ABOLITION OF ALLOGENEIC INHIBITION OF HEMATOPOIETIC STEM CELLS BY TREATMENT OF RECIPIENT MICE WITH CYCLOPHOSPHAMIDE

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After transplantation of bone marrow from C57BL/6 mice into lethally irradiated (CBA × C57BL/6)F_1 hybrids, the degree of allogeneic inhibition of the stem cells was 92.5%. Cyclophosphamide, given to the recipients in a dose 200 mg/kg 24 h before bone marrow transplantation (4 h after irradiation) reduced the degree of allogeneic inhibition to 24%. Administration of a similar dose of cyclophosphamide did not affect the number of colonies in the spleen of lethally irradiated syngeneic recipients. It is postulated that cyclophosphamide, if injected into lethally irradiated recipients, acts on the population of the recipient's radioresistant cells, which are responsible for allogeneic inhibition of the stem cells.

KEY WORDS: allogeneic inhibition; hematopoietic stem cells; cyclophosphamide.

The mechanism of allogeneic inhibition has not yet been adequately studied. According to some workers, allogeneic inhibition is due to the absence of syngeneic (relative to the donor) lymphocytes, essential for proliferation of transplanted hematopoietic stem cells, in the lethally irradiated recipient mice [4, 11, 12]. Experiments have shown that allogeneic inhibition is based on the immune response of radioresistant lymphocytes of irradiated F_1 hybrid recipients on antigens of the stem cells of mice of the parental line controlled by recessive Hh genes [7, 8, 16].

In this investigation an attempt was made to evaluate the role of immune and nonimmune factors in the manifestation of the phenomenon of allogeneic inhibition of hematopoietic stem cells. For this purpose the action of treatment of the recipient mice with cyclophosphamide, with a well-marked immunodepressive action, on the manifestation of allogeneic inhibition of bone marrow stem cells was investigated.

EXPERIMENTAL METHOD

Adult (weight 22-24 g) female inbred C57BL/6 mice and (CBA × C57BL/6)F_1 hybrids were used. Whole-body Ce^{137} γ-ray irradiation of the recipient mice was given on the "Stebel' 3A" apparatus (dose rate 900 R/min) in a dose of $LD_{100/13}$, namely 765 R for the C57BL/6 mice and 900 R for the (CBA × C57BL/6)F_1 hybrids.

An intraperitoneal injection of cyclophosphamide in a dose of 25-200 mg/kg body weight was given to the mice 4 a after irradiation. An intravenous injection of $0.5\text{-}1 \cdot 10^5$ viable nucleated bone marrow cells was given to the mice 24 h after injection of cyclophosphamide. Nine days after bone marrow transplantation the number of colonies in the recipients' spleens was counted by the method of Till and McCulloch [14], using a mixture of absolute ethanol and glacial acetic acid in the ratio of 3:1 as the fixative.

Irradiated recipients, treated or not treated with cyclophosphamide, and not receiving transplantation of bone marrow (irradiation control), and irradiated recipients receiving an injection of distilled water instead of cyclophosphamide before bone marrow transplantation (cyclophosphamide control) were used as the controls.

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TABLE 1. Number of Colonies in Spleen of Lethally Irradiated C57BL and (CBA × C57BL)F_1 Mice Treated and Not Treated with Cyclophosphamide, after Transplantation of Bone Marrow Cells from Syngeneic Donors ($0.5 \cdot 10^5$ from F_1 hybrids, $1 \cdot 10^5$ from C57BL mice); M ± m

Cyclophosphamide (mg/kg)	Bone marrow	$F_1 \rightarrow F_1$		C57BL → C57BL	
		No. of mice	No. of colonies	No. of mice	No. of colonies
—	—	8	0,1±0,3	7	1,6±1,3
200	—	—	—	6	0,2±0,4
—	+	10	7,9±1,9	10	13,6±2,5
25	+	10	7,8±2,1	10	12,8±3,2
100	+	10	8,1±0,9	—	—
200	+	8	8,0±1,2	9	12,6±3,3

TABLE 2. Number of Colonies in Spleen of Lethally Irradiated (CBA × C57BL/F_1 Hybrids, Treated and Not Treated with Cyclophosphamide, after Transplantation of $1 \cdot 10^5$ Bone Marrow Cells from C57BL Mice

Cyclophosphamide (mg/kg)	Bone marrow	No. of mice	No. of colonies $M \pm I_P$
—	—	19	0,5±0,3
200	—	9	0
—	+	30	1,4±0,4
25	+	25	4,0±0,6
50	+	16	5,1±0,8
100	+	25	6,8±1,1
200	+	25	9,1±0,8

The cyclophosphamide solution was made up in distilled water immediately before use. Methods of preparing the cell suspensions and assessing the viability of the cells with trypan blue were described earlier [3].

