ABOLITION OF THE ALLOGENEIC INHIBITION OF HEMATOPOIETIC STEM CELLS BY MEANS OF SYNGENEIC LYMPHOCYTES

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Transplantation of bone marrow cells from C57BL mice into lethally irradiated (CBA × C57BL/6)F_1 hybrids was followed by allogeneic inhibition of colony-forming units. Lymph gland or thymus cells of C57BL/6 mice, injected together with or 24 h before the transplantation of bone marrow stimulated colony formation in the spleen of irradiated genetically foreign recipients.

The writers have shown previously that combined transplantation of bone marrow cells and lymph gland cells from CBA mice into lethally irradiated (CBA× C57BL)F_1 hybrids is accompanied by a decrease in the number of colonies of hematopoietic cells formed by the donors' bone marrow in the recipients' spleen [1, 2].

In the investigation described below the colony-forming activity of bone marrow cells of C57BL mice was studied after their transplantation into lethally irradiated (CBA × C57BL)F_1 hybrids together with syngeneic (C57BL) lymph gland or thymus cells.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 hybrid mice weighing 22-24 g and irradiated with Ce^{137} γ-rays in a dose of 900 R ($LD_{100/13}$) were used as recipients. The recipients were given an intravenous injection of 300,000 viable nucleated bone marrow cells from C57BL mice 24 h after irradiation. Injections of 0.3-20 million lymph gland cells of C57BL mice were given to the experimental mice 20 h before, concurrently with, or 24 h after the injection of bone marrow. In some experiments 2-20 million platelets were injected along with the bone marrow.

On the 9th day after transplantation of the bone marrow the number of macroscopically visible colonies was counted in the recipients' spleen [5]. The number of colonies in the group of mice irradiated only (irradiation control) or the group of irradiated recipients injected with lymph gland or thymus cells but without bone marrow (lymphocyte control) averaged 0.2. On the basis of the results obtained in each experiment the coefficient of realization of colony-forming ability was calculated as the ratio between the number of colonies in the recipients' spleen after combined administration of bone marrow and lymphoid cells and the number of colonies observed after the transplantation of an isolated bone marrow suspension, for the number of colonies in the "bone marrow control" group of animals varied in different experiments from 3.5 to 8.7.

EXPERIMENTAL RESULTS

The degree of allogeneic inhibition of bone marrow stem cells of the parental genotype in this C57BL→ F_1 model reached 90-95%. In other words, only 5-10% of the transplanted bone marrow stem cells were able

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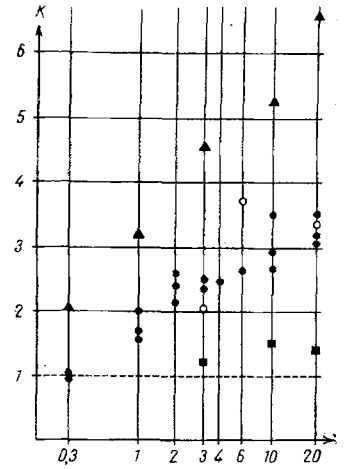

Fig. 1. Coefficient of realization of colony-forming ability (K) of bone marrow cells of C57BL mice transplanted together with syngeneic lymphocytes into lethally irradiated (CBA × C57BL) F_1 mice. Abscissa, number of cells injected (in millions); ordinate, number of colonies in spleen of recipients receiving injection of bone marrow only was taken as 1. Filled circles denote injection of lymph gland cells together with bone marrow cells; triangles – 20 h before, squares – 24 h after transplantation of bone marrow; empty circles denote injection of thymocytes together with bone marrow.

to form colonies in the spleen of the foreign recipients compared with the syngeneic combination C57BL → C57BL.

The simultaneous injection of lymph gland cells and bone marrow cells stimulated the colony-forming activity of the hematopoietic stem cells of the bone marrow. With an increase in the dose of lymphocytes there was an increase in the number of colonies formed in the spleen by the donor's bone marrow. Thymus cells also had similar activating power (Fig. 1).

The most marked effect of stimulation of the colony-forming ability was observed when lymph gland cells were transplanted 20 h before the bone marrow. Under these experimental conditions, depending on the dose of lymph gland cells, the number of colonies in the spleen of the irradiated F_1 hybrids was 2-7 times higher. Even with the maximal stimulation of colony formation achieved, however, the number of colonies in the spleen of the F_1 hybrids was only half that observed in the syngeneic combination C57BL → C57BL.

Injection of lymph gland cells 24 h after transplantation of bone marrow had a weak action on colony formation in the spleen of the foreign recipients.

Viable lymphocytes had the ability to stimulate colony-forming activity. Destruction of the lymph gland cells by repeated freezing and thawing abolished their colony-stimulating function. Changes in the colony-forming ability of the stem cells under the influence of syngeneic lymphocytes were observed only after the transplantation of cells into foreign recipients. If donor and recipient were syngeneic, the lymphocytes did not affect the number of hematopoietic colonies formed.

The donor's lymphocytes, colonizing the spleen of the irradiated foreign recipients, possibly produced a syngeneic "microenvironment" [3, 4], or alternatively, the suspension of lymphocytes may have contained cells capable of forming the syngeneic stroma essential for the proliferation and differentiation of hematopoietic stem cells.

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