Main Lymphoid Subpopulations and Their Mitogenic Response after Lethal Irradiation and Transplantation of Syngeneic Bone Marrow from Young and Old Donors

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The recovery of T-lymphocyte subpopulations depends on the age of the stem cell donor. After transplantation of bone marrow cells from old donors, the $CD4^+$ and $CD8^+$ cell counts decrease, while the number of cells with the memory cell phenotype ($CD4^+CD44^{high}$) in the $CD4^+$-lymphocyte population increases. Lymphocyte proliferation in irradiated mice does not depend on the age of the stem cell donor. Our findings indicate that bone marrow stem cells change with age and transfer information regarding the donor's age.

Key Words: *aging; bone marrow; irradiation; T lymphocytes*

The function of the immune system noticeably declines with age. The major changes occurring in T cells have been attributed to thymic involution. Age-related changes in hemopoietic stem cells draw much less attention. Experiments with young and old irradiated animals showed that T cells originating from the stem cells of the bone marrow (BM) grafted from old donors have a lower functional activity [1,6,8]. In this study we attempted to find out whether subpopulations of T cells growing from the stem cells of young and old donors are different.

MATERIALS AND METHODS

Young (4 months) and old (22 months) female CBA/Ca mice were used in experiments. The animals were divided into 4 groups: 1) young intact mice; 2) young irradiated recipients of BM cells from young donors; 3) young irradiated recipients of BM cells from old donors; 4) old intact mice. The recipient mice were irradiated in a RUM-7 x-ray installation (dose intensity 0.72 Gy/min, total dose 8.5 Gy). Bone marrow cells were injected within the first 4 h after exposure. Mature T cells were removed from the BM suspension as described previously [9]. Bone marrow cells were incubated for 30 min at 37°C in culture medium conditioned by HO-13-4 hybridoma producing antibodies (IgM) to murine Thy 1.2 antigen, washed, resuspended in serum-free medium, and injected intravenously in a dose of 2×10^7 cells per mouse. Young and old animals were used 3 months after exposure, at the age of 7 and 25 months, respectively. It was shown that during this period 90-95% of T cells and 100% of B cells in the recipient are grown from donor cells [1,7,9]. The splenocyte population was analyzed by direct immunofluorescence using antibodies to different murine lymphocyte antigens: fluorescein-conjugated anti-Thy 1.2, anti-IgM, anti-Lyt2 (CD8), anti-Pgp-1 (CD44), and phycoerythrin-conjugated anti-L3T4 (CD4) (Becton Dickinson, Sigma). The splenocyte suspension was stained and fixed according to the manufacturer's instructions and sorted in a Becton Dickinson FACStar Plus cytofluorimeter in the LYSIS II mode. $CD4^+$ lymphocytes with high and low density of CD44 membrane marker were designated $CD4^+CD44^{high}$ and $CD4^+CD44^{low}$, respectively. These cells were differentiated by the expres-

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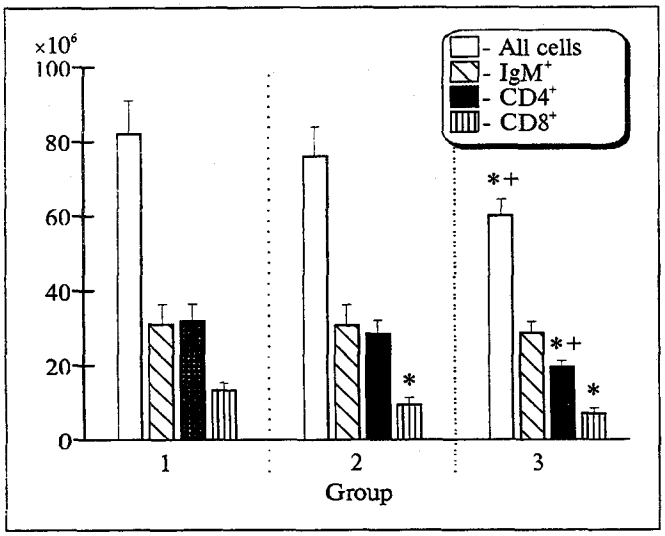

Fig. 1. Different lymphocyte populations in the spleen of intact (group 1) and irradiated recipients of bone marrow cells from young (group 2) or old (group 3) donors. Here and in Fig. 2: $p<0.05$: *compared with group 1, +compared with group 2.

