INHIBITION OF HEMATOPOIESIS AND OF OSTEOBLAST FORMATION IN THE BONE MARROW OF LETHALLY IRRADIATED MICE AFTER TRANSPLANTATION OF ALLOGENEIC LYMPHOCYTES

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From 7 to 9 days after transplantation of 2 million lymphocytes of CBA mice into lethally irradiated (CBA×C57BL) F_1 mouse hybrids, inhibition of osteoblast formation and of foci of hematopoiesis was observed in the femoral marrow of the recipients. Colony-forming cells and precursors of antibody producers were inactivated in the spleen, and osteoblasts and foci of hematopoiesis in the bone marrow of the irradiated recipients.

Combined transplantation of a mixture of hematopoietic cells and lymphocytes of two incompatible genotypes into lethally irradiated recipients is followed by inactivation of the hematopoietic stem cells of the transplant: no colony-forming units (CFU) appear in the spleen of such animals [3, 6]. The phenomenon of inactivation of nonsyngeneic stem cells is manifested not only in relation to exogenous CFU, but also in relation to endogenous stem cells. Transplantation of lymphocytes from CBA mice into sublethally irradiated (CBA×C57BL) F_1 mouse hybrids suppresses endocolonization of the recipients' spleen [4, 5, 8]. It has also been shown that interaction between nonsyngeneic cells is characterized not only by suppression of colony-forming elements; transplantation of a mixture of genetically foreign lymphocytes into irradiated recipients is followed by inactivation of precursors of antibody producers, so that antibody-forming cells do not accumulate in the spleen of the irradiated mice [2, 7]. These results suggest that the effect of inactivation of nonsyngeneic stem cells by lymphocytes is of general importance and extends to a wide variety of proliferating precursor cells [1]. The possibility cannot be ruled out that inactivation of nonsyngeneic cells of different nature may take place in any part of the hematopoietic system.

In the investigation described below the effect of transplanted nonsyngeneic lymphocytes was studied on osteoblast formation and on hematopoietic foci in the bone marrow of irradiated mice.

EXPERIMENTAL METHOD

(CBA×57BL) F_1 mouse hybrids aged 4-5 months, of both sexes, irradiated on a type ÉGO-2 apparatus in a dose of 850 R (Co^{60}; dose rate 409 R/min) were used as the recipients.

The day after lethal irradiation each recipient received an intravenous injection of $2 \cdot 10^6$ cells obtained from the inguinal, axillary, submandibular, and mesenteric lymph glands of CBA mice. The method of obtaining the cell suspension was described previously [2]. On the 1st, 4th, 7th, and 9th days after transplantation of the lymphocytes (the 2nd, 5th, 8th, and 10th days after irradiation), the animals were sacrificed. The femora were fixed in Bouin's fluid, which acts simultaneously as a decalcifying agent. The material was embedded in paraffin wax. Longitudinal series of sections, 5-7 μ in thickness, were stained with hematoxlin-eosin.

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TABLE 1. Effect of Transplantation of Lymphocytes of CBA Mice on Number of Osteoblasts and Hematopoietic Foci Counted in Femoral Marrow of Lethally Irradiated (CBA×C57BL) F_1 Mouse Hybrids

Experimental conditions	Day		Number of experiments	Percent of animals with osteoblasts	Mean number in medullary cavity		Ratio between erythroid and myeloid foci	Student's criterion of significance (P)
	after irradiation	after transplantation			osteoblasts	foci of hematopoiesis		
Irradiation	2	–	3(15)	80	33±11	0	–	0.5
	5	–	3(10)	70	11±4	10.8±1.5	1 : 7	0.1
	8	–	2(7)	100	79±7	2.8±0.5	1 : 1	0.01
	10	–	3(9)	100	104±14	3.8±0.8	1 : 1.4	0.01
Irradiation and transplantation of lymphocytes from CBA mice	2	1	3(15)	80	34±11	0	–	0.5
	5	4	3(15)	40	7±20	7.3±0.4	1: 7	0.05
	8	7	3(12)	0	0	1.5±0.2	–	0.01
	10	9	2(8)	0	0	0	–	0.01

Note. Number of animals used is shown in parentheses.

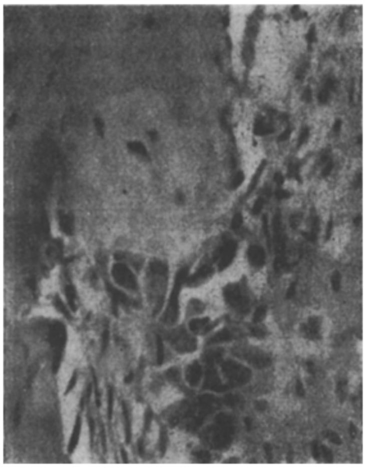

Fig. 1. Proliferation of osteoblasts forming osteoid in the diaphysis of an irradiated animal (10 days). Hematoxylin-eosin, 400×.

