ROLE OF THE BONE MARROW STROMA IN THE HYBRID RESISTANCE PHENOMENON

I. L. Chertkov, O. A. Gurevich, and G. A. Udalov

UDC 612.419.014.2

An irradiated fragment of bone marrow was implanted beneath the capsule of the kidney. Karyologic analysis showed that all hematopoietic cells in the newly formed focus were of recipient origin. Meanwhile, resistance to the bone marrow graft was of donor type. Cells of hematopoietic origin capable of migration, including lymphocytes and macrophages, play no part in the phenomenon of hybrid resistance.

KEY WORDS: bone marrow; heterotopic transplantation; hybrid resistance; chromosomal markers.

Hematopoietic cells transplanted into an irradiated xenogeneic, allogeneic, or semiallogeneic recipient regenerate more slowly than in a syngeneic organism. This phenomenon, which has been called genetic resistance to a hematopoietic graft [4], is particularly marked during transplantation of the bone marrow of certain lines of mice into their first generation hybrids, when it is known as hybrid resistance [2, 5, 9].

The question of the nature of the recipient's cells which interact with the transplanted hematopoietic cells has not yet been decided. However the answer to it is important for the understanding of the mechanisms of regulation of hematopoiesis.

In the present investigation the nature of cells responsible for the phenomenon of hybrid resistance were studied in a model heterotopic focus of hematopoiesis in which the stromal and hematopoietic cells were of different genotypes.

EXPERIMENTAL MATERIAL AND METHOD

Experiments were carried out on female CBA-H, CBAT6T6, C57BL, (C57BL × CBA)F_1 (later referred to as CBF_1), and (C57BL × CBAT6T6)F_1 mice. To obtain a focus of heterotopic hematopoiesis, femoral bone marrow was implanted beneath the capsule of the kidney. In some experiments the primary focus was retransplanted 1 month later into a second recipient. The bone marrow was irradiated in vitro with ^{137}Cs γ-rays with a dose rate of 500 rad/min. The nature of the dividing cells in the heterotopic focus was established karyologically by means of the T6 marker, which differentiated the donor from the recipient. To determine hybrid resistance

TABLE 1. Hybrid Resistance to Hematopoietic Transplant in Territory of Focus of Heterotopic Hematopoiesis

Expt.	Recipient of implant	Donor of implant	Dose of irradiation of implant, rad	Time after implantation, months	Dose of C57BL cells injected into recipient of implant	No. of CFU/implant, M ± m	
						7 days after irradiation and transplantation of bone marrow	10 days after irradiation and transplantation of bone marrow
1	CBF_1	CBF_1	—	1	2	3±0,8	
		C57BL	—	1	2	17±3,4	
2	CBF_1	CBF_1	1000	$8^1/_2$	1	36±4,3	
		C57BL	1000	$8^1/_2$	1	98±11,7	
3	CBF_1	CBF_1	1500	$3^1/_2$	1		8±1,0
		C57BL	1500	$3^1/_2$	1		47±8,5
4	CBF_1	CBF_1	1500	$3^1/_2$	2	24±4,0	68±12,0
		C57BL	1500	$3^1/_2$	2	49±3,8	395±45,0
	C57BL	C57BL	1500	$3^1/_2$	2	50±10,4	350±27,5

Laboratory of Bone Marrow Culture and Transplantation, Central Institute of Hematology and Blood Transfusion, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 337-340, April, 1979. Original article submitted April 14, 1978.

0007-4888/79/8704-0343$07.50

TABLE 2. Origin of Hematopoietic Cells in Focus of Heterotopic Hematopoiesis

Expt.	Donor of implant	Recipient of implant	Dose of irradiation of implant, rad	Time after implantation, months	No. of implants	No. of metaphases of recipient and donor	Fraction of recipient's metaphases
1	C57BL	CBFI-T6	—	1—4*	23	751/114	86% ±9,3
			—	6	2	58/6	94%
			—	14	11	983/17	98% ±3,0
2	C57BL	CBFI-T6	1000	2	6	246/16	94% ±4,0
			1000	6	6	400/0	100%
3	CBA	CBAT6T6	—	1—4*	37	1039/546	66% ±7,8
			—	6	2	72/27	73%
			—	14	6	568/34	94% ±6,8
4	CBA	CBAT6T6	500	1	3	136/115	54% ±12,1
			500	3	4	203/197	51% ±7,2
5	CBA	CBAT6T6	1000	1	1	70/14	83%
			1000	2	5	220/16	93% ±4,3
			1000	3	6	462/22	95% ±4,0
			1000	5	3	300/0	100%
			1500	3	5	480/0	100%
6	CBAT6T6	CBA †	—	1 ‡	4	632/78	89% ±8,7
			—	4 ‡	5	500/0	100%

*Some of these results were published in [1].

†The focus of hematopoiesis was formed in an intermediate CBA T6 T6 recipient and regrafted 1 month later.

‡Time after retransplantation.

