PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 33-35 (1976)

Impaired Thymic Regeneration in Lethally Irradiated Mice Given Bone Marrow from Aged Donors[1]

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Both humoral and cell-mediated immune responses decline with advancing age as the result of complex changes in the immune system and its environment (1). In part this decline can be attributed to a decrease in the proliferative capacity of mature T-cells. This conclusion is based on observations demonstrating that although the number of θ positive cells in mouse lymphoid tissues remains relatively constant (1), the proliferative responses of these cells to T-cell mitogens (2), allogeneic cells (3), and to thymus-dependent antigens (4) decrease markedly with age.

To date, studies on age-related changes in cellular immunity have focused on the function of mature post-thymic T-cells obtained primarily from peripheral lymphoid tissues, and little or no information has been developed on the effects of aging on the precursors of these cells. Reported here are observations suggesting that with age there is an absolute decrease in bone marrow T-cell progenitors and/or an impairment of their proliferative capacity.

Materials and methods. Mice. Male (C57BL/6 × A/HeJ)F_1, B6AF_1, mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. Bone marrow recipients were 12- to 16-weeks old; they were given 900 R of whole-body γ radiation on the day of cell transfer (^{60}Co; 100 R/min; target-to-source, 80 cm; 100% lethal to unprotected controls in 11–14 days). After irradiation the mice were housed five per cage, and for 21 days they were given water containing neomycin. Bone marrow donors were 10- to 143-week-old B6AF_1 males.

Bone marrow. Donors were killed by cervical dislocation. Bone marrow cells were flushed from the femoral cavity with Hank's solution, and they were washed twice. The recovered cells were counted in triplicate in a hemocytometer. Cell viability was assayed by the trypan blue exclusion test, and in all cases 90 to 93% of the cells excluded the dye. After appropriate adjustments in cell concentration, the cells were injected iv in 0.2 ml into the lateral tail vein of irradiated recipients.

Regeneration of thymus and spleen. It is generally accepted that only T-cell precursors repopulate the thymus of lethally irradiated, bone marrow-reconstituted recipients (5). Therefore, irradiated mice were injected iv with 2.8 to 3.2 × 10^6 viable nucleated marrow cells obtained from 10- to 143-week-old syngeneic donors, and 3 weeks later their spleens and thymuses were removed and weighed individually. This time period was selected because preliminary work showed that repopulation of the thymus by donor cells was nearly maximal by 21 days after injection and host marrow recovery, if any, was minimal at this time (unpublished data). The results of these experiments have been expressed in terms of milligrams of organ weight per 10^6 cells injected. The data have been analyzed by means of Student's t test.

Colony-forming units (CFU) assay (6). Previous studies have shown that with most strains of mice there is little or no change with age in the number of hematopoietic stem cells (CFU) in the marrow (7, 8). Therefore, as a check on the reliability of the total cell counts and cell viability determinations, irradiated mice were injected iv with 1 to 1.5 × 10^5 marrow cells from the preparations used to measure lymphoid organ regeneration. Nine days later the mice were killed, and their spleens were fixed in Bouin's solution. CFU were counted under 4× magnification.

Results and discussion. Although the number of CFU varied somewhat between experiments, total CFU per femur and per 10^5 cells remained relatively constant between 14 and 127 weeks (Table I). These

[1] This work was supported by USPHS Research Grant Nos. HD 07708, DE 02668, and RR 5333.

TABLE I. SPLEEN CFU AND THYMUS AND SPLEEN WEIGHTS OF LETHALLY IRRADIATED B6AF$_1$ MICE GIVEN MARROW CELLS FROM AGING SYNGENEIC DONORS.[a]

Age of donor (weeks)	CFU			Organ weight (mg ± SD/10^6 cell)		
	Number of mice	Per femur	Per 10^5 cells	Number of mice	Thymus	Spleen
16	5	1545	13.5	7	9.9 ± 1.1	21.6 ± 3.6
63	5	1512	14.6	9	10.1 ± 0.9	19.2 ± 2.5
16	5	1612	11.5	5	9.8 ± 1.2	16.0 ± 2.7
71	5	1555	12.7	5	8.9 ± 1.1	16.1 ± 3.1
10				4	10.4 ± 0.8	21.6 ± 2.2
117				5	7.1 ± 0.7[b]	17.7 ± 2.6
14	6	2220	15.7	5	10.1 ± 1.2	23.7 ± 1.8
122	5	1884	14.8	5	6.8 ± 1.1[b]	24.1 ± 3.0
14	5	1643	11.3	5	10.3 ± 0.7	23.6 ± 2.4
124	5	1795	13.6	5	6.4 ± 0.9[b]	20.1 ± 1.8
16	5	2209	13.7	5	9.8 ± 0.9	23.3 ± 1.9
127	5	2034	14.5	5	5.2 ± 0.7[b]	23.6 ± 2.1
16				5	9.4 ± 0.9	24.6 ± 2.4
143				5	4.3 ± 0.3[b]	16.8 ± 2.6[b]

[a] The mice were exposed to 900 R ^{60}Co γ-radiation and given 1.0–1.5 × 10^5 or 2.8–3.2 × 10^6 marrow cells iv. Nine days later CFU were counted in the spleens of mice given 10^5 cells, and 21 days after irradiation, spleen and thymus weights were determined in recipients of 10^6 cells.

[b] $P < 0.01$, Student's t test.

findings are consistent with previous work which demonstrated that in healthy mice total marrow cellularity and the hematopoietic stem cell population were little affected by aging (7, 8).

Similarly, the regeneration of spleen weight was not consistently or significantly affected by the age of the marrow donor. The one exception to this was found in the group given marrow from 143-week-old donors; here splenic regeneration was significantly diminished. However, the reproducibility and the biological significance of this finding remain to be determined.

By contrast, thymic regeneration was significantly impaired when the irradiated recipients were given marrow cells from donors 117 weeks of age or older; further, there appeared to be an inverse relationship between the age of the donor and the ability of the marrow to repopulate the thymus.

Although it has been shown that marrow from aged donors has a diminished ability to repopulate the thymus of irradiated hosts, a function reserved to precursors of T-cells, it is not clear whether this is the result of a decrease in the size of the stem cell pool and/or an impairment of proliferative capacity. The resolution of these questions may await the discovery of a unique cell marker for the precursors of T-cells. In experiments not reported here, it has been found that although the graft-vs-host activity of spleen cells from 126-week-old mice was significantly diminished, 10 × 10^6 marrow cells from these aged donors produced the same mortality among irradiated recipients (4/20) as did equal numbers of cells from 12-week-old mice. This suggests that the numbers of post-thymic effector T-cells in the marrow inoculae were not grossly different.

Summary. Mice were given a lethal dose of whole-body γ radiation and injected with 10^5 or 10^6 marrow cells from 10- to 143-week-old syngeneic donors. Nine days later, colony-forming units (CFU) were counted in the spleens of mice given 10^5 cells, and 21 days after irradiation, spleen and thymus weights were determined in the recipients of 10^6 cells. It was found that there were no significant changes with age in marrow CFU or in the ability of marrow cells to repopulate host spleens. In contrast, thymic regeneration was significantly impaired when the recipients received marrow cells from donors 117 weeks of age or older. These observations suggest that with aging there is a decrease in marrow T-cell progenitors and/or a decline in their proliferative capacity.

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Received December 8, 1975. P.S.E.B.M. 1976, Vol. 152.