CHROMOSOMAL ANALYSIS OF INACTIVATION OF STEM CELLS BY TRANSPLANTATION OF MIXED BONE MARROW FROM GENETICALLY DIFFERENT DONORS

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UDC 612.419+612.42] : 612.6.02 : 576.312.32

A mixture of equal volumes of bone marrow cells from donor mice of lines A and CBAT6T6 was transplanted into lethally irradiated F_1 (A × CBAT6T6) mice, and a mixture of F_1 (A × CBAT6T6) + F_1 (CBA × C57BL) cells was injected into lethally irradiated CBAT6T6 recipients. Cytogenetic analysis of colonies in the spleen and bone marrow of the recipients showed that transplantation of the mixture of bone marrow cells from genetically incompatible donors is accompanied by inactivation of the stem cells of one of the genotypes. Not only the ability of the stem cells to form colonies, but also their ability to proliferate in the recipient's bone marrow is inhibited.

It has recently been found that transplantation of a mixture of hematopoietic or lymphoid cells from intact mice of different genotypes into lethally irradiated recipients leads to considerable or complete blocking of transplanted hematopoietic stem cells [1, 2, 5]. Maximum inactivation as regards the number of colony-forming units (CFU) is observed after transplantation of lymph gland cells from mice of one genotype (95-100%). After combined transplantation of bone marrow cells or a mixture of embryonic hematopoietic tissues of two different genotypes, the inactivation index is smaller (35-36%). It may be asked what is responsible for this difference. It is also uncertain whether only the ability of the bone marrow stem cells to form splenic colonies is inhibited, or whether their ability to proliferate in other organs of the hematopoietic system is also inhibited.

An attempt was therefore made to examine this problem in experiments on inbred mice the cells of which contain marker chromosomes.

EXPERIMENTAL METHOD

Male inbred mice of lines A and CBAT6T6, and first generation hybrids F_1 (A × CBAT6T6) and F_1 (CBA × C57BL) were used in the experiments. In other words cells of three different karyotypes were used: cells with two T6 markers among their chromosomes – CBAT6T6 (T6+T6+), cells with one T6 chromosome – F_1 (A × CBAT6T6) (T6+T6–), and a karyotype with no T6 chromosome in the cells – A and F_1 (CBA × C57BL) (T6–T6–).

By the method used previously [6], $5 \cdot 10^4$ nucleated bone marrow cells from one of the two donor genotypes were transplanted into two groups of recipients (control, groups 1 and 2), and a mixture of equal numbers of cells of the two donor genotypes (a total of $10 \cdot 10^4$ karyocytes) was transplanted into the recipients of group 3 (experimental).

The recipient mice were irradiated 24 h before transplantation on a type EGO-2 apparatus in a dose of 850-900 R.

Institute of Biophysics, Ministry of Health of the USSR. (Presented by Academician P. D. Gorizontov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 6, pp. 88-91, June, 1970. Original article submitted November 5, 1969.

TABLE 1. Identification of Colonies in Spleens of Lethally Irradiated F_1 (A × CBAT6T6) Mice Receiving Mixture of Bone Marrow Cells of Two Genotypes: CBAT6T6 and A

Group of animals	Genotype of transplanted bone marrow cells	No. of col. in spleen of ind. rec.	No. of colonies of corr. karotype		Mean no. of CFU/ $5 \cdot 10^4$ injected cells		Index of inactivation (in percent)		Significance of inactivation, P	
			T6+T6+	T6—T6—	T6+T6+	T6—T6—	T6+T6+	T6—T6—	T6+T6+	T6—T6—
1	CBA (T6+T6+)	12; 18; 14; 13; 15	72	—	14,4± 1,03	—				
2	A (T6—T6—)	11; 10; 12; 11; 12; 8	—	64		10,6±0,61				
3	CBA+A (T6+T6+ и T6—T6—)	13; 17; 16; 14; 15; 16; 11; 12	26	88	3,2± 0,45	11,0±0,68	77,4	Inactivation absent	<0,001	Inactivation absent

