ORIGIN OF HEMATOPOIETIC CELLS IN SYNGENEIC AND SEMISYNGENEIC FOCI OF HETEROTOPIC HEMATOPOIESIS

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

UDC 612.119

Foci of heterotopic hematopoiesis were obtained by transplanting bone marrow of C57BL/6 or (CBA × C57BL)F_1 mice beneath the kidney capsule of (CBA/T6T6 × C57BL)F_1 mice carrying a chromosomal translocation. Cytogenetic analysis of hematopoietic cells from these foci 20-120 days after transplantation showed that only the recipient's hematopoietic cells proliferate in 40% of the grafts, whereas the rest are mosaic and contain on average less than 20% of the donor's cells in both syngeneic and semisyngeneic systems. These characteristics remain stable for at least 4 months. It is concluded that the heterotopic focus is populated in one stage by a very small number (fewer than 10) hematopoietic stem cells. The stability of the clones is evidence that in the dynamic equilibrium of hematopoiesis the exchange of cells between different parts of the hematopoietic system is extremely small or is absent altogether.

KEY WORDS: bone marrow; heterotopic transplantation; hematopoietic stem cells; chromosomal labeling.

After heterotopic transplantation of bone marrow a focus of hematopoiesis consisting of bone and hematopoietic cells is formed and persists for a long time. It has been shown that the bone in such a focus is of donor origin [4]. The question of the origin of the hematopoietic cells in the focus is less clear. There are few experimental data on this question and they are contradictory. According to some evidence [4], the hematopoietic cells of the heterotopic focus belong to the recipient alone, whereas according to others [1], most of the foci are mosaic in character and contain cells of both donor and recipient origin. However, both these investigations were carried out on a very small quantity of material (only 52 and 102 metaphases from 3 and 14 foci, respectively, were analyzed), and this is insufficient for quantitative analysis of the system. Consequently it is not yet clear whether the heterotopic focus is a mosaic of the hematopoietic cells of the donor and recipient, and if it is so, what is the fate of the donor's cells – do they continue all the time in the graft until an adequate hematopoietic microenvironment has been created to induce their proliferation or do they survive longer than this in the recipient's hematopoietic system and give rise to secondary repopulation of the hematopoietic focus after the formation of a stroma capable of supporting hematopoiesis in it. Meanwhile the question of the origin of hematopoietic cells in a heterotopic graft is of fundamental importance, because this model provides important advantages for the study of relations between stromal and hematopoietic cells, the intensity of cell migration between different parts of the hematopoietic system, the nature of the phenomenon of hybrid resistance, and so on.

In this investigation the problem of the origin of the hematopoietic cells was studied in mice at various times after heterotopic transplantation of bone marrow beneath the kidney capsule of syngeneic or semisyngeneic recipients carrying a chromosomal translocation.

Laboratory of Culture and Transplantation of Bone Marrow, 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. 83, No. 5, pp. 584-586, May, 1977. Original article submitted November 1, 1976.

TABLE 1. Cytogenetic Analysis of Hematopoietic Cells in Focus of Heterotropic Hematopoiesis

System	Indices of karyological analysis	Time after transplantation, days				
		20	55—60	90—100	115—120	mean
Syngeneic (CBA × C57BL)F_1 in (CBA/T6T6 × C57BL)F_1	Recipient's metaphases/donor's metaphases in graft, percentage of recipient's mitoses in parentheses	98/32 (75±8,7)	57/29 (66±8,1)	170/53 (76±8,7)	213/63 (77±8,8)	538/187 (74±8,6)
	The same, in bone marrow	225/0 (100)	190/0 (100)	300/0 (100)	400.0 (100)	1115/0 (100)
	Number of grafts with recipient's cells only/number of mosaic grafts	5/3	1/5	3/4	2/4	11/16
Semisyngeneic C57BL/6 in (CBA/T6T6 × C57BL)F_1	Recipient's metaphases/donor's metaphases in graft, percentage of recipient's mitoses in parentheses	66/10 (87±9,3)	142/28 (84±9,2)	328/27 (92±9,5)	215/49 (81±9,0)	751/114 (87±9,3)
	The same, in bone marrow	145/0 (100)	250/0 (100)	300/0 (100)	400/0 (100)	1095/0 (100)
	Number of grafts with recipient's cells only/number of mosaic grafts	2/2	4/4	3/2	3/3	12/14

EXPERIMENTAL METHOD

Experiments were carried out on C57BL/6, (CBA × C57BL)F_1, and (CBA/T6T6 × C57BL)F_1 mice. A fragment of the content of the femur of mice without a T6 chromosome was transplanted beneath the capsule of the left kidney of mice with a T6 chromosome anesthetized intraperitoneally with hexobarbital (200 mg/kg). After an interval of 20-120 days, the mice (four to eight animals at each time) were given an intraperitoneal injection of colcemid (2 mg/kg). The mice were killed 40-50 min later and cells of the heterotopic focus and of the femoral marrow were subjected to hypotonic treatment in 0.7% solution of trisodium citrate for 50 min. After fixation with ethanol:acetic acid (3:1) preparations for karyological analysis were made by burning off the fixative. The films were stained by the Romanovsky–Giemsa method. The recipient's cells were identified from the presence of the T6 chromosome in the metaphase plate. The absence of a T6 chromosome when all 40 chromosomes were present in the set showed that the cell belonged to the donor of the marrow.

