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Abstract

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—— BRIEF NOTE ——

ACTIVATION OF NK ACTIVITY AND AUTO-REACTIVE CYTOTOXICITY AFTER HEPATECTOMY

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Abstract. Spleen cells serially sampled from normal mice following partial hepatectomy were tested for antibody-dependent cellular cytotoxicity (ADCC) and natural killer (NK) activity. There was a marked augmentation of these activities of spleen cells from the hepatectomized animals, compared to cells from controls with a simple laparotomy. The augmentation of ADCC in the hepatectomized mice was largely attributable to the activity of T lymphocytes. When cultured with interleukin-2 (IL-2), the spleen cells from hepatectomized mice exhibited cytotoxicity to syngeneic lymphoblasts, which was found to be effected by T cells.

Key words : antibody-dependent cellular cytotoxicity (ADCC), natural killer (NK) activity, auto-reactive cytotoxicity, interleukin-2 (IL-2), hepatectomy.

Partial hepatectomy (HEP) triggers DNA synthesis not only in the remaining liver but also in lymphoid organs (1, 2). It has also been shown that regenerating liver cells stimulate (3) a proliferative response of syngeneic lymphocytes *in vitro.* Considering the hypothesis of Bunch and Burwell (4) that the essential function of the lymphoid system is to establish and maintain morphostatis for many types of differentiated tissues, these phenomena seem to suggest that the process of hepatic regeneration is controlled by the lymphoid system. This report describes the activation of effector lymphocytes such as killer (K) cells, natural killer (NK) cells and auto-reactive cytotoxic T cells following HEP.

Materials and Methods

Mice and hepatectomy. Male inbred mice of the C3H/He strain were obtained from Shizuoka Experimental Animal Farm (Hamamatsu) and used when they attained the age of 6 to 8 weeks. Approximately 40 % of the liver was removed under anesthesia with ether by the Higgins-Anderson method (5). The hepatectomy group of mice was compared with a simple laparotomy group and normal control group.

Preparation of effector cells. The spleen was excised, cut into small pieces in culture medium and filtered through #150 platinum mesh. Lymphocytes were separated by using Percoll density gradient centrifugation, and washed 3 times with culture medium solution. The cells were suspended in RPMI-1640 with 25 mM

HEPES, containing 10 % heat-inactivated fetal bovine serum (FBS, GIBCO, U.S.A.), 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 5×10^{-5} M 2-mercaptoethanol. The effector cells (5×10^6 cells/ml) were cultured with 10 units/ml of IL-2 in tissue culture plates (2 ml/well, Falcon 3047,U.S.A.) at 37 ℃ under 5 % CO_2 in air for 4 days. As a control, the cells were cultured in the absence of IL-2.

Antibody-dependent cellular cytotoxicity (ADCC) assay. One hundred thousand cells/100 μl of $Na_2[^{51}Cr]O_4$(NEN)-labeled chicken red blood cells (CRBC) and 50 μl of appropriately diluted rabbit anti-CRBC antibody were placed in each well of a microtest III type plate (Falcon 3072), and were incubated at 37 ℃ for 30 min. Then, 5×10^5 cells/100 μl of effector cells were added and incubated at 37 ℃ under 5 % CO_2 in air for 12 h. One hundred μl of supernatant was collected and radioactivity was measured with a γ-spectrometer (experimental cpm). The radioactivity of supernatant without effector cells was considered spontaneous cpm.

NK and auto-reactive cytotoxicity assay. YAC-1, Moloney virus-induced lymphoma cells of A/Sn mice and M-HeLa, measles virus infected HeLa cells were used as target cells in the NK assay. Syngeneic blastoid lymphocytes induced by phytohemagglutinin (PHA-P, 20 μg/ml), concanavalin A (Con A, 10 μg/ml) and pokeweed mitogen (PWM, 40 μg/ml) were used as target cells for the auto-reactive cytotoxicity. After the target cells were labeled with ^{51}Cr,effector cells and target cells were incubated for 6 or 12 h at an effector cell to target cell (E : T) ratio of 50 : 1 or 100 : 1.

Percent specific lysis in ADCC, NK and auto-reactive cytotoxicity assays was calculated as follows ;

$$\% \text{ specific lysis} = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100$$

Treatment of lymphocytes with antisera and complement. The effector cells were incubated for 30 min at 4 ℃ with appropriately diluted monoclonal IgM anti-Thy1.2 antibody (F7D5, Olac, England) and then centrifuged at 1000 rpm for 5 min. The cell pellet was resuspended in fresh medium containing low-toxic dried guinea pig complement (C', 1 : 5) and incubated for 40 min at 37 ℃. Afetr the incubation cells were washed twice and used as effector cells. The degree of reduction of ADCC activity by the elimination of the T cells was expressed as % reduction :

$$\% \text{ reduction} = \left(1 - \frac{\% \text{ cytotoxicity of cells treated with anti-Thy 1 plus C'}}{\% \text{ cytotoxicity of cells treated with C' only}}\right) \times 100$$

