FUNCTIONAL ACTIVITY OF T AND B LYMPHOCYTES IN THERMAL BURNS

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UDC 617-001.17-07:616.155.32-008.1-07

KEY WORDS: burns; T lymphocytes; B lymphocytes.

Thermal trauma acts variously on immunologic reactivity: injury to small areas of skin regularly induces immunostimulation, more extensive burns immunodepression [2]. The onset of these states may be connected with changes in the activity of cells interacting with each other in the immune response: B lymphocytes and immunoregulatory subpopulations of T cells (helpers and suppressors), and macrophages [3, 5].

This paper gives the results of a study of the function of T and B lymphocytes in mice with thermal burns of varied severity.

EXPERIMENTAL METHOD

Experiments were carried out on CBA and (CBA × C57BL)F_1 mice weighing 20-22 g. A thermal burn affecting 10 or 30% of the body surface was inflicted on the previously epilated skin by means of a special instrument (exposure 10 sec). To assess the function of B lymphocytes, a mixture of lymph node cells of intact donors and of bone marrow of burned syngeneic donors was injected intravenously into irradiated mice. To determine helper activity, syngeneic bone marrow cells from intact mice and from lymph nodes of burned mice were injected into irradiated animals. The control for these experiments was provided by results obtained in mice receiving lymph node and bone marrow cells from healthy donors. To prepare the various mixtures, the same number of bone marrow cells (the source of B lymphocytes) was taken. Since in burned mice T lymphocytes appear in the bone marrow, but their number in the lymph nodes falls [2], different numbers of lymph node cells (the source of T lymphocytes) were added to the mixture, calculated on the basis of the total number of T lymphocytes. Sheep's red blood cells (SRBC), injected in a dose of 2×10^8 cells per mouse, were used as the antigen. On the 8th day after transplantation the number of antibody-forming cells (AFC) in each recipient's spleen was determined by Jerne's method [4]. The mice were irradiated in a dose of 850 R on a gamma-apparatus with four cesium sources.

The number of T lymphocytes was determined in the complement-dependent cytotoxic test [3] with antihymocytic globulin (obtained from the Laboratory of Immunology, Moscow Research Institute of Experimental Medicine).*

The suppressor activity of the spleen cells on antibody production was tested on the model suggested by Möller [1, 5]. Spleen cells from intact or burned CBA mice in a dose of 5×10^7 were transplanted into intact (CBA × C57BL)F_1 mice. Seven days after the transplantation the recipients were given an injection of SRBC in a dose of 5×10^8. On the 4th day after immunization the number of AFC was determined in the spleen by the method mentioned above.

The results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that in mice with burns covering an area of 10% of the body surface, regularly causing stimulation of the immune response to SRBC, the functional activity of the B lymphocytes was somewhat depressed during the first few hours after trauma, after which it rose again to reach a maximum by

*The authors are grateful to N. K. Kraskina for providing the antithymocytic globulin.

Department of Microbiology, Chelyabinsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 75-77, July, 1980. Original article submitted July 10, 1979.

0007-4888/80/8907-0952$07.50

TABLE 1. Functional Activity of T and B Lymphocytes in (CBA × C57BL)F_1 Mice After Thermal Burn Affecting 10 and 30% of the Body Surface (M ± m)

Time of removal of cells from donors after trauma	Number of recipients	Source and number of transplanted cells (× 10^6)				Number of AFC in spleen
		bone marrow of intact mice	lymph nodes of intact mice	bone marrow of burned mice	lymph nodes of burned mice	
Control I	12	10	—	—	—	143±40
Control II	53	10	1 (0,6)	—	—	1591±159
Burn affecting 10% of body surface						
2 h	10	10	—	—	1,2 (0,6)	1394±332
	13	—	1 (0,6)	10	—	813±120†
1st Day	15	—	—	10 (0,1)	—	398±93*
	13	10	—	—	1 (0,4)	2514±422†
	12	—	0,8 (0,5)	10 (0,1)	—	2380±345†
3rd Day	11	10	—	—	1 (0,6)	2920±463†
	12	—	1 (0,6)	10 (0,06)	—	3120±495†
6th Day	10	10	—	—	1 (0,6)	2037±332†
	10	—	1 (0,6)	10	—	1785±312
Burn affecting 30% of body surface						
1st Day	7	10	—	—	1,2 (0,6)	2640±425†
	15	—	1 (0,6)	10 (0,07)	—	733±144†
3rd Day	12	10	—	—	1,5 (0,6)	1150±196
	14	—	1 (0,6)	10 (0,05)	—	1795±280
6th Day	13	10	—	—	1,2 (0,6)	880±210†
	15	—	0,8 (0,5)	10 (0,08)	—	2360±296†
12th Day	13	10	—	—	1,5 (0,6)	770±183†
	12	—	1 (0,6)	10	—	1378±340
24th Day	10	10	—	—	1 (0,6)	3245±306†
	11	—	1 (0,6)	10	—	3348±491†

Legend. *) Values differing significantly from those of control I; †) differing significantly from values of control II. Number of T lymphocytes in parentheses.