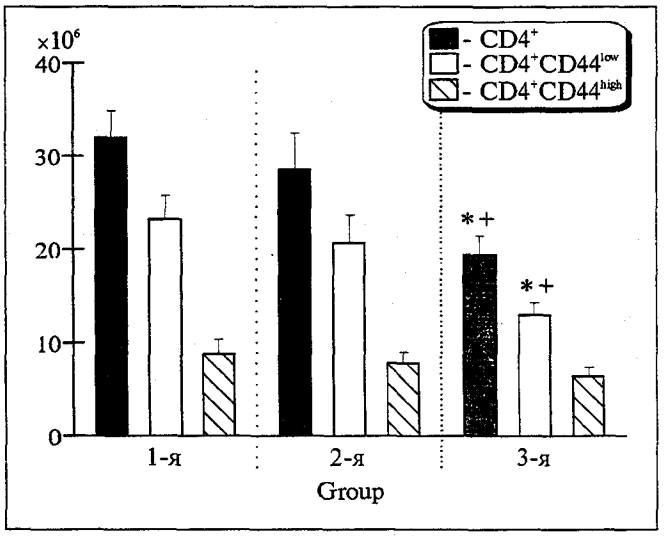

Fig. 2. Count of CD4⁺ lymphocytes differing by the expression of CD44 in the spleen of intact (group 1) and irradiated mice grafted BM cells from young (group 2) or old (group 3) donors.

sion of CD44 antigen as described previously [13]. Proliferation of splenocytes *in vitro* after stimulation with phytohemagglutinin (PHA), ConA, and LPS was assessed by the increment in the number of metabolically active cells in the NBT reduction test [12]. Intergroup differences were evaluated using Student's t test or, if the sample dispersions were reliably unequal, Wilcoxon—Mann—Whitney's U test.

RESULTS

In order to assess the radiation-induced changes in the immune system we compared animals of groups 1 and 2. Young mice receiving BM cells from young donors had a smaller thymus and less Thy 1.2⁺ lymphocytes in the spleen (Table 1). The number of peripheral blood T cells in group 2 decreased mainly at the expense of CD8⁺ cells (Fig. 1), as evidenced by an increase in the CD4/CD8 ratio (Table 1). The number and percentage of IgM⁺ cells did not differ considerably in mice of groups 1 and 2.

More pronounced changes in the splenocyte population were observed in irradiated mice with BM cells transplanted from old donors: in group 3 mice, the counts of CD4⁺ and CD8⁺ cells continued to decline (Fig. 1), which resulted in a decrease in the total number of splenocytes and in the percentage of Thy 1.2⁺ cells (Table 1).

TABLE 1. Effect of the Age of BM Cells on the Recovery of the Immune System in Young Irradiated CBA/Ca Mice

Parameters	Group of animals			
	1 (n=8)	2 (n=10)	3 (n=14)	4 (n=7)
Weight of thymus, mg	33.9±2.1	19.5±1.2*	18.9±0.8	12.1±1.0*
Total count of splenocytes, ×10⁻⁶	82.0±7.9	76.0±7.4	59.7±4.1*+	109.1±11.6
Thy 1.2⁺, %	56.0±1.1	50.3±2.1*	43.9±2.0*+	34.7±2.9*
CD4⁺, %	39.5±1.1	37.9±1.5	32.6±1.5*+	21.7±1.9*
CD8⁺, %	16.5±0.5	12.4±0.8*	11.3±1.0*	13.0±1.5*
IgM⁺, %	37.8±1.8	39.8±1.9	48.7±2.5*+	59.1±4.1*
CD4⁺CD44^{high}, % of total CD4 cells	27.5±2.0	27.4±0.7	33.0±2.5*+	69.7±5.2*
CD4⁺/CD8⁺	2.41±0.12	3.09±0.12*	3.12±0.26*	1.74±0.17*
PHA, units optical density	0.22±0.02	0.24±0.04	0.20±0.04	0.01±0.02*
ConA, units optical density	0.27±0.03	0.35±0.02*	0.33±0.01	0.15±0.03*
LPS, units optical density	0.25±0.02	0.30±0.03	0.28±0.02	0.14±0.03*

Note. $p<0.005$: *compared with group 1, +compared with group 2.