Results

The ADCC activity of CRBC changed slightly in the laparotomy group from the 5th to the 10th postoperative days, but showed no significant difference com-

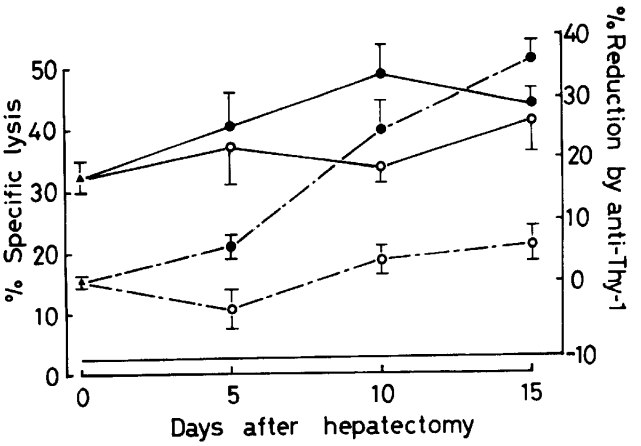

Fig. 1. Changes in ADCC activity of spleen cells after hepatectomy and % reduction of the activity by treatment with anti-Thy 1.2 antibody and complement. E/T ratio of 5 : 1. Solid lines indicate % specific lysis ; broken lines indicate % reduction. ▲, normal ; ○, simple laparotomy, and ●, hepatectomy.

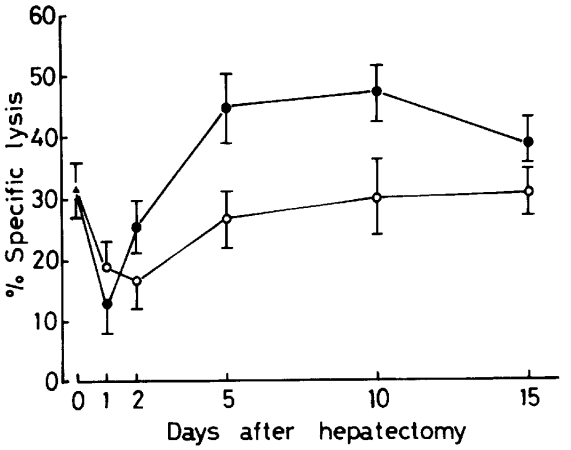

Fig. 2. Changes in NK activity of spleen cells after hepatectomy. E/T ratio of 100 : 1. ▲, normal ; ○—○, simple laparotomy, and ●—●, hepatectomy group.

pared to the activity of the normal group. On the other hand, the ADCC activity of the hepatectomy group increased from the 5th day and reached a peak of activity (48.7 %) on the 10th day which was maintained till the 15th day. When lymphocytes were treated with anti-Thy 1.2 antibody and complement to exclude the influence of T cells, the ADCC activity of the normal group showed a little change, and the laparotomy group showed a 6 % reduction on the 15th day. On the other hand, in the hepatectomy group the % reduction of ADCC activity

by T cell elimination was 6.2 % on the 5th day, 24.9 % on the 10th day and 36.3 % of the 15th day. This reduced activity was nearly equal to that of the normal group (Fig. 1).

The NK activity to YAC-1 was examined. In the laparotomy group, the activity decreased markedly on the 1st and 2nd postoperative days to nearly 50 % of the preoperative value. The reduced activity began to increase on the 5th postoperative day, and nearly reached the preoperative value on the 10th day. In the hepatectomy group, the NK activity decreased on the 1st day more markedly than in the laparotomy group, but began to increase on the 2nd day and showed a 150 % augmentation of the preoperative value on the 5th day, and then tended to decrease after the 10th day (Fig. 2). The natural killer activity to the syngeneic lymphoblasts induced by PHA, ConA or PWM was not detected in either the laparotomy or hepatectomy group.

When IL-2 was added to lymphocytes of each of these groups and incubated for 4 days, the NK activity to YAC-1 and M-HeLa was augmented markedly from the 5th day after hepatectomy, and this augmentation lasted till the 10th postoperative day. In the hepatectomy group, cytotoxicity to syngeneic lymphoblasts was detected in the cultured lymphocytes from the 5th to the 10th postoperative days, but such auto-killing was not detected in the normal and laparotomy groups (Table 1).

TABLE 1. ACTIVATION OF NK ACTIVITY AND AUTO-REACTIVE CYTOTOXICITY BY IL-2

Target	Effector		Days after operation					
			1	3	5	7	10	14
YAC-1	Normal	48.6± 3.3						
	Laparotomy		26.4	29.2	37.6	38.9	47.5	49.0
	Hepatectomy		21.5	44.3	63.2	68.1	69.6	52.8
M-HeLa	Normal	35.5± 5.2						
	Laparotomy		17.8	18.1	23.4	31.7	36.2	36.9
	Hepatectomy		8.6	28.5	50.2	56.6	57.0	40.1
PHA-Lymphoblasts	Normal		0	0	0	0	0	0
	Laparotomy		0	0	1.8	0	0	0
	Hepatectomy		0	1.4	8.7	14.6	15.7	5.7
ConA-Lymphoblasts	Normal		0	0	0	0	0	0
	Laparotomy		0	0	2.4	2.2	0	0
	Hepatectomy		0	3.6	10.3	15.5	15.3	7.6
PWM-Lymphoblasts	Normal		0	0	0	0	0	0
	Laparotomy		0	0	0.8	1.1	0	0
	Hepatectomy		0	1.9	7.4	11.9	11.2	6.0

Effector to target cell ratio 50 : 1.

