ABOLITION OF ALLOGENEIC INHIBITION AND CHANGES IN DIFFERENTIATION OF MOUSE BONE MARROW COLONY-FORMING CELLS BY SYNGENEIC LYMPHOCYTES

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A mixture of bone marrow cells and lymph gland lymphocytes of C57BL mice was injected intravenously into F_1(A × C57BL) mice irradiated in a dose of 850 R. Under the influence of syngeneic lymphocytes of the C57BL mice colony-forming cells (CFUs) of the bone marrow proliferated in the F_1 mouse hybrids as if they had been in a syngeneic organism. Besides restoration of the number of CFUs capable of forming colonies in a genetically foreign organism, under the influence of lymphocytes the colonies changed their direction of differentiation, with an increase in the number of granulocyte colonies. This process was accompanied by the development of a blast transformation reaction and by hyperplasia of the lymphoid tissue of the recipients' spleens.

KEY WORDS: allogeneic inhibition; differentiation of colony-forming cells; syngeneic lymphocytes.

This investigation is a continuation of work in the writers' laboratory to study interaction between lymphocytes and hematopoietic stem cells [2-5].

Its object was to study the ability of syngeneic lymphocytes to affect the proliferative activity and differentiation of colony-forming cells (CFUs) in a genetically foreign organism.

EXPERIMENTAL METHOD

F_1(A × C57BL) recipient mice were irradiated with Co^{60} γ rays in a dose of 850 R (dose rate 300 R/min). Mice of the C57BL parental strain were used as donors of bone marrow cells and lymphocytes. Twenty-four hours after irradiation of the recipients they were given an intravenous injection of bone marrow cells or a mixture of these cells with lymph gland lymphocytes. On the ninth day after this injection the spleen was removed from the recipients. Material was embedded in paraffin wax and series of histological sections were cut to a thickness of 5-7 μ. The morphological composition of cells in colonies of different types was determined in sections stained with hematoxylin–eosin. Details of the methods were described previously [4, 5].

EXPERIMENTAL RESULTS

It will be clear from the results given in Table 1 that as the number of lymphocytes mixed with a constant number of bone marrow cells from C57BL mice increased, the ability of the hematopoietic cells to form colonies in F_1 hybrid mice also increased. The number of colonies growing in the recipients' spleens reached the same number as in the syngeneic

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TABLE 1. Effect of Syngeneic Lymphocytes of C57BL Mice on Proliferative Activity and Direction of Differentiation of Bone Marrow CFUs (M ± m)

Transplantation of cells of C57BL mice		Recipient	Type of hematopoietic colonies					E/M	Mean number of colonies per spleen
bone marrow cells	lymph gland cells		erythroid (E)	myeloid (M)	megakaryocytic	undifferentiated	mixed		
10^5	—	C57BL	18,0±1,7	10,0±2,2	5,0±1,0	1,2±0,5	1,0±0,5	1,8	34,2±3,0
10^5	—	F_1(A×C57BL)	8,0±2,2	1,8±1,0	2,0±1,0	0,2±0,2	0	4,4	12,2±5,0
10^5	10^6	»	7,8±1,4	3,2±1,0	1,6±0,4	0,4±0,2	0,2±0,2	2,4	13,4±1,8
10^5	$5\cdot10^6$	»	13,0±1,4	13,0±1,0	4,0±0,6	0	0	1,0	30,0±0,7
10^5	10^7	»	14,0±2,3	16,0±2,0	4,0±0,7	0	0	0,9	34,0±3,1
10^5	$5\cdot10^7$	»	16,0±1,8	25,0±2,7	5,0±0,4	0	0	0,6	46,0±3,6

*From eight to ten animals were investigated at each time.

experiments. The result indicated that syngeneic lymphocytes abolished the effect of allogeneic inhibition, as a result of which the CFUs can proliferate in a nonsyngeneic environment just as if they had been in a syngeneic organism. In the absence of lymphocytes, or in the presence of a few lymphocytes, the typical effect of allogeneic inhibition of CFUs, as described for various cells of different tissues [6, 7], was manifested.

It will also be clear from Table 1 that activation of the colony-forming process was accompanied by a change in the ratio between the numbers of colonies of erythroid and myeloid types, just as was observed after transplantation of a mixture of bone marrow cells and lymphocytes of CBA mice into an F_1 hybrid [5]. If the ratio between the number of bone marrow cells and lymphocytes of the CBA mice was appropriate, only colonies of myeloid type appeared in the spleens of the F_1 recipients, and the increase in their number matched the decrease in the number of erythroid colonies. This phenomenon has been called the effect of redifferentiation of stem cells from the erythroid to the myeloid type [4]. In the case described above, a similar effect evidently was observed. The possibility cannot be ruled out that a change in the ratio between the erythroid and myeloid colonies was due not only to redifferentiation, but also to stimulation of the precursor cells of granulocytopoiesis. This is shown by the fact that a number of erythroid colonies increased not more than to the level of the same colonies in the syngeneic experiments, whereas the number of myeloid forms increased to approximately twice the number of erythroid and the total number of colonies also increased.

It is not clear what role must be ascribed to the graft versus host reaction (GVHR) developed by the lymphocytes in this case. Morphological analysis of the spleen of the F_1 hybrid recipient mice showed absence of any clearly defined GVHR: The development of a blast-transformation reaction and hyperplasia of the lymphoid tissue of the spleen were found. Similar results, demonstrating changes in the lymphoid tissue and an increase in the number of CFUs, have been obtained by injecting phytohemagglutinin into donors. Transplantation of spleen cells from these donors led to an increase in the number of colonies in the spleen of the syngeneic recipient [8].

If these data are compared with the results of the present investigation a parallel can be drawn between interaction of lymphocytes with CFUs and the effect of phytohemagglutinin on CFUs. In both cases the presence of the blast-transformation reaction and of hyperplasia of lymphoid tissue leads to the same effect: an increase in the CFU population. The hypothesis put forward by Golovistikov [1] that the mechanism of action of lymphocytes of C57BL mice is aimed at adjusting the substrate (stroma) of the spleens of the F_1 recipients to the syngeneic type, with a consequent abolition of allogeneic inhibition, can accordingly be accepted. The GVHR evidently does not play an active role in this case. However, this conclusion requires further precise experimental verification.

The principle by which interaction between bone marrow cells and lymphocytes takes place is not yet known: whether

it is of the lymphocyte—CFU or the lymphocyte—stroma—CFU type. In both cases the presence of direct or indirect contact between lymphocytes and CFUs is assumed through a biologically active substance, a factor stimulating granulocytopoiesis and produced by the stroma of the spleen or by the lymphocytes.

It can be concluded from these facts and others obtained previously [2-5] that two different processes take place as a result of cooperation between CFUs and lymphocytes: 1) redifferentiation of the CFUs from the erythroid to the myeloid type in the case of interaction between lymphocytes and CFUs of CBA mice in the body of F_1(CBA × C57BL) mice [4]; 2) abolition of allogeneic inhibition accompanied by considerable stimulation of the cell population and, in particular, of the precursors of granulocytopoiesis.

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