EFFECT OF NONSYNGENEIC LYMPHOCYTES ON DIFFERENTIATION OF STEM CELLS IN THE SPLEEN OF IRRADIATED MICE

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Transplantation of mixed spleen cells of two different genotypes (CBA and C57BL) into lethally irradiated (CBA × C57BL) F_1 hybrids leads to inactivation of the colony-forming units of one of the genotypes (C57BL). The colony-forming and differentiating ability of the stem cells of the other genotype (CBA) was unchanged. Interaction of sensitized C57BL mouse lymphocytes with stem cells of CBA mice was accompanied by changes in differentiation of the uninactivated stem cells from the erythroid to the myeloid path.

Transplantation of a mixture of spleen cells of mice from two parent lines into lethally irradiated hybrid recipients leads to inhibition of the colony-forming ability of the stem cells of one genotype [3, 4].

The object of the present investigation was to determine the effect of combined transplantation of spleen cells from two genetically different donors on the direction of differentiation of uninactivated stem cells, having regard to the scarcity of literature on this subject [1].

EXPERIMENTAL METHOD

CBA × C57BL F_1 hybrid mice irradiated on a cobalt apparatus in a dose of 850-900 R ($LD_{100/30}$) were used for the experiments. The F_1-recipient/mice received an intravenous injection of 2×10^6 spleen cells of mice of the parent lines (CBA and C57BL) separately or as a mixture, or 2×10^6 lymph gland and spleen cells, separately and as a mixture, 24 h after irradiation. The cell suspensions were compared and their viability determined by the method described previously [2]. In some experiments the donor C57BL mice were sensitized against tissues of CBA mice by skin grafting [7]. The mice were killed 12-14 days after grafting (always after rejection of the grafts), and a cell suspension was prepared from their regional lymph glands. On the 9th day after transplantation of hematopoietic and lymphoid cells the recipients' spleen was removed, fixed in Bouin's fluid, and the number of colonies was counted macroscopically by the method of Till and McCulloch [9]. The material was then embedded in paraffin wax. Series of sections were cut to a thickness of 5-7 μ and stained with hematoxylin-eosin. The following types of hematopoietic colonies were then counted by a microscopic method: erythroid (E), myeloid (My), megakaryocytic (Mk), undifferentiated (U), and mixed (Mx). The ratio between the number of erythroid colonies and the number of myeloid colonies (E/My) and between the number of myeloid and megakaryocytic colonies (My/Mk) was used as the index of differentiating ability of the stem cell. The index of inactivation (II) of colony-forming units (CFU) was calculated by the equation proposed previously [3].

EXPERIMENTAL RESULTS

As Table 1 shows, an equal number of spleen cells of CBA and C57Bl mice, transplanted separately into irradiated (CBA × C57BL) F_1 hybrids, formed different numbers of colonies in the recipients' spleen:

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TABLE 1. Histological Analysis of Colonies in the Spleen of Lethally Irradiated (CBA × C57BL) F_1 Recipient Mice Appearing after Transplantation of a Mixture of Spleen Cells of Mice of the Parent Lines * (M ± m)