EXPERIMENTAL RESULTS

The results are given in Table 1. Of the 53 foci studied 23 (43 ± 6.5%) contained only the recipient's cells, whereas the other foci were mosaic and contained both recipient's and donor's cells. No focus contained only donor's cells. At all times of investigation (until 4 months) both the ratio between the purely recipient and the mosaic foci and the fraction of the donor's cells in the mosaic foci remained unchanged. Two important conclusions can be drawn from this fact. First, repopulation of the grafts with hematopoietic cells takes place in one stage, evidently after organization of a suitable microenvironment for the maintenance of hematopoiesis. Second, subsequent exchange of hematopoietic cells between individual parts of the hematopoietic system is on a small scale or is absent altogether, at least during steady-state hematopoiesis.

The absence of donor's cells in the recipient's marrow throughout the period of investigation (0 among 2210 metaphases studied) shows that donor's hematopoietic cells present in most foci remained at the site of transplantation and did not recolonize the focus after primary repopulation of the recipient's hematopoietic system. It is logical to suppose that the process of retention of the hematopoietic stem cells at the site of the transformed graft is random in character and obeys a Poisson distribution. If this hypothesis is true, it is possible to calculate the mean number of donor's stem cells remaining in the focus until the time of its population by hematopoietic cells from the fraction of grafts in which no donor's stem cells were preserved. Given that on average one donor's stem cell remained in the graft, the fraction of "empty" (containing only the recipient's cells) grafts (P_0) must be 0.37. Since this value, determined experimentally, was 0.43, on average about one of the donor's hematopoietic stem cells remained in the focus. Since the mean percentage of recipient's metaphases (1289 of 1590 studied) was 82 ± 9.0%, one stem cell was the precursor of about 18% of all dividing cells of the hematopoietic focus. Hence it follows that a heterotopic focus, equivalent in the number of its hematopoietic cells ($10 \cdot 10^6$–$15 \cdot 10^6$) to the femur of an adult mouse, is repopulated by only five or six hematopoietic stem cells settling in it. Later, hematopoiesis is maintained at a stable level in the focus by the progenies of these stem cells settling in it initially, and no appreciable replacement of cells in the focus takes

place on account of migration of the recipient's hematopoietic cells. The findings indicating a small number of cells repopulating extensive areas of the hematopoietic system and a small scale of renewal of the hematopoietic clones on account of hematopoietic stem cells arriving from the blood during steady-state hematopoiesis are in full agreement with results [6] obtained recently in experiments with radiation chimeras.

As Table 1 shows, the character of repopulation by the recipient's hematopoietic cells was the same in the syngeneic and semisyngeneic systems. At all times of investigation no increase in the fraction of the donor's hematopoietic cells could be found in the heterotopic focus. Hence, it follows that hematopoietic cells of the C57BL/6 genotype have no selective advantage over the cells of mice with a T6 chromosome in the territory of the hematopoietic focus formed by stromal precursors of the C57BL/6 genotype. On the other hand, the absence of any decrease in their fraction in the focus with the passage of time indicates that the phenomenon of hybrid resistance is not involved in this case [3, 5]. Hence it follows that hematopoietic cells of the (C57BL × CBA)F_1 hybrid capable of repopulation are not responsible for the phenomenon of hybrid resistance, as has been suggested [2].

LITERATURE CITED

1. S. Amsel and E. S. Dell, Proc. Soc. Exp. Biol. Med., 138, 550 (1971).
2. G. Cudkowicz and M. Bennett, J. Exp. Med., 134, 83 (1971).
3. G. Cudkowicz and J. H. Stimpfling, Immunology, 7, 291 (1964).
4. A. Ya. Friedenshtein (A. J. Friedenstein) et al., Transplantation, 6, 230 (1968).
5. E. A. McCulloch and J. E. Till, J. Cell. Physiol., 61, 301 (1963).
6. H. S. Micklem, C. E. Ford, E. P. Evans, et al., Cell Tissue Kinet., 8, 219 (1975).

ACTION OF X-RAY RADIATION ON DNA SYNTHESIS IN THE EPITHELIUM OF THE UTERINE GLANDS AND ITS DEPENDENCE ON THE PHASE OF THE MITOTIC CYCLE

É. E. Ogandzhanyan and S. A. Mkrtchyan

UDC 612.627.015.36:547.963.32/:612.6/014.481.1

The action of x-ray irradiation on DNA synthesis in the epithelium of the uterine glands of ovariectomized mice stimulated by dihydrostilbestrol and the dependence of its action on the phase of the mitotic cycle were studied by autoradiography with thymidine-^{3}H. After local irradiation of the region of the uterus in a dose of 400 rad the decrease in the index of labeled nuclei was found to differ depending on the phase of the mitotic cycle in which most cells were.

KEY WORDS: x-ray irradiation; epithelium of uterine glands; DNA synthesis.

During irradiation a decrease in the number of labeled cells is observed in the reproductive organs and its degree depends on the state of the cells at the moment of irradiation [2, 4-6, 8].

The object of this investigation was to study the action of x-ray irradiation on the entry of the epithelial cells of the uterine glands into the phase of DNA synthesis and the dependence of this action on the phase of the mitotic cycle in which most of the cells were.

Laboratory of Pathomorphology and Histochemistry, Sector of Radiobiology, Ministry of Health of the Armenian SSR, Erevan.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 5, pp. 586-588, May, 1977. Original article submitted December 14, 1976.