Note. Numerator, number of recipient's metaphases; denominator, number of donor's metaphases.

in the territory of the focus of ectopic hematopoiesis the mice were irradiated (CBF_1 in a dose of 1300 rad, C57BL in a dose of 1100 rad) with ^{137}Cs γ-rays with a dose rate of 21 rad/min, after which the mice received an intravenous injection of 1×10^6 to 2×10^6 C57BL bone-marrow cells. At various times after this injection the number of hematopoietic stem cells (CFU) in the foci of the C57BL genotype was determined by the method in [10] and compared with the number in foci of the CBF_1 genotype. Cells from 7-12 foci were mixed at each point. In these experiments the intermediate recipients were CBF_1 mice, each with two grafts — of the C57BL genotype on one kidney and CBF_1 on the other. The dimensions of the foci of the different genotypes were virtually identical.

EXPERIMENTAL RESULTS AND DISCUSSION

After implantation of the bone marrow fragment the hematopoietic cells leave the graft, but the donor's stromal precursors "build" cancellous bone [3] which is repopulated by the recipient's hematopoietic cells [6, 7]. It was important to discover whether conditions favoring hybrid resistance are produced in such a newly formed focus. For this purpose the kinetics of growth of CFU was determined after injection of 2×10^6 bone-marrow cells of the C57BL genotype into irradiated C57BL and CBF_1 recipients carrying syngeneic bone-marrow implants (Fig. 2). The kinetics of CFU in the femoral marrow and heterotopic focus was found to be identical. In the syngeneic system the exponential phase of growth was observed after the 4th day, but in the semiallogeneic system 3 days later, reflecting marked hybrid resistance, which was manifested equally in the bone marrow and in the focus of heterotopic hematopoiesis.

After these results had been obtained it was possible to move on to the main experiment in which bone marrow of the C57Bl and CBF_1 genotypes was transplanted into the same CBF_1 recipient. After formation of the focus the mice were irradiated, C57BL hematopoietic cells were injected into them, and during the exponential phase of growth the number of CFU was compared in the two foci. As Table 1 (experiment 1) shows, 7 days after transplantation the number of CFU in the focus of C57BL genotype was considerably greater than in the hybrid focus. Hence it follows that the hematopoietic cells of the hybrid repopulating the implant did not cause hybrid resistance on its territory. However, it must be remembered that not all hematopoietic cells of a heterotopic focus are of recipient origin [1, 6]. In the first 6 months both in a semiallogeneic (Table 2, experiment 1) and, rather more so, in a syngeneic system (Table 2, experiment 3), many dividing cells of donor origin still remain; even 1 year after implantation a determinable number of donor's cells still remains in the foci. Replacement took place more rapidly in the case of retransplantation of the focus, for 4 months later no dividing donor's cells remained in it (Table 2, experiment 6). After preliminary irradiation of the implants in a dose of 500 rad the number of donor's cells in

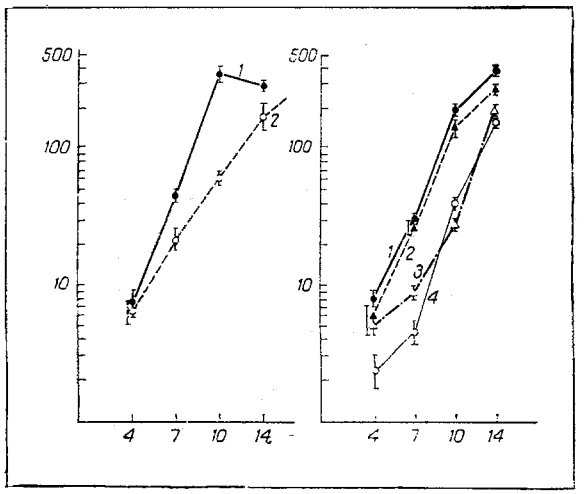

Fig. 1 Fig. 2

Fig. 1. Kinetics of growth of CFU of C57BL genotype in irradiated CBF_1 recipients. Abscissa, time after irradiation and transplantation of hematopoietic cells (in days); ordinate, number of CFU in heterotopic focus of C57BL (curve 1) or CBF_1 (curve 2) genotype.

Fig. 2. Kinetics of growth of CFU of C57BL genotype in irradiated CBF_1 (curves 3 and 4) and C57BL (curves 1 and 2) recipients. Abscissa, time after irradiation and transplantation of hematopoietic cells (in days); ordinate, number of CFU in femur (curves 1, 4) or in heterotopic focus (curves 2 and 3).

them did not diminish during the first 3 months after implantation (Table 2, experiment 4). After irradiation in a dose of 1000 rad the donor's cells did not disappear completely until 5 or 6 months (Table 2, experiments 2 and 5). No donor's hematopoietic cells could be found in the graft 3 months after irradiation in a dose of 1500 rad (Table 2, experiment 5). Accordingly hybrid resistance in the heterotopic focus of hematopoiesis was determined 8.5 months after implantation of bone marrow irradiated in a dose of 1000 rad (Table 1, experiment 2) or 3.5 months after retransplantation of a hematopoietic focus formed by bone marrow irradiated in a dose of 1500 rad (Table 1, experiments 3 and 4).