Source of transplanted cells		Number of animals	Type of hematopoietic colonies							Mean number of colonies per spleen (inactivation index)			
CBA mice	C57BL mice		E	My	Mk	U	Mx	E/My	My/Mk	Microscopic analysis	II	Macroscopic analysis	II
Spleen	—	13	10,6±1,1	9,5±0,9	3,0±0,7	2,1±0,4	1,2±0,3	1,1	3,2	26,6±1,6	—	13,7±1,5	—
—	Spleen	10	28,8±2,1	4,0±2,5	2,3±0,5	5,3±0,9	0,7±0,3	7,2	1,7	41,3±5,0	—	21,5±1,1	—
Spleen	Spleen	16	10,5±1,7	8,0±2,0	2,4±0,8	3,5±0,8	0,3±0,1	1,3	3,3	25,0±4,5	63,30	11,3±0,8	67,90
Lymph gland	Spleen	15	0	0	0	0,1±0,1	0	—	—	0,1±0,1	100,0	0	100,0
Spleen	Lymph gland	10	12,4±3,8	7,3±0,6	1,8±0,3	4,0±0,8	0,4±0,1	1,7	4,0	26,0±4,6	2,300	14,5±1,3	0
Spleen	Lymph gland†	22	0,1±0,1	0,5±0,1	0,3±0,1	0,2±0,1	0,1±0,1	0,2	1,6	1,2±0,4	95,90	2,5±0,3	81,80
—	Lymph gland	10	1,6±0,1	0,4±0,1	0,2±0,1	0,2±0,1	0,2±0,1	4,0	2,0	2,4±1,0	0	0,8±0,2	0
—	—	10	0,1±0,1	0	0	0,1±0,1	0	—	—	0,2±0,1	86,00	0	100,0
		20	0,6±0,2	0,2±0,1	0,4±0,2	0,1±0,1	0,1±0,1	3,0	0,5	1,4±0,3	—	0,8±0,6	—

*Combined results of two to three experiments.

† Sensitized lymphocytes.

26.6 and 41.3 respectively. Transplantation of a mixture of the same numbers of spleen cells of mice of genotypes CBA and C57BL led to the formation of 25 colonies.

Histological analysis of the hematopoietic foci showed that the distribution of different types of colonies in the spleen of the F_1 recipients receiving a mixture of cells was that characteristic of CBA mice. In fact, in the case of transplantation of spleen cells from CBA mice into the F_1 hybrid the ratio E/My and My/Mk was 1.1 and 3.2 respectively; in the case of transplantation of cells of C57BL mice these ratios were 7.2 and 1.7. After combined transplantation of spleen cells the ratios E/My and My/Mk were 1.3 and 3.3; i.e., the same as the ratio between the number of colonies when spleen cells of CBA mice were transplanted separately.

When the number of colonies in the spleen of recipients receiving a mixture of spleen cells from CBA and C57BL mice was assessed (by macroscopic and microscopic methods) the number of colonies recorded corresponded to the number of CFU contained in the suspension of spleen cells of CBA mice. In this case inactivation of colony-forming cells of C57BL mice was found histologically. The ability of the stem cells of CBA mice to differentiate toward erythropoiesis, myelopoiesis, and thrombocytopoiesis was unchanged. Lymphoid cells of the CBA-genotype exhibited an inactivating action. This is clear from the fact that combined transplantation of spleen cells of C57BL mice with lymph gland cells of CBA mice led to blocking of the proliferative activity of the stem cells contained in the suspension of C57BL spleen cells (inactivation index 100%). Lymphoid cells of the C57BL genotype did not affect colony-forming and differentiating properties of the stem cells of CBA mice (it will be recalled that no colony-forming cells are present in lymphoid tissue [6]), and it can accordingly be assumed that during transplantation of a mixture of spleen cells of CBA and C57BL mice the lymphoid cells of CBA mice will inactivate multiplication of the stem cells of C57BL mice. This is in good agreement with the results of chromosome analysis of the inactivation effect [5, 8]. In experiments in which lymphocytes of C57BL mice sensitized against the CBA genotype were used, a "killing effect" was observed on the CFU, as a result of which differentiation of the remaining uninactivated stem cells was changed toward predominance of myeloid colonies.

To sum up the results described above it can be concluded that interaction between nonsyngeneic lymphocytes and hematopoietic cells of the transplanted mixture leads to elimination of the CFU of one of the genotype (C57BL). The process of colony formation and the direction of differentiation of the stem cells along the path of erythropoiesis, myelopoiesis, and thrombocytopoiesis of the other genotype (CBA) were unchanged. Interaction between sensitized lymphocytes of C57BL mice with hematopoietic cells of CBA mice leads to marked inactivation of the colony-forming properties of the latter and changes the differentiation of the uninactivated cells from the erythroid path to the myeloid.

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