HYBRID RESISTANCE IN LONG-TERM BONE MARROW CULTURE AND IN FOCI OF ECTOPIC HEMATOPOIESIS FORMED BY IT

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The phenomenon of hybrid resistance to a bone marrow graft [8] consists of delayed growth of hematopoietic stem cells of certain strains of mice in an irradiated F_1 hybrid recipient [5]. The phenomenon has aroused great interest because it provides a model with which to study regulation of stem cell proliferation. However, its nature is not yet clear. A method of long-term bone marrow culture suitable for the analysis of this problem *in vitro* has recently been developed [6]. Practically all directions of differentiation of stem cells are realized in such a culture on an intricately organized adherent cell layer (ACL) and their proliferation is controlled. Meanwhile the phenomenon of hybrid (allogenic) resistance could not be reproduced in culture [7].

In this connection it was interesting to determine whether cells of cultures, on reimplantation *in vivo*, can construct an ectopic focus of hematopoiesis in which the phenomenon of hybrid resistance would take place, as happens in ectopic foci formed by bone marrow which has not been cultured [4]. The investigation described below was devoted to a study of this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male C57BL/6 (hereafter B6) male mice and also on (CBA × C57BL/6)F_1 hybrids (CBF_1) aged 8-12 weeks at the beginning of the investigation. Long-term bone marrow cultures of Dexter type were grown in Fisher's medium with 20% horse serum and 10^{-6} M hydrocortisone hemisuccinate [1]. To study hybrid resistance *in vitro*, 3-week cultures were irradiated in a dose of 10 Gy (dose rate 5 Gy/min) with ^{137}Cs γ-rays on an IPK apparatus [4], after which the medium was completely changed and a second application of a suspension of syngeneic or semisyngeneic bone marrow cells was made. The ability of adherent cells of the cultures to exhibit the phenomenon of hybrid resistance *in vivo* was studied by implantation of ACL (8-12 cultures in the group) beneath the kidney capsule, as described in [1]. Cultures of B6 genotype were implanted in syngeneic and CBF_1 recipients. In the latter case, a culture of CBF_1 genotype was implanted beneath the capsule of the second kidney. After 7 or 10 days the number of CFUc was counted in the ectopic foci of hematopoiesis and also in bone marrow by the splenic colonies method [9] in B6 recipients (10 in a group), irradiated in a dose of 11 Gy. The number of colonies was determined 7-8 days after injection of the hematopoietic cells.

EXPERIMENTAL RESULTS

Since delay of growth of the parental cells in the F_1 hybrid *in vivo* took place only during the first 10 days after transplantation, data on hematopoietic cell content of the cultures 7 days after application of cells to the syngeneic or semisyngeneic ACL are given in Table 1. Explantation of bone marrow cells of the B6 genotype on an irradiated underlayer of syngeneic (B6) or semisyngeneic (CBF_1) cultures led to equal hematopoiesis in cultures of both types. The identical character of hematopoiesis when cultures with both cell combinations were used was observed regardless of the number of hematopoietic cells used for the second application, over the range from 1×10^6 to 10×10^6 cells. Meanwhile, B6 cells are inhibited most strongly in a recipient of CBF_1 genotype [2].

Consequently, these results confirm the absence of hybrid resistance in culture [7]. Absence of inhibition in this case cannot be attributed to "overwhelming" of resistance by

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TABLE 1. Number of Hematopoietic Cells In Culture 1 week after Explantation of Bone Marrow Cells on an Irradiated Syngeneic or Semisyngeneic Sublayer

Expt. No.	Genotype of irradiated sublayer	Genotype of explanted cells	Dose of explanted cells ($\times 10^{-6}$)	Cell content of culture in 1 ml ($\times 10^{-6}$)
1	B6	B6	7	2,45
	CBF1	B6	7	2,58
2	B6	B6	5	1,55
	CBF1	B6	5	2,85
	B6	CBF1	5	4,65
	CBF1	CBF1	5	2,65
3	B6	B6	1	2,83
	CBF1	B6	1	2,98
	B6	B6	10	0,4
	CBF1	B6	10	0,6

TABLE 2. Number of CFUc in Ectopic Foci of Hematopoiesis 10 days after Transplantation of B6 Cells Into Irradiated Recipient

Expt. No.	Genotype of irradiated stroma	Source of irradiated stroma	Genotype of recipient of irradiated stroma	Number of CFUc per organ
1	B6	ACL of culture	B6	12,2
	B6	The same	CBF1	7,9
	CBF1	» »	CBF1	2,5
2	B6	ACL of culture	B6	65,2
	B6	The same	CBF1	5,1
	CBF1	» »	CBF1	1,1
	B6	Femoral bone marrow		142,4
	CBF1	The same		1,7

the large number of cells explanted the second time (10^7), as is found *in vivo* on injection of 10^7 parental hematopoietic cells or more into a hybrid recipient [3]. In fact, when doses of cells 2-10 times smaller were used, hybrid resistance still was not detected (Table 1). With a high cell dose (10^7), incidentally, the second explantation led initially to less intensive hematopoiesis in the culture, the cell content of which after 1 week was much less than when only 10^6 hematopoietic cells were used (Table 1). On continued culture these differences disappeared within the next 1 or 2 weeks (data not given). Hence it follows that the structural organization of the sublayer of the culture could not support the phenomena of hybrid and allogeneic resistance, and certain components essential for these phenomena to take place were evidently lost from the culture.