The number of CFU in foci of the C57BL genotype, colonized by CBF_1 hematopoietic cells, 7-10 days after irradiation and injection of hematopoietic cells was 2-6 times greater than the number of CFU in foci of the CBF_1 genotype and corresponded strictly to the number of CFU in foci of the C57BL genotype located in a syngeneic environment (Table 1, experiment 4). To obtain more detailed information on the phenomenon the kinetics of growth of CFU in foci of hematopoiesis of C57BL and CBF_1 genotypes, formed by retransplanted bone marrow irradiated in a dose of 1500 rad, was studied. Just as in the previous experiments, C57BL bone marrow was implanted on one side and CBF_1 marrow on the other side into each recipient. An equal number of CFU in the foci was observed only 4 days after irradiation and injection of hematopoietic cells, i.e., before the beginning of the exponential phase of growth of the CFU. Later the kinetics of growth of CFU in foci of the CBF_1 genotype showed a delay of 3-7 days compared with foci of the C57BL genotype (Fig. 1).

An attempt to use the model of a heterotopic hematopoietic focus to analyse the mechanism of hybrid resistance was undertaken on a model of implantation of the spleen of newborn mice [8]. Hybrid resistance was preserved in the implants, but the absence of data on the genotype of the hematopoietic cells in such spleens, together with a shortage of quantitative data, does not allow any final conclusions to be drawn regarding the nature of the cells causing resistance to the hematopoietic graft.

This investigation showed that hybrid resistance was absent in foci of the C57BL genotype in which the hematopoietic cells had been destroyed by a high dose of irradiation. In

such foci, repopulated twice with hematopoietic cells of the CBF_1 hybrid, stem cells of the C57BL genotype regenerated the same way as in syngeneic hematopoietic tissue. These results show that cells of hematopoietic origin capable of repopulation, including lymphocytes and macrophages, do not participate in the mechanism of hybrid resistance.

Another important conclusion is that regulation of regeneration of hematopoietic stem cells is local in character and is due to cooperate interaction between the injected hematopoietic stem cells and the stromal microenvironment of the hematopoietic organs and not to their interaction with cells of myeloid origin or their progeny, capable of repopulation.

LITERATURE CITED

1. G. A. Udalov, O. A. Gurevich, and I. L. Chertkov, Byull. Éksp. Biol. Med., No. 5, 584 (1977).
2. I. L. Chertkov, L. N. Lemeneva, and O. A. Gurevich, Probl. Gematol., No. 1, 3 (1972).
3. W. H. Crosby, in: Symposium on Hemopoietic Cellular Proliferation. Proceedings, F. Stohlman, ed., New York (1970), p. 87.
4. G. Cudkowicz, Transplant. Proc., 7, 155 (1975).
5. G. Cudkowicz and M. Bennett, J. Exp. Med., 134, 1513 (1971).
6. W. Fried, S. Husseini, W. H. Knospe, et al., Exp. Hematol., 1, 29 (1973).
7. A. J. Friedenstein, K. V. Petrakova, A. I. Kuralesova, et al., Transplatation, 6, 230 (1968).
8. A. Lengerova, V. Matousek, and V. Zeleny, Transplant. Proc., 5, 1389 (1973).
9. E. A. McCulloch and J. E. Till, J. Cell. Physiol., 61, 301 (1963).
10. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).

RADIOSENSITIVITY OF COLONY-FORMING UNITS OF DOG BONE MARROW IN AGAR CULTURES

I. A. Gorbunova

UDC 612.119.612.112.12

The radiosensitivity of colony-forming units in dog bone marrow was determined by a modified method of cloning hematopoietic cells in semisolid agar gel in diffusion chambers in vivo. The dose of radiation whose action was followed by preservation of 37% of the objects (D_o) was 144 ± 14.8 rad ($n = 0.8$) for committed precursor cells of granulocytopoiesis (CFUc) and 468 ± 35.8 rad ($n = 0.9$) for precursor cells forming "stellate" colonies of fibroblast-like cells (CFUf). It is concluded that the CFUf belong to the class of stromal precursors of hematopoietic organs. This system is suitable for the simultaneous study of hematopoietic and stromal precursor cells in dogs.

KEY WORDS: hematopoietic precursor cells; stromal precursor cells; bone marrow; radiosensitivity.

The agar method of culture of mammalian bone marrow cells can be used to obtain information about colony-forming units, i.e., about precursor cells committed in the granulocytic direction (CFUc).

During culture of dogs' bone marrow in an agar medium, besides colonies of granulocytes and monocytes, "stellate" colonies consisting of elongated cells resembling fibroblasts also were observed [1]. However, it was not clear whether these colonies were formed by stromal mechanocytes (fibroblasts) or by hematopoietic cells (histiocytes, for example). Since analysis of the morphological data and the kinetics of the "stellate" colonies [1] did not answer this question, the investigation described below was undertaken to determine

Laboratory of Bone Marrow Culture and Transplantation, Central Institute of Hematology and Blood Transfusion, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 340-342, April, 1979. Original article submitted February 27, 1978.

0007-4888/79/8704-0346$07.50