to cause maximal killing of lymph node cells was 0.2 µg/ 5×10^6 cells. This antibody was purchased from Cedarlane Lab. Ltd., Canada.

Preparation of cell suspension. Preparation of cell suspension is described in detail elsewhere[11,12,16]. Briefly, mesenteric lymph nodes were taken from male mice infected with 1000 eggs 4 days before cell collection. The nodes, stripped of fat and connective tissue, were squashed between frosted microscope slides in Medium 199 (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan). The suspension was filtered through a 150-gauge stainless wire mesh, centrifuged at 200 × g for 10 min and resuspended in fresh medium at a suitable concentration for the experiments described below. This cell suspension is called immune MLNC. Non-immune cell suspensions were similarly prepared from age and sex matched non-infected mice.

Treatment of cells with monoclonal antibody plus complement. Suspension of 5×10^7 cells (1 ml) was incubated at 37 °C for 30 min with 0.1 mg of antibody. After washing, the pelleted cells were resuspended in 1:10 diluted Low Tox Rabbit Complement (Cedarlane Lab. Ltd., Canada) and incubated at 37 °C for further 45 min. After incubation, the cells were washed twice with Medium 199 and resuspended at a suitable concentration for subsequent experiments.

Preparation of milk extract. Pregnant mice, 6 weeks of age, were orally inoculated with 5000 eggs at day 15 of pregnancy. Day 1 of pregnancy denotes the day on which we could find a vaginal plug. At 7 days after delivery, the neonates were killed under ether anesthesia and the stomach contents were collected. Clotted materials were placed into phosphate-buffered saline at pH 7.0, and homogenized with a glass homogenizer for 15 min in an ice-cold water bath. The homogenized materials were then centrifuged at 1000 × g for 30 min at 4 °C. The supernatant fluid, which had a final protein concentration of 10 mg/ml, as determined by Bradford's method[17], was used as aqueous milk extract. Milk from neonates nursed by non-infected mothers provided the control aqueous milk extract.

Preparation of serum. Male donor mice, 6–8 weeks of age, were orally inoculated with 5000 eggs. After 5 weeks blood samples were taken by cardiac puncture under ether anesthesia, clotted and spun at 500 × g for 10 min. Serum was collected, stored at −40 °C and inactivated at 56 °C for 30 min before use. This serum, called the immune serum, was used as humoral factor (antibody) positive control. Non-immune serum was prepared from age and sex matched non-infected donor mice.

Assessment of immunity to H. nana. The criterion used for immunity was inhibition of larval growth from the oncosphere to the cysticercoid stage. Neonates were challenged at 7 days with 500 eggs. Four days after challenge they were autopsied, and cysticercoids present within the intestinal villi were counted under × 40 magnification[9]. If the intestines harbored no cysticercoids, they were carefully examined for the presence of earlier stage larvae under × 200 magnification. To examine for the presence of humoral factor related to immunity in milk and in serum, a slight modification of the procedure described by Di Conza[13] was used. Briefly, a 0.1-ml sample containing 500 eggs was injected subcutaneously into the back of an etherized 6-week-old mouse. This was followed by daily injection of 0.1-ml samples not containing eggs at the location of the parasites from day 1 – day 6 after inoculation. On the seventh day all mice were autopsied and the numbers of developing larvae were counted under × 200 magnification.

