COMPARISON OF ADOPTIVE IMMUNITY THROUGH SENSITIZED CELLS FROM PRIMARY AND SECONDARY LYMPHOID ORGANS IN MICE INFECTED WITH *HYMENOLEPIS NANA*

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ABSTRACT

Singly-sensitized spleen, thymus, bone narrow and thymus + bone marrow cell combination from donors infected with 2000 *Hymenolepis nana* eggs were injected into the respective recipients and then challenged seven days after cell transfer. It was found that recipients which received sensitized spleen and mixture of thymus and bone marrow cells produced the highest immune response.

INTRODUCTION

REPORTS on the passive transfer of immunity directed against the highly immunogenic cestode, *Hymenolepis nana* (the human dwarf tapeworm) are very few. Sensitised cells from secondary lymphoid organs such as spleen[1] and mesenteric lymphnodes[2], peritoneal exudate cells[3,4] and those of the bone marrow[5] have been successfully employed to transfer adoptive immune response in the *H. nana*—mouse model. In other parasitic models, cells from the primary lymphoid organs, the thymus and bone marrow either separately[6,7] or together[8,9] have been implicated in the successful transfer of immune response. In this communication, immune response obtained through sensitized cells from both the primary, (thymus, bone marrow and thymus-bone marrow combination) and secondary (spleen) lymphoid organs were compared to determine their comparative efficacy in the adoptive transfer of immune response.

MATERIALS AND METHODS

Isogenic female Swiss albino mice (6–8 weeks old, 20–25 g wt) were used in all experiments. Donor mice were sensitized with a single dose of 2000 *H. nana* eggs by oral administration and cells were transferred to their respective recipients four days after the immunizing dose using the sterile technique. Intact thymuses and spleens were removed from donors after cervical dislocation and processed in Ringer's solution to release the cells. Bone marrow cells were collected from humerus and femur of the same donor according to the method of Larsh *et al*[10].

Approximately 90×10^4 thymus, spleen and bone marrow cells and a mixture of 60×10^4 each of thymus and bone marrow cells were transferred within 4 hr after collection. Syngeneic female recipients of the same body weight and age were divided into four groups — A, B, C and D of 40 mice each. Group A was divided into 4 subgroups: A_1, a_1, A_2 and a_2, group B into subgroups B_1, b_1, B_2 and b_2, group C divided into C_1, c_1, C_2 and c_2 and group D into D_1, d_1, D_2 and d_2. Subgroup A_1, a_1 received sensitized spleen cells, A_2, a_2 received non-sensitized spleen cells. B_1, b_1 received sensitized thymus cells B_2, b_2 received non-sensitized (control) thymus cells, C_1, c_1 received sensitized bone marrow cells, C_2, c_2 received non-sensitized bone marrow cells, D_1, d_1 received thymus and bone marrow cell mixture and groups D_2, d_2 received non-sensitized thymus and bone marrow cell mixture. All recipients were challenged with a single dose of 1000 *H. nana* eggs on the 7th day after cell transfer and necropsied on the 4th (for cysticercoids) and 21st day (for adults) after challenge and the degree of immunity was assessed by cysticercoid and adult recoveries.

RESULTS AND DISCUSSION

Results of recoveries from different groups of recipients are shown in table 1 and their statistical analyses are presented in table 2. On 21st day the recovery of adults showed that recipients injected with mixed population of sensitized thymus and bone marrow cells exhibited the maximum response with an average recovery of only 1.6 (group D_1) compared with 23.8 cycticercoids (group d_1) which had developed on the 4th day; recipients with sensitized spleen cells also showed appreciable low average recovery of 1.9 adults (group A_1) and 22.7 cycticercoids (group a_1). Recipients with sensitized thymus and bone marrow cells separately showed an average of 2.4 (group B_1) and 10.1 (group C_1) adults and 23.9 (group b_1) and 31.0 (grup c_1) cysticercoids respectively.

The only other parasite where adoptive transfer of immunity has been investigated at some length is

Table 1 *Mean recovery of H. nana cysticercoids and adults from experimental and control recipients after a challenge infection of 1000 H. nana eggs given 7 days after cell transfer*

Recipients with cells of	Cysticercoid recovery Group-Experimental		Group-Control		Adult recovery Group-Experimental		Group-Control	
Spleen	a_1	22.7	a_2	37.8	A_1	1.9	A_2	21.7
Thymus (T)	b_1	23 9	b_2	38 0	B_1	2 4	B_2	22 0
Bone marrow (B)	c_1	31.0	c_2	38 8	C_1	10.1	C_2	22.0
T + B	d_1	23.8	d_2	39.0	D_1	1 6	D_2	21.5

Readings are based on mean recoveries made from 10 animals.