To examine these possibilities, the sublayer of the cultures was retransplanted into a syngeneic (B6 into B6 or CBF_1 into CBF_1) or a semisyngeneic (B6 into CBF_1) recipient. After the cultured cells had formed ectopic foci of hematopoiesis, the presence of mechanisms of hybrid resistance in them was studied (Table 2). In foci of CBF_1 genotype strong hybrid resistance to the injected B6 hematopoietic cells developed. In these foci 10 days after irradiation and transplantation of B6 cells there were just as many CFUc as in the femoral marrow of CBF_1 recipients, and about 100 times fewer CFUc than in bone marrow of the B6 recipients. Consequently, as a result of retransplantation of CBF_1 cells from culture *in vivo*, their ability to effect hybrid resistance to B6 bone marrow cells was fully restored. This can be explained by restoration of the hematopoietic microenvironment essential for manifestation of the phenomenon of hybrid resistance, lost in the culture, into the ectopic focus.

The results with transplantation of ACL of cultures of the B6 genotype enable the first hypothesis to be rejected. In fact, the structural organization in these foci was the same whether they were reimplanted into a syngeneic (B6) or semisyngeneic (CBF_1) recipient. In both cases perfectly normal hematopoietic territories about equal in size appeared and maintained proliferation of hematopoietic cells differentiating in all directions, as well as hematopoietic stem cells. Yet in foci formed by cells from B6 cultures in a semisyngeneic CBF_1 recipient, the number of injected CFUc of B6 genotype was always less than in the same foci formed in a syngeneic (B6) recipient (Table 2). This makes it probable that a cell component of the hematopoietic microenvironment migrated from the CBF_1 recipient into the focus of ectopic hematopoiesis formed by ACL of cultures of B6 genotype.

Results such as these were not observed after implantation of bone marrow which had not been cultured [4]. In that case growth of B6 hematopoietic cells was the same and developed similarly in a syngeneic (B6) or semisyngeneic (CBF_1) recipient.

Certain important conclusions can be drawn from these results. A component of the microenvironment essential for appearance of the hybrid resistance phenomenon is lost in culture. This component can be restored by reimplantation of ACL of the culture into a recipient, thereby proving directly for the first time the ability of a certain component of the hematopoietic microenvironment essential for regulation of hematopoietic stem cell proliferation, at least in a semisyngeneic system, to migrate.

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COMPARATIVE STUDY OF PROLIFERATIVE ACTIVITY OF HUMAN LYMPHOCYTES FROM DIFFERENT PAIRS OF DONORS IN MIXED CULTURE *IN VITRO*

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Mixed lymphocyte culture (MLC) is a widely used method of studying functional properties of human immunocompetent cells. The level of proliferative response in MLC is characterized by marked individual variability [9]: This phenomenon has received little study and is usually associated with the degree of difference between the partner cells with respect to HLA antigens. However, participation of spontaneous regulator cells in determination of the intensity of the proliferative response in MLC is highly probable [4].

In the investigation described below an attempt was made to analyze the causes of differences in the intensity of the proliferative response of cells in MLC. For this purpose the proliferative response of the lymphocytes in a two-way MLC with the partner cells in different ratios was first investigated. MLC in which the partner cells from two donors were alternately responders and stimulators also were studied in pairs. The effect of factors modulating the functional properties of human lymphocytes (preincubation of lymphocytes *in vitro*; addition of thymosin) also was analyzed in this investigation.

EXPERIMENTAL METHOD

Mononuclear cells (MC) were isolated by centrifugation of heparinized venous blood from healthy blood donors in a Ficoll—Verografin density gradient [1].

Macro- and micromodifications of the two-way MLC technique were used. In the first case separated MC from two different donors were cultured as described previously [4]. When the micromodification of the MLC method was used, MC were cultured in wells in plates used for immunologic tests (from the Leningrad Medical Polymers Factory) in culture medium consisting of 80% medium RPMI-1640 (from Serva, West Germany), 20% human group IV (AB) serum inactivated by heating, and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml) and in atmosphere of air with 5% CO_2. The total number of cells in the two-way MLC was $0.2 \cdot 10^6$ MC in 0.15 ml of culture medium in each well. The number of responding and stimulating

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