Experimental design. Four types of experiments were performed in this study. In Experiment I, pregnant, 6-week-old mice were orally inoculated with 5000 eggs each at day 15 of pregnancy and placed in separate cages. Soon after delivery, each litter size was adjusted to five or six neonates per mother. For cross-fostering experiments, immune mice were established as above, but immediately after birth, neonates born of non-immune and immune mothers were exchanged and cross-fostered. All neonates were challenged with 500 eggs when 7 days old. Worm counts were made 4 days after challenge. In Experiment II, eggs of *H. nana* were suspended in aqueous milk extract prepared as described above at a concentration of 5000 eggs per ml. A 0.1-ml sample was injected subcutaneously into the backs of normal mice. The same dose of a sample not containing eggs was then injected into the locale of the parasites daily for 6 days. On the seventh day all mice were autopsied and developing larval were counted. Experiments using immune serum were performed as humoral factor (antibody) positive control using the same dosage and injection schedule employed with aqueous milk extract. In Experiment III, aliquots of 3×10^8 cells were injected intravenously into naive lactating recipients on day 1 postpartum. All neonates, at 3 days old, were challenged with 500 eggs, and autopsied 4 days later to perform worm counts. In Experiments IV, neonates born of naive mothers were fed a total of 3×10^8 cells in a single dose of 1×10^8 cells (50 µl) on days 1, 3, and 5 after birth. All neonates were challenged with 500 eggs 2 days after the last feeding, and killed 4 days later to perform worm counts.

Table 1. Transfer of immunity to *Hymenolepis nana* from mother to neonates

Group	No. of neonates Examined	Harbored onco.	Harbored cyst.	No. of worms (mean ± SD) onco.	cyst.
Neonates nursed by own naive mothers	11 (2)	0	11	0 ± 0	32 ± 5.6
Neonates nursed by own immune mothers	29 (5)	19	10	42 ± 14.0	2 ± 1.3
Neonates born of immune mothers and nursed by naive mothers	20 (4)	0	20	0 ± 0	42 ± 10.3
Neonates born of naive mothers and nursed by immune mothers	28 (6)	25	3	54 ± 17.1	2 ± 0.9

onco.: oncospheres, cyst.: cysticercoids; figures in parentheses represent number of mother mice.

Results

Transfer of immunity to H. nana during lactation. Experiment I was designed to examine whether female mice immunized to *H. nana* by oral egg inoculation would transfer immunity to their neonates. From table 1, it can be seen that control neonates nursed by two naive mothers showed no evidence of immunity. However, neonates nursed by immune mothers were significantly immune; 19 of the 29 neonates from five immune mothers harbored oncospheres and the remaining 10 neonates each harbored a smaller number of cysticercoids than the control neonates. Neonates born of immune mothers and foster-nursed by naive mothers showed no immunity, and 20 neonates nursed by four naive mothers were all positive for cysticercoids. Of the 28 neonates born of five non-immunized mothers and nursed by five immune mothers, 25 neonates harbored oncospheres. The remaining 3 neonates harbored a small number of cysticercoids.

Influence of aqueous milk extract on subcutaneous larval growth. Experiment II was designed to examine whether a humoral factor responsible for inhibition of larval growth was present in aqueous milk extract. The data in table 2 show that immune serum was positive for a humoral factor that inhibits subcutaneous larval growth. However, aqueous milk extract did not influence subcutaneous larval development. Control and experimental mice had approximately equal numbers of fully developed cysticercoids.

Influence of maternal injection of MLNC on transfer of immunity to neonates. Experiment III was designed to examine whether maternal injection of MLNC would lead to passage of immunity to their neonates. The intravenous injection with immune MLNC, non-treated, or treated with complement alone, into lactating recipients led to passage of immunity to their neonates, and all neonates harbored oncospheres but no cysticercoids. However, maternal injection of immune MLNC, treated with anti-Thy-1.2 monoclonal antibody, did not result in passage of immunity to their neonates; these neonates were positive for cysticercoids, harboring numbers that were approximately equal to those in controls that had received non-immune MLNC (table 3).

Table 2. Effect of aqueous milk extract and serum on subcutaneous larval growth of *Hymenolepis nana*

H. nana infection (donor)	No. of mice examined	No. of cysticercoids (mean ± SD)
	Serum	
–	3	123 ± 22.2
+	4	0 ± 0
	Aqueous milk extract	
–	5	132 ± 21.0
+	5	152 ± 27.0

Effect of MLNC administered orally to neonates on transfer immunity. Experiment IV was carried out to examine the influence of immune MLNC on transfer of immunity by their oral administration to suckling neonates prior to challenge. From data in table 4, it can be seen that neonates fed immune MLNC, untreated, or treated with complement alone showed immunity as strong as that of the controls. However, neonates that were administered anti-Thy-1.2 monoclonal antibody-treated immune MLNC showed no immunity. All neonates harbored fully developed cysticercoids in the intestinal villi.

