HYBRID RESISTANCE OF BONE MARROW STROMA TO PRECURSOR CELLS

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The focus of hematopoiesis arising after transplantation of a fragment of bone marrow from a C57BL mouse beneath the capsule of the kidney of a syngeneic mouse contains more hematopoietic cells than if transplanted into a semisyngeneic (CBA × C57BL)F_1 recipient. Experiments with repopulation of the graft, when depopulated by irradiation, by injected hematopoietic cells of the same genotype as the graft (C57BL) showed that these differences are due to the smaller size of the hemotopoietic microenvironment in the focus formed in the hybrid than in the syngeneic system. Hybrid resistance is thus manifested not only against hematopoietic cells, but also against stromal precursors transferring the hematopoietic microenvironment.

KEY WORDS: *heterotopic transplantation; bone marrow; stromal precursor cells; hybrid resistance.*

After transplantation of bone marrow from mice of some strains to their F_1 hybrids proliferation of the injected hematopoietic cells is strongly inhibited, as is shown by the reduced formation of hematopoietic colonies in the spleen [9], the smaller number of DNA-synthesizing cells in the spleen [4], and the reduced number of hemoglobin-synthesizing cells in the spleen and bone marrow [7]. This phenomenon has been called hybrid resistance, allogeneic inhibition, repression of colony-forming units (CFU), etc. Its nature has not been established. The suggestion has been made that the phenomenon is connected with the presence of products of a recessive gene, which is nonfunctioning in the heterozygous state, on the surface of the hematopoietic cells of homozygous animals, as a result of which these products are not present on cells of the F_1 hybrid. Since hybrid resistance is clearly manifested against transplanted hematopoietic cells, it is postulated that the gene functions only in hematopoietic stem cells and in their progeny, and it is therefore known as Hh (hematopoietic histocompatibility) [5].

When studying the phenomenon of hybrid resistance on a model of a heterotopic hematopoietic focus, the writers unexpectedly found that this phenomenon is also manifested against cells building the stroma of the hematopoietic organs, i.e., against nonhematopoietic cells. These results are described below.

EXPERIMENTAL METHOD

C57BL and (CBA × C57BL)F_1 mice aged 8-12 weeks were studied. To obtain a focus of heterotopic hematopoiesis, a fragment of bone marrow expressed by means of a stilet from the femur was transplanted beneath the kidney capsule of mice anesthetized with hexobarbital. The size of the focus thus formed was estimated 1-1.5 months after transplantation from the number of hematopoietic cells (after flushing out with medium No. 199 from the bone) and the number of hematopoietic stem cells (CFU) contained in it. At each point cells from 8 to 10 foci of ectopic hematopoiesis were mixed. The number of CFU was determined by cloning in the spleen of irradiated mice [11]. The animals were irradiated with ^{137}Cs γ rays in doses of 1100 (C57BL) or 1300 rad (CBA × C57BL)F_1. During the 2 h after irradiation, hematopoietic

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TABLE 1. Splenic and Medullary Fractions of CFU (f 24 h) from a Heterotopic Hematopoietic Focus

Experiment	Number of CFU/10^4 cells in initial suspension of hematopoietic cells	Number of CFU injected into intermediate recipient	Material injected into final recipient	Colonies/spleen in final recipient	CFU/organ in intermediate recipient	f
1	8,0±0,8	400	Cells from 1/3 spleen	11,7±1,5	35,1/spleen	0,081
			Cells from two femora	7,1±1,4	3,55/femur	0,009
2	8,9±1,0	445	Cells from 1/4 spleen	7,7±0,7	30,8/spleen	0,069
			Cells from one femur	2,0±0,6	2,0/femur	0,004

TABLE 2. Size of Focus of Heterotopic Hematopoiesis Formed by Bone Marrow of C57BL Mice in Syngeneic and Semisyngeneic Systems

Experiment	Graft	Recipient	Number of cells (number of CFU in parentheses) per focus	Recipient	Number of cells (number of CFU in parentheses) per focus
1	Bone marrow from 1/4 femur	C57BL	$3,8\times10^6$ (712±66)	(CBA×C57BL) F1	$1,4\times10^6$ (88±28)
	Bone marrow from 1 femur	C57BL	$8,1\times10^6$ (625±101)	(CBA×C57BL) F1	$5,3\times10^6$ (554±106)
2	Bone marrow from 1 femur	C57BL	$3,4\times10^6$	(CBA×C57BL) F1	$2,6\times10^6$

cells were injected into the mice (10 to 12 in each group) in a dose of $4 \cdot 10^4$. The spleens were fixed 8 days later in Bouin's fluid and the number of colonies was counted under a magnifying glass. The number of endogenous colonies did not exceed 0.2 per spleen. The fractions (f) of stem cells settling in the spleen and bone marrow were determined [10]. Hematopoietic cells were injected into irradiated intermediate recipients. Some of the splenic or femoral cells were injected 24 h later into irradiated final recipients. The value of f was calculated as the fraction of stem cells contained in the original suspension that gave colonies in the final recipients.