TABLE 2. Suppressor Activity of Spleen Cells of CBA Mice after Thermal Burn Affecting 10 or 30% of the Body Surface (M ± m)

Donors of spleen cells (CBA mice)	Time of removal of spleen after trauma	Number of (CBA × C57BL) recipients	Number of AFC in recipients' spleen	P
—	—	23	37 454±2 341	
Intact (control)	—	27	7 762±884	
Burn affecting 10% of body surface	2 h	22	5 632±796	>0,05
	1st day	20	12 362±1 132	<0,001
	3 rd "	14	13 344±2 132	<0,05
	6 th "	19	4 932±836	<0,05
Burn affecting 30% of body surface	1 st "	13	11 321±1 337	<0,05
	3 rd "	12	12 867±1 148	<0,001
	6 th "	13	15 323±2 022	<0,001
	12 th "	13	9 310±1 135	>0,05
	24 th "	9	6 945±995	>0,05

the 3rd day. By the end of 1 week the ability of bone marrow cells of the burned mice to produce AFC was virtually back to normal. The helper activity of the lymph node cells also had increased after a brief fall, and it remained high throughout the first week after burning. After transplantation of bone marrow cells from burned donors only into the irradiated mice, significantly more AFC were formed than after transplantation of the same number of bone-marrow karyocytes from intact animals. This is evidence that the bone marrow of the burned animals contained T lymphocytes with helper properties. The ability of the spleen cells to suppress AFC production during the first few hours after a burn covering 10% of the body surface was somewhat increased, but by the end of the first day it was significantly reduced, and it rose again toward the end of the week (Table 2).

After thermal injury affecting 30% of the body surface (a burn of this size regularly leads to the development of lasting immunodepression) the state of the B cells was depressed only on the first day. By the middle of the week the cooperative ability of these cells had increased and it remained high throughout the month. Properties of the T lymphocytes changed differently. The function of the T helpers was considerably depressed

by the 3rd day and recovered only at the end of the month. The suppressor activity of the spleen cells fell toward the end of the 1st day after trauma and returned to normal at the beginning of the 3rd week after burning.

The results thus indicate that activity of B lymphocytes and T helpers increases in mice after burns affecting 10% of the body surface, but activity of T suppressors declines. After an extensive burn functional insufficiency of the immunoregulatory T lymphocytes develops, whereas the B cells retain their power to form antibodies against SRBC. It can accordingly be concluded that the development of immunodepression in severe burns is connected with injury to the T system of lymphocytes.

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SUPPRESSIVE ACTION OF XENOGENEIC BONE MARROW CELLS ON ANTIBODY FORMATION IN SPLEEN CELL CULTURES in vitro

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UDC 612.411.017.1-064:612.419.014.2]-085.23

KEY WORDS: bone marrow suppressor; immunodepression.

Recent investigations have demonstrated the suppressive action of cells of bone marrow origin on the immune response [1, 7, 9]. The addition of syngeneic bone marrow cells to cultures of spleen cells depresses the primary response to sheep's red blood cells (SRBC) practically completely [2-5, 10]. It has been suggested that bone marrow suppressor cells "prohibit" development of the immune response on the territory of the bone marrow [3]. The role and mechanisms of action of these regulatory cells has not been finally explained. It has been shown in vitro that suppression of the primary immune response to SRBC can be effected by allogeneic bone marrow cells [5].

The object of this investigation was to study the effect of xenogeneic bone marrow cells on induction of the primary immune response in cultures of mouse spleen cells in vitro.

EXPERIMENTAL METHOD

(CBA $\times$ C57BL)F_1 mice aged 2-4 months were used. Noninbred piglets aged 3 months, White Leghorn hens aged 4 months, guinea pigs aged 2 months, and Wistar rats aged 3 months served as donors of bone marrow cells. Induction of the primary immune response in cultures of spleen cells to SRBC was carried out by a modified Click's method. Spleen and bone marrow cells were cultured in the ratio of 1:1 in "GIBCO Serumless medium" in concentrations of 2.5×10^6 and 5×10^6 cells/ml. Additional substances added included fetal serum (10%), glutamine (200 mM), and 2-mercaptoethanol (10^{-5}M). The viability of the cells was determined by staining with trypan blue and eosin. SRBC kept in Alsever's solution served as the antigen. The number of 19S antibody-forming cells (AFC) was counted after culture for 4 days in vitro by Jerne's method [8]. The experimental data was subjected to statistical analysis.

(Presented by Academician of the Academy of Medical Sciences of the USSR R. V. Petrov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 77-79, July, 1980. Original article submitted October 12, 1979.

0007-4888/80/8907-0954$07.50