Discussion

The results obtained from Experiment I indicate that immunity is passed from mother to neonates via their milk.

Table 3. Ability of mesenteric lymph node cells injected into mothers to transfer immunity to neonates during lactation

H. nana infection (donor)	Treatment of cells injected	No. of neonates Examined	Harbored onco.	Harbored cyst.	No. of worms (mean ± SD) onco.	cyst.
–	None	30 (6)	0	30	0 ± 0	32 ± 8.0
	None	30 (6)	30	0	69 ± 4.3	0 ± 0
	Complement alone	25 (5)	25	0	65 ± 4.5	0 ± 0
+	Anti-Thy1.2 + complement	25 (5)	0	25	0 ± 0	47 ± 4.1

onco.: oncospheres, cyst.: cysticercoids; figures in parentheses represent number of mother mice.

Table 4. Transfer of immunity to *Hymenolepis nana* by oral inoculation of sensitized mesenteric lymph node cells prepared from *H. nana*-infected donor mice into suckling neonates

H. nana infection (donor)	Treatment of cells inoculated	No. of neonates Examined	Harbored onco.	Harbored cyst.	No. of worms (mean ± SD) onco.	cyst.
–	None	5	0	5	0 ± 0	33 ± 2.7
	None	10	6	4	35 ± 11.0	1 ± 0
	Complement alone	11	11	0	49 ± 8.8	0 ± 0
+	Anti-Thy1.2 + complement	17	0	17	0 ± 0	31 ± 6.5

onco.: oncospheres, cyst.: cysticercoids.

Neonates born of normal mothers and fostered by immune mothers were as immune as those born of and nursed by immune mothers. Little or no immunity is passed in utero, or, if it is, it is lost rapidly, since neonates that were born of immune mothers and fostered by naive mothers showed no immunity. These results agree with those of Larsh[1] who reported that most immunity to *H. nana* was passed from mother to their neonates via milk with the passage of immunity through the placenta being minor. The same characteristics have been found in the transmission of immunity to *T. spiralis* in rats[18] and in mice[19,20], and *T. taeniaeformis* in rats[21]. These kinds of immunity were described as being passed more in milk than in utero. Several studies have proposed that secretory Ig A[2,20] and Ig G[3,4] are responsible for maternal transfer of immunity to parasitic infections. However, our results (table 2) did not demonstrate the presence of the humoral factor in milk that is responsible for immunity; aqueous milk extract from neonates nursed by immune mothers did not inhibit larval growth in ectopic tissue.

Rose et al.[22] demonstrated that mesenteric T lymphocytes prepared from *T. spiralis*-infected rats migrated to the mammary grand when injected into syngeneic recipients. The presence of cells that were reactive to a wide variety of antigens has been demonstrated in the milk of many animal species[23–27], and it has been shown that fluorescent-stained lymphocytes from milk actively invade the intestinal mucosa, when fed to suckling neonates[28]. These reports and the results obtained from Experiments I and II prompted us to explore maternal to neonatal transmission of sensitized T cells during lactation. The next experiments, therefore, were carried out to clarify the transfer of sensitized T cells from mothers to their neonates via milk. The result of Experiment III showed that MLNC, especially T cells, served an active role(s) for passage of immunity to *H. nana* from mothers to their neonates; neonates nursed by mothers who were injected with immune MLNC treated with anti-Thy-1.2 monoclonal antibody were all positive for cysticercoids (table 3). Furthermore, the oral feeding experiment using MLNC, which mimics the normal route of entry for lymphocytes in milk also showed T-cell participation in transferring immunity to neonates (table 4). These results, when considered together with the above reports may be interpreted to indicate passage of T cells in milk, representing the maternal immune state, to neonates during lactation and delivery of specific cell-mediated immunity to naive neonates.