Interestingly, a positive correlation was established between the thymus weight and splenic T-cell count in irradiated animals grafted BM cells from both young and old donors. The strongest correlation (r=0.56, p=0.004) was observed between the thymus weight and the $CD8^+$-cell count (Fig. 3). This is consistent with the hypothesis that the differentiation of $CD8^+$ cells in the thymus is more vulnerable than the production of $CD4^+$ lymphocytes [2,5].

In addition to the drop in the total number of $CD4^+$ cells in group 3 mice, the content of cells with high and low expression of CD44 changed. CD44 is a tissue adhesion receptor reacting with the hyaluronic acid residues [11]. "Naive" $CD4^+$ lymphocytes not stimulated with an antigen occur mainly among cells with a small number of CD44 ($CD4^+CD44^{low}$ phenotype). Generally, memory cells formed during the immune response markedly stimulate the expression of these receptors [3]. Moreover, in the majority of mouse strains, including CBA (Table 1), the share of $CD4^+CD44^{high}$ lymphocytes increases 2- to 3-fold with age, whereas the share of $CD4^+CD44^{low}$ cells decreases [10]. In mice grafted BM cells from young donors, the splenic content of "naive" $CD4^+CD44^{low}$ lymphocytes is 20×10^6 cells, whereas in the recipients of BM from old donors it is 8×10^6 cells lower (Fig. 2). This difference is not only quantitative, i.e., caused by a decrease in the total count of $CD4^+$ cells in group 3 mice, but also qualitative: the number of $CD4^+CD44^{high}$ memory cells among $CD4^+$ lymphocytes originating from the old BM cells is higher (33.0 *vs.* 27.4%; $p(U)$=0.026, Table 1). It can be suggested that an increase in the share of $CD4^+CD44^{high}$ cells in group 3 was indeed caused by changes in the stem cell population but not by expansion of mature T lymphocytes, which were removed from the suspension of BM cells.

Previously, it was shown that $CD4^+CD44^{high}$ lymphocytes from young animals less actively proliferate in response to ConA [10]. It was suggested that the high predominance of these cells in old animals (along with other changes) markedly attenuates the response to the T-cell mitogens with aging [10]. In remote periods (8 months) after irradiation, a decrease in proliferative responses to PHA and ConA was observed in young animals grafted BM cells from old donors [6]. In the present study, proliferative responses in group 2 and 3 mice did not differ very much. Moreover, a slightly increased proliferation of ConA-stimulated splenocytes was observed in irradiated animals (Table 1), which might be related to an increase in the $CD4^+/CD8^+$ ratio in them (groups 2 and 3).

Thus, old stem cells differentiating in the thymus of young irradiated mice form less mature peripheral T cells (both $CD4^+$ and $CD8^+$). In addition, the number of cells with the memory cell phenotype

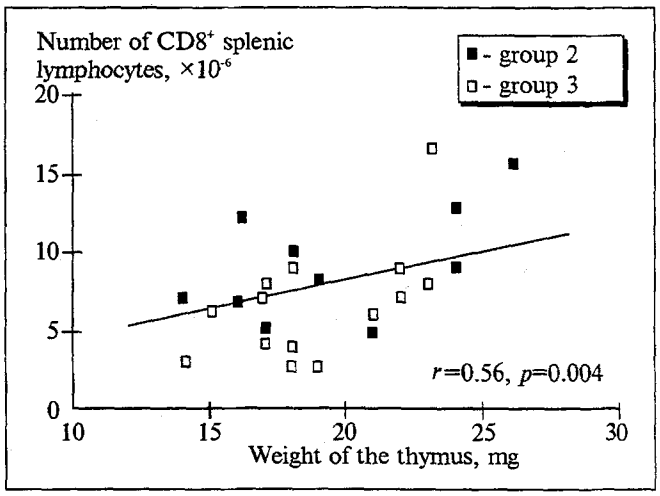

Fig. 3. Correlation between the weight of the thymus and the $CD8^+$-lymphocyte count in lethally irradiated mice grafted BM from young (group 2) or old (group 3) donors.

($CD4^+CD44^{high}$) is slightly increased in the population of $CD4^+$ lymphocytes in the recipients of old BM; predominance of such cells is characteristic of old animals. Nevertheless, changes in the main splenic lymphocyte populations did not modulate functional characteristics of these cells, specifically, their proliferative activity. Previously, it was shown that the age of BM donors has no appreciable effect on primary immune response in young irradiated CBA mice [4]. Our findings indicate that the BM stem cells undergo certain changes during aging, which may cause certain changes in the population of mature peripheral blood T lymphocytes.

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