TABLE 2. Identification of Colonies in Spleens of Lethally Irradiated CBAT6T6 Mice Receiving Mixture of Bone Marrow Cells of Two Genotypes: F_1 (A × CBAT6T6) + F_1 (CBA × C57BL)

Group of animals	Genotype of transplanted bone marrow cells	No. of col. in spleen of ind. rec.	No. of colonies of corr. karotype		Mean no. of CFU/ $5 \cdot 10^4$ injected cells		Index of inactivation (in percent)		Significance of inactivation, P	
			T6+T6—	T6—T6—	T6+T6—	T6—T6—	T6+T6—	T6—T6—	T6+T6—	T6—T6—
1	F_1(A×CBA) (T6+T6—)	14; 12; 14; 16; 15; 8; 7; 5; 4	95	—	10,5±1,49	—				
2	F_1(CBA×C57BL) (T6—T6—)	9; 8; 10; 11; 8; 6; 8; 11; 13; 12; 13; 7	—	116	—	9,6±0,74				
3	F_1(A×CBA) F_1(CBA×C57BL) (T6+T6—) и (T6—T6—)	16; 18; 18; 20; 10; 15; 7; 9; 15	91[1]	30	10,1±0,63	3,3±0,33	4,7	65,5	>0,5	<0,001

*Of 128 colonies of group 3, 7 contained cells of karyotype T6+T6+, i.e., these were spontaneous colonies of recipient nature.

TABLE 3. Chromosomal Analysis of Bone Marrow of Lethally Irradiated Recipients 9-10 Days After Mixed Transplantation

Donors of bone marrow	No. of identified metaphase plates	Karotype of cells		
		T6+T6+	T6−T6−	T6+T6−
CBAT6T6+A	127	23(18,2%)	99(77,8%)	5(4%)
F_1(A+CBAT6T6) + F_1(CBA×C57BL)	149	42(28,1%)	26(17,4%)	81(54,5%)

The recipients were sacrificed 9 and 10 days after transplantation and the number of colonies in the spleens was counted by the method of Till and McCulloch [7]. By means of Ford's methods, cytogenetic studies were made of the bone marrow [4] and the colonies in the recipients' spleens were identified [3].

The standard error of the arithmetic mean was calculated and values of P were determined using Student's criterion.

EXPERIMENTAL RESULTS

The results of the experiment in which a mixture of bone marrow cells of mice CBAT6T6+A was transplanted into lethally irradiated F_1 (A × CBAT6T6) recipients are given in Table 1. The results of this experiment show that some stem cells of the transplanted mixture of bone marrow cells were inactivated. The colony-forming activity of one of the genotypes was inactivated: a marked decrease in the number of colonies of genotype CBAT6T6 was observed compared with the corresponding control 1 (index of inactivation 77.4%). The number of colonies of genotype A after combined transplantation with CBAT6T6 cells was not reduced.

Since in the experiment described above the results of transplantation could be explained by a "graft versus host" reaction, it was decided to investigate whether inactivation of the stem cells of the mixture takes place in the absence of such a reaction. A mixture of bone marrow cells from F_1 (A × CBAT6T6) + F_1 (CBA × C57BL) donors was transplanted into lethally irradiated CBAT6T6 recipients. In these experiments (Table 2) the effect was accompanied by inactivation predominantly (index of inactivation 65.5%) of the colony-forming cells of the F_1 (CAB × C57BL) mice. Inactivation of colony-forming activity of the F_1 (A × CBAT6T6) gentotype was not statistically significant ($P > 0.05$).

After transplantation of a mixture of bone marrow cells of different genotypes into lethally irradiated recipients, a proportion of the stem cells of the graft is inactivated; not only the colony-forming elements, but also cells proliferating in the recipient's bone marrow are inhibited. Stem cells of one of the two transplanted genotypes are inactivated.

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