The numerical results (the number of colonies in the recipients' spleens) were subjected to statistical analysis with calculation of the arithmetic mean (M) and confidence limits (I_P) with a level of significance of $P = 0.05$.

EXPERIMENTAL RESULTS

After transplantation of bone marrow cells from C57BL mice into lethally irradiated (CBA × C57BL/6)F_1 hybrids the degree of allogeneic inhibition reached 90-95%. In other words, only 5-10% of transplanted stem cells formed colonies in the spleen of the F_1 hybrids by comparison with the syngeneic combination C57BL→C57BL, in agreement with previous observations [1, 2].

Before assessing the effect of cyclophosphamide on the manifestation of allogeneic inhibition, it was necessary to show that the compound itself had no direct effect on the transplanted stem cells under the conditions of its administration. For this purpose, cyclophosphamide was injected into C57BL and F_1 mice 4 h after irradiation, and bone marrow cells were transplanted from syngeneic donors 24 h later.

The lethal doses of irradiation used caused virtually total suppression of endogenous colony formation (irradiation control; Tables 1 and 2). Treatment of the irradiated recipients into which no bone marrow was transplanted with cyclophosphamide in a dose 200 mg/kg body weight caused total suppression of endogenous colony formation, as other workers also have observed [6].

The results given in Table 1 show that cyclophosphamide, if injected 24 h before transplantation of bone marrow, had no direct effect on the transplanted cells: The number of colonies in the spleen of the recipients of the syngeneic bone marrow was the same whether or not they were treated with cyclophosphamide.

The inability of cyclophosphamide, injected 24 h before transplantation of syngeneic bone marrow, to prevent the formation of exogenous colonies can most probably be explained by the rapid elimination of the compound. According to available data [13], for instance, cyclophosphamide is eliminated from the body after 3 h.

After transplantation of the equivalent dose of bone marrow cells from C57BL mice ($1 \cdot 10^5$) the number of colonies formed in the spleen of the lethally irradiated F_1 hybrids was 13.3 times smaller than the number formed in the spleen of syngeneic recipients (Tables 1 and 2). The degree of allogeneic inhibition was 92.5%.

Treatment of the lethally irradiated F_1 hybrids with cyclophosphamide 24 h before transplantation of bone marrow from C57BL mice led to partial abolition of allogeneic inhibition of the stem cells. With an increase in the dose of the compound, more of the stem cells with the C57BL phenotype performed their colony-forming function in the spleen of the F_1 hybrids (Table 2). With cyclophosphamide in a dose of 200 mg/kg the degree of allogeneic inhibition of the stem cells was only 24% (compared with 92.5% in the control). According to data obtained by other workers [8], depression of the degree of allogeneic inhibition (hybrid resistance) occurred in recipients treated with cyclophosphamide 14 and 7 days before irradiation and transplantation of spleen cells from mice of a parental line into F_1 hybrids.

The results of these experiments show that survival and proliferation of stem cells in the allogeneic organism can take place in the absence of a syngeneic (relative to the donor) microenvironment (syngeneic lymphocytes, stroma). Abolition of allogeneic inhibition is not the result of the action of cyclophosphamide on the injected cells, but is due to its action on the irradiated recipient itself. Although the precise mechanism of the immunodepressive action of cyclophosphamide is unknown, its activity is nevertheless due to its effect on lymphocytes [5]. Presumably cyclophosphamide, if injected into lethally irradiated animals, acts on the population of radioresistant lymphocytes that is responsible for allogeneic inhibition of the stem cells.

The results of this investigation thus support the hypothesis that allogeneic inhibition is based on the immune response of radioresistant lymphocytes to nonsyngeneic stem cells. After transplantation of bone marrow from mice of a parental line into lethally irradiated F_1 hybrids the source of antigenic stimulation could be isoantigens of the stem cells, which, unlike codominance, are inherited as a recessive, i.e., they can appear in homozygous parents but are absent in the F_1 heterozygote [7, 8]. The experiments with cyclophosphamide do not shed light on the origin of the lymphocytes responsible for the manifestation of allogeneic inhibition, for the compound can act on both T and B cells [9, 10, 14].

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