EXPERIMENTAL RESULTS

Since one of the criteria used to estimate the size of the heterotopic focus was the number of CFU it contained, it was necessary to make sure that the splenic colonies method is suitable for determination of the number of CFU in a heterotopic focus, i.e., that the CFU from a focus of ectopic hematopoiesis do not have altered affinity for the spleen (Table 1). As Table 1 shows, after 24 h 7-8% of injected CFU was found in the spleen, compared to 0.4-0.9% in the marrow of one femur, corresponding to the distribution of normal hematopoietic stem cells [2, 8].

In the case of heterotopic transplantation of bone marrow into the syngeneic recipient the size of the resulting focus, based on the number of hematopoietic cells, was 1.5-2.5 times greater than in the semisyngeneic recipient resistant to the donor strain (Table 2). Significantly less clear results were obtained on the basis of the number of CFU. When these differences are analyzed, two factors must be taken into account. First, the bone marrow contains cells capable of abolishing or sharply reducing the hybrid resistance of the recipient to hematopoietic cells when injected intravenously [1]. It can be accepted that bone marrow cells can abolish resistance when transplanted beneath the kidney capsule also. This hypothesis was confirmed by the experimental results. The ability of F_1 hybrids to inhibit growth of the hematopoietic cells of the parental strain was assessed by determining the number of CFU formed in an irradiated hybrid in the course of 7 days after transplantation of $2 \cdot 10^6$ hematopoietic cells into it (Table 3). Clearly heterotopic transplantation of parental bone marrow sharply reduced the ability of the hybrid to inhibit the proliferation of injected hematopoietic stem cells from the same parental strain, i.e., the C57BL genotype. The second factor that can influence the determination of size of the heterotopic focus was connected with the fact that the newly formed hematopoietic organ is a chimera in which the donor's cells are gradually replaced by the recipient's hematopoietic cells [3, 6]. Depending on the fraction of donor's cells that have succeeded in being replaced by recipient's cells at the time of testing, the results of testing will vary: The longer the donor's cells

TABLE 3. Effect of Heterotopic Transplantation of Bone Marrow from C57BL Mice on Hybrid Resistance of (CBA × C57BL)F_1 Mice

Recipient	Donor of heterotopic graft	Number of CFU 7 days after transplantation of 2×10^6 C57BL bone marrow cells		
		in graft	in spleen	in femur
C57BL	C57BL	72±2,8	175±9,6	35,6±1,4
(CBA×C57BL) F1	—	—	39±13,8	4,4±0,8
The same	C57BL	57±2,8	178±8,4	17,2±1,7

TABLE 4. Size of Focus of Hematopoiesis of C57BL Genotype in Syngeneic and Semisyngeneic Recipient

Experiment	Recipient	Number of CFU 7 days after transplantation of 2×10^6 C57BL bone marrow cells	
		in graft	in femur
1	C57BL	133±5,5	88±2,3
	(CBA×C57BL) F_1	45±3,4	7,8±0,8
2	C57BL	81±3,2	—
	(CBA×C57BL) F1	26±1,4	—

(C57BL) have been preserved in the focus, the smaller the number of stem cells detectable in the test recipient (F_1 hybrid), because of the marked hybrid resistance to them.

In order to abolish the effect of both these factors the experiments were carried out as follows. Bone marrow previously irradiated *in vitro* in a dose of 340 rad was used for heterotopic transplantation. After irradiation in this way the ability of the hematopoietic cells to abolish hybrid resistance disappears completely [1], whereas the ability of stromal cells to form a focus is virtually not reduced. To avoid chimerism in the graft the following method uas used. After formation of the heterotopic focus the mice were irradiated in a dose of 1300 rad and they were then grafted with bone marrow ($2 \cdot 10^6$ cells) of the C57BL genotype, i.e., syngeneic with the stroma of the graft. The number of stromal cells was counted 7 days later by transplanting cells from the focus of ectopic hematopoiesis into irradiated C57BL mice; under these conditions its value was determined entirely by the size of the stroma of the heterotopic focus suitable for repopulation by syngeneic hematopoietic cells. The results showed (Table 4) that the hybrid carrying such a graft completely preserved its hybrid resistance (in the course of 7 days of proliferation the number of newly formed stem cells, counted per femur, was only one-tenth that in the syngeneic system) and, consequently, preliminary irradiation abolished the depressive activity of the graft. The size of the focus in the semiallogeneic system or, more exactly, the size of the hematopoietic microenvironment suitable for repopulation of syngeneic hematopoietic cells was found to be between 25 and 29% of that in the syngeneic system (Table 4).

The results thus show that the phenomenon of hybrid resistance is manifested not only in hematopoietic, but also in the other cell populations which are not derivatives of hematopoietic stem cells. This applies at least to cells capable of transferring the hematopoietic microenvironment and it thus suggests that products of the Hh gene are not confined to hematopoietic cells.

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