The NK activity of post-hepatectomy spleen cells cytotoxic to the syngeneic lymphoblasts became enhanced in this activity following passage through a Nylon wool column. Further treatment of the spleen cells with anti-Thy 1.2 antibody and complement led to an almost complete disappearance of the activity (Table 2).

TABLE 2. EFFECTS OF PASSAGE THROUGH NYLON WOOL COLUMN OR TREATMENT WITH ANTI-THY-1.2 PLUS C′ ON AUTO-REACTIVE CYTOTOXICITY AFTER HEPATECTOMY

Effector cells	Cells with treated	% specific lysis	
		C3H-PHA-lymphoblasts	C3H-hepatoblasts
IL-AK	—	11.0	11.5
	Nylon column	14.1	13.7
	Anti-Thy-1+C′	1.2	1.5
	C′ control	9.2	10.3

Effector to target cell ratio 50 : 1.
Spleen cells 7 days after hepatectomy were used as effector cells.
IL-AK : IL-2 activated killer cells.

Discussion

Participation of K cells, cytotoxic T cells and NK cells in the destruction of liver cells associated with chronic hepatic disorders is generally assumed. There have been many reports which indicated that ADCC by K cells plays a principal part in the mechanism that underlying the liver cell destruction (6). The post-hepatectomy activation of K cells, as demonstrated in this study, could facilitate liver cell destruction by ADCC, and thus create a serious clinical problem in the practice of surgery especially for the treatment of hepatoma with chronic disorders of the liver.

Evidence has been accumulating that NK cells serve an important function in host resistance to tumors. Recent observations have led to the speculation that NK cells may also have a physiological role *in vivo* since they are closely associated with the rejection of bone marrow grafts (7). While the target "antigen" of NK cells has not been fully established as yet, a wide range of malignant and non-transformed cells, including normal thymocytes (8), bone marrow cells and cultured macrophages (9) are also sensitive to low levels of NK-mediated cytolysis.

Macrophages or Kupffer cells, which make up about 30 % of the total cell population of the liver, have been shown to undergo DNA synthesis and mitosis during restorative hyperplasia of the liver following partial hepatectomy in the rat (1). Furthermore, hemopoietic colony-forming cells in the regenerating liver markedly increased in number, reaching a peak between 5 to 7 days after the hepatectomy (10). It would be intriguing if the augmentation of NK activity in the partially hepatectomized animals aided in the control of macrophage pro-

liferation.

It was noted in this study that killer cells to syngeneic lymphoblasts were induced *in vitro* in the presence of IL-2, in association with the post-hepatectomy augmentation of K cells and NK cells activation. Human and murine autocytotoxic lymphocytes have been identified in many studies. However, their physiologic roles *in vivo* remain unclear. Initially, murine autocytotoxic cells were detected *in vitro* in cultures of normal spleen cells with irradiated syngeneic fibroblasts (11). Naturally occurring autocytotoxic lymphocytes more recently have been demonstrated in old NZB mice (12) as well as in mice infected with lymphocytic choriomeningitis (LCM) virus (13). Normal mouse spleens contain both autocytotoxic and suppressor lymphocytes, and it appears likely that the reactivity of the former becomes detectable when the suppressor cell population diminishes (14).

Autoreactivity of normal lymphocytes has been observed with testicular cells (15), and thymus reticulum cells (16). These data seem to indicate that the cells endowed with the ability to evoke autosensitization are in a dynamic state of differentiation or of a high mitotic activity. A 70 % HEP in the rat triggers DNA synthesis not only in the remaining liver tissue but also in a variety of lymphoid organs as well (2). Lymphocyte stimulation to proliferation *in vitro* was noted in mixed cultures of normal mouse lymphocytes with liver cells from hepatectomized mice, and it reached a peak at 6 days after HEP (3). The primed lymphocytes harvested from the mixed cell culture on the tenth day of incubation showed a typical secondary response to restimulation with freshly isolated regenerating liver cells, with a peak of responsiveness on the second day of culture. Furthermore, mouse lymphocytes collected after HEP exhibited a secondary response *in vitro*, thus suggesting activation of lymphocytes *in vivo* in such surgically treated mice (17).

Little is known as to what particular type or types of effector are induced from such lymphocyte activation occurring in the regeneration of the liver following a hepatectomy. The present study has shown autocytotoxicity of post-hepatectomy spleen cells to syngenic lymphoblasts in the presence of IL-2 *in vitro*. This findings suggests that the post-hepatectomy activation of lymphocytes serves an autoreguratory function in the process of hepatic regeneration.

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