IMPORTANCE OF THE TIME OF BONE MARROW TRANSPLANTATION FOR MANIFESTATION OF ALLOGENEIC INHIBITION OF STEM CELLS

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The effect of duration of the interval (4-96 h) between irradiation of F_1(CBA × C57BL/6) hybrids and transplantation of bone marrow from C57BL/6 mice on manifestation of allogeneic inhibition of the stem cells was studied. In this particular donor—recipient model the degree of allogeneic inhibition was 90%. Transplantation of bone marrow carried out 4-48 h after irradiation had no effect on the number of colonies in the spleen of the F_1 hybrids. Considerable abolition of allogeneic inhibition (33%) was observed if the parental cells were injected 96 h after irradiation. Remote transplantation had no effect on the number of colonies in the spleen of syngeneic recipients.

KEY WORDS: *allogeneic inhibition; hematopoietic stem cells; transplantation.*

The mechanism of allogeneic inhibition is unknown: No general agreement has yet been reached on the immune and nonimmune factors responsible for the manifestation of this phenomenon. Recent experimental data suggests that a possible basis of allogeneic inhibition is the response of radioresistant lymphocytes of irradiated F_1 hybrids on recessively inherited hypothetical Hh-isoantigens of the stem cells of bone marrow transplanted from mice of the parental strain [4, 9]. In fact, after irradiation of mice in a dose of the order of 1000 R, nondividing lymphocytes capable of participating in the immune response remain viable [3, 5]. Since radioresistant lymphocytes remaining after irradiation are nondividing cells, it is to be expected that their lifespan is limited and that after their death allogeneic inhibition may possibly be abolished.

With these considerations in mind, the effect of the duration of the interval between irradiation and transplantation of bone marrow on manifestation of allogeneic inhibition of stem cells was investigated.

EXPERIMENTAL METHOD

Adult C57BL/6 and F_1(CBA × C57BL/6) hybrid mice weighing 22-24 g were totally irradiated with Ce^{137} γ-rays on the "Stebel'-3A" apparatus (dose rate 900 R/min) in doses of 765 and 900 R respectively. The recipients were irradiated 4-96 h before intravenous injection of 300,000 bone marrow cells from female C57BL/6 mice. Bone marrow injected into the experimental mice irradiated at different times was taken from the same suspension. The number of colonies of hematopoietic cells in the recipients' spleen 8 days after transplantation of the cells was determined by the method of Till and McCulloch [8], using a mixture of absolute ethanol and glacial acetic acid (3:1) as the fixative. The control for the number of endogenous colonies (the recipient's phenotype) consisted of lethally irradiated mice into which no bone marrow was transplanted. The methods of preparing the cell suspensions and estimating cell viability with the aid of trypan blue were described earlier [2].

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TABLE 1. Number of Colonies in Spleen of Lethally Irradiated F_1(CBA × C57BL/6) Hybrids when Bone Marrow Cells of C57BL/6 Mice Were Transplanted at Various Times after Irradiation

Interval betw. irradiation and transplantation (in h)	Bone marrow injected	Number of mice	Number of colonies ($\bar{x} \pm ts_{\bar{x}}$)	Degree of allogeneic inhibition (in %)
4	—*	13	0,46±0,34	—
4	+	25	3,40±0,51	89,6
24	—	9	0,66±1,01	—
24	+	19	3,47±0,65	89,4
48	—	15	0,46±0,32	—
48	+	24	3,91±0,64	88,0
72	—	17	0,58±0,27	—
72	+	24	7,70±0,97	76,4
96	—	5	1,20±2,42	—
96	+	17	21,82±3,71	33,1

*Irradiation control

The numerical results (number of colonies in the recipients' spleen) was subjected to statistical analysis, with calculation of the arithmetic mean ($\bar{X}$) and the confidence interval ($tS_{\bar