Although it is clear that the immunity transferred during lactation is directed to parasites present in the intestinal villi and result in inhibition of larval growth, the precise mechanisms are not well understood. Our previous report[16], and unpublished data have shown production of inflammatory cytokines by immune MLNC when cultured in vitro with soluble egg antigen of *H. nana*, and proposed correlation between acute inflammatory responses and inhibition of larval growth in the intestinal villi[12,16]. Other mechanisms effected by immune T cells in the intestine, such as increased activity of phospholipase[29] as a result of eosinophil influx may be part of cell-mediated response involved in inhibition of larval growth. Study of whether these changes are brought about by T cells transferred to neonates will provide valuable information concerning cellular mechanisms mediated by maternal derived T cells in the neonate.

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Shape change of human blood platelets: Reliable and fast detection by quasi-elastic light scattering

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Abstract. Quasi-elastic light scattering has been used for the first time to obtain reliable information about the morphology of platelets under physiological conditions within a short time. By measuring two independent parameters (electrophoretic mobility and diffusion coefficient) it is possible to distinguish between different stages of shape change on the one hand, and between shape change and binding of particles to the platelet surface without shape change on the other hand.
Key words. Light scattering; electrophoresis; platelets; shape change; hyperlipaemia.

The initial events in the genesis of disorders like atherosclerosis[1,2], hypertension[3] or Raynaud's[4] disease are thought to be closely related to platelet shape change and release reaction. Although turbidimetric measurements of platelet aggregability proved to be very suitable in determining haemostatic disorders in routine clinical examinations[5], the investigation of platelet shape change requires more complicated techniques. The measurement of light transmission under the undefined optical conditions of the aggregometer cannot give quantitative results[6]. Even the simultaneous measurement of 90° light scattering in a lumi-aggregometer does not improve shape change measurement, since the intensity of the scattered ligth I decreases rapidly with the increase of the angle: for $\Theta = 10°$:$I = 2.8 \times 10^5$ pulses/s, $\Theta = 20°$:$I = 2.9 \times 10^4$ pulses/s, $\Theta = 90°$:I = 360 pulses/s, measured with a 30 mW HeNe-laser. Another disadvantage of aggregometry is the fact that hyperlipaemic plasma, i.e. plasma containing chylomicra, cannot be investigated. Furthermore, contamination of the sample with larger cells such as leukocytes or erythrocytes may cause big errors from the optical point of view regardless of their physiological interaction.

Quasi-elastic light scattering (QELS) does not suffer from these disadvantages. As it is a method of measuring the Doppler shift frequency of the light scattered by moving particles the basic theory differs very much from that underlying turbidimetric experiments[7].

It is possible to identify discoid, resting platelets in buffer solutions after gel-filtration or washing of the platelets, or directly in platelet-rich plasma (PRP), even in the presence of chylomicra or contaminations with other cells. The quasi-simultaneous measurement of electrophoretic and diffusion light scattering (EDLS) allows shape changes to be followed, i.e. to distinguish between different stages of bleb and pseudopod formation, and concomitant conversion from discoid to spherical bodies. The resultant parameters are the electrophoretic mobility μ, which is proportional to the surface charge density σ_e and to the zeta potential ζ, and the diffusion coefficient D, which is reversely proportional to the hydrodynamic radius R_H or the effective size of the cell. Both parameters change significantly during shape change. The electrophoretic mobility decreases by 40% and the diffusion coefficient decreases by about 50%. The change in electrophoretic mobility is caused by the formation of pseudopods, i.e. other parts of the membrane (open canalicular system) are exposed[8], which also increase the effective size. Therefore the thermal motion of activated cells is slowed down compared to that of discoid cells, which leads to a decreased diffusion coefficient. A similar effect on the electrophoretic mobility could be caused by simple binding of nonactivating substances to the platelet membrane, but in contrast to shape change this will only slightly affect the diffusion coefficient. Distinguishing between these two effects makes the simultaneous measurement of both parameters necessary.

Materials and methods

EDLS was applied to compare gel-filtered platelets (GFP) with PRP, discoid platelets at 37 °C with activated platelets at 20 °C, and to investigate the influence of chylomicra on platelets.