Table 2 *Statistical analysis (difference between means, student's t-test)*

Groups	*t*-value	Groups	*t*-value	Groups	*t*-value
a_1 vs a_2	4.435*	a_1 vs A_1	21.579*	a_1 vs d_1	0.458
A_1 vs A_2	10.361*	d_1 vs B_1	12.413*	A_1 vs D_1	0.369
b_1 vs b_2	4 284*	c_1 vs C_1	7.485*	b_1 vs c_1	2.569*
B_1 vs B_2	15.592*	d_1 vs D_1	7.367*	B_1 vs C_1	4.328*
c_1 vs c_2	2 200*	a_1 vs b_1	0.828	b_1 vs d_1	0.029
C_1 vs C_2	5.929*	A_1 vs B_1	0.638	B_1 vs D_1	0.916
d_1 vs d_2	2.613*	a_1 vs c_1	3.621*	c_1 vs d_1	1.947
D_1 vs D_2	16.638*	A_1 vs C_1	4 687*	C_1 vs D_1	4.743*

P value at 5% level of significance is 2.101. *Statistically significant values.

Ancylostoma caninum—mouse model[11-15]. Both are (*H. nana* and *A. caninum*) tissue invading forms; however, they present quite contrasting features with regard to their intensity of infection in adoptively immunized host. Sensitized thymus and bone marrow cells employed separately gave an intensity of 2.39% and 3.10% respectively in the cestode model in contrast to 14.0% and 16.3% respectively in nematode model[6] at 96 hours; presumably wide variations and differences in the type of parasite (one a non-migrating cestode, the other a migrating nematode), period of sensitization (4 days in cestode model and 21 days in nematode model), challenging dose (500 in nematode model) and the number of cells transferred may have also contributed to these differences. Thus, the cestode model exhibits greater immune response in short sensitization period with a greater number of cells transferred. The influx of senzitized cells[6] and the immune pressure on the parasite's main-stay loci are also important factors; and early migration from the intestine to the other organs of the adoptively immunized host[9] provides a suitable cover to free themselves from a possible attack, a situation not obtained in the case of non-migrating cestode like *H. nana* which also faces a hostile intestinal environment thus unable to establish, getting dislodged, destroyed and/or eventually expelled.

The B-memory cells which have migrated from spleen into the thymus and bone marrow in donors[16] differentiated in the presence of specific T-cells in recipients causing interactions with antigens favouring the significant expulsion of worms. There is no significant difference in the adult counts within the recipients of spleen cells and T + B cells. This suggests that though spleen is the seat of blastogenesis of T and B precursor cells, not all of them stimulate in antibody forming cells in spleen[17], however, the intensity of expulsion of adult worms was greater than the thymus and the bone marrow cells exerting separately in their recipients. During cysticercoid recovery, the maximum immune response conferred by sensitized spleen cells indicates the insufficient time for the circulation of specific antigen sensitive lymphocytes from spleen to thymus and bone marrow[13] resulting in higher recovery of worms by thymus and bone marrow recipients. The specific antigen sensitive lymphocytes probably recirculate from spleen to enrich the primary lymphoid organs since significant immune response was found in all the experimental groups at the time of adult recovery in comparison to respective controls.

Also, contamination of thymus cell suspension with sensitized cells of parathymic lymph nodes[19] and by sensitized blood lymphocytes to bone marrow cell suspension may have also contributed in conferring adoptive immunity.

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NEWS

PREDICTING EARTHQUAKES IN JAPAN

. . . "A very extensive high-quality digital network for micro-earthquake and grount-tilt observation has recently been completed in the south Kanto-Tokai area surrounding Tokyo. The first scientific results are now appearing and allow, among other things, a remarkable three-dimensional picture of the earthquake hypocentre distribution to be produced. The distribution maps out the boundaries of the three plates that meet under this region of Japan. . . . Routine work for hypocentre determination began as soon as the first parts of the system were completed in July 1979. Every day around twenty earthquakes are located, most of them micro-earthquakes with magnitude around two. [A] vertical section [through 36.25°N] clearly shows the Pacific Plate diving down underneath the Eurasian Plate upon which the main Japanese island stands. The precise study of three-dimensional hypocentral distribution and focal mechanisms based on a large amount of accurate data leads us to an excellent image of the plate configuration in this complex region. Also we have been able to measure a precursory tilt change in the 18 days preceding an earthquake that was of magnitude six and thirty-one km away from the station."

[Y. Okado (Natl. Research Ctr. for Disaster Prevention, Sakuramura, Japan) in *Nature* 312(5994): 500-1, 6 Dec. 84. Reproduced with permission from Press Digest, *Current Contents* ®, No. 8, February 25, 1985, p. 12, (Published by the Institute for Scientific Information ®, Philadelphia, PA, USA.)]