The number of hematopoietic foci was counted in series of sections passing through the whole of the femur with the bone marrow. The presence or absence of osteoblasts in the metaphysis and diaphysis was assessed from visual observations. The number of osteoblasts was counted in an area of diaphysis 2500 μ in length starting from its boundary with the metaphysis.

Animals irradiated only and irradiated animals into which syngeneic lymphocytes were transplanted were used as the control.

EXPERIMENTAL RESULTS

Intact Animals. An average of 21 osteoblasts was found on the inner surface of the bone in a distance of 2.5 mm (in the region of the metaphysis near its border with the diaphysis) in the intact (CBA×C57BL)F_1 mouse hybrids. Osteoblasts were observed in all the animals. The distinguishing features of the morphology of these cells are the peripheral position of the regular, round nucleus, with its one or two nucleoli, and the basophilia of the cytoplasm. The medullary cavity was filled with hematopoietic tissue, with more myeloid than erythroid cells.

Irradiated Animals. The number of osteoblasts and hematopoietic foci counted in the femoral marrow of the irradiated mice is shown in Table 1. Osteoblasts were seen in 70-80% of animals 2-5 days after irradiation. From 11 to 33 osteoblasts were counted in the marrow of these animals. On the 8th and 10th days their number was significantly increased (to 79-104). Osteoblasts were found in all mice examined; they were most numerous in the metaphysis and diaphysis.

On the 2nd day after irradiation no foci of hematopoiesis were present in the bone marrow of the mice. The venous sinuses of the marrow were dilated and filled with a gelatinous mass and with erythrocytes, and solitary fat cells were seen. Starting from the 5th day after irradiation, foci of hematopoiesis containing 1000-50,000 myelois cells, mainly promyelocytes, appeared in the marrow of the irradiated mice. The mean number of hematopoietic foci per femur was 10.8. The ratio between erythroid and myeloid foci was 1 : 7. On the 8th-10th day after irradiation, proliferation of the endosteum took place in some parts of the diaphysis, so that it consisted of 2-4 rows of fibroblast-like cells with zones of osteoid (Fig. 1).

Fig. 2. Transplantation of allogeneic lymphocytes into an irradiated animal. Osteoblasts absent in metaphysis (7 days). Hematoxylin-eosin, 200×.

The hematopoietic foci contained 10^5-10^6 cells, mainly immature forms. The mean number of foci of hematopoiesis per femur was 2.8-3.8. The ratio between erythroid and myeloid foci was 1 : 1.

Irradiated Animals Receiving Transplanted Nonsyngeneic Lymphocytes. On the 1st day after transplantation of lymphocytes from CBA mice into lethally irradiated (CBA×C57BL) F_1 mouse hybrids, or on the 2nd day after irradiation, the indices were the same as in the irradiated mice not receiving injections of parental cells (Table 1). However, on the subsequent days after transplantation of lymphocytes, a marked decrease in the number both of osteoblasts and of foci of hematopoiesis was observed in the medullary cavity of the irradiated animals. By the 4th day after transplantation of the cells (5th day after irradiation), for example, the first signs of the action of the allogeneic lymphocytes on the osteoblasts and hematopoietic foci of the recipients were observed. At this period the recipients' marrow exhibited marked edema of the venous sinuses, erythrophagia, and the presence of giant karyocytes. The percentage of mice with osteoblasts was reduced from 80 (the 1st day after transplantation of the lymphocytes) to 40. By the 7th day after transplantation (the 8th day after irradiation) complete inhibition of osteoblast formation (Fig. 2) and a reduction in the number of foci of hematopoiesis by half were observed in the marrow of the irradiated animals. By the 9th day after transplantation of the lymphocytes (the 10th day after irradiation) no foci of hematopoiesis could be seen in the marrow of the irradiated mice. Variations in the structure of the marrow were observed at these times. In some cases there was severe aplasia of hematopoiesis, while in others the bone marrow contained a large number of small cells of the macrophage type and cells of the lymphoid series.

In the group of control (irradiated) mice receiving transplanted syngeneic lymphocytes, no inhibition of osteoblast formation could be observed on the 10th day after irradiation (the 9th day after transplantation of the cells). Osteoblasts were found in all mice examined. The hematopoietic bone marrow tissue was indistinguishable in structure from that of the irradiated animals not receiving transplanted cells.

By the 8th-10th day after lethal irradiation, restoration of the hematopoietic function of the bone marrow and increased osteoblast formation were thus observed in the marrow of the mice. Injection of allogeneic lymphocytes into these mice inhibited these processes. This shows that the action of lymphocytes on nonsyngeneic cells possesses a broad spectrum: not only the colony-forming and antibody-producing cells in the spleen of the irradiated recipients are inactivated, but so also are the foci of hematopoiesis and the process of osteoblast formation in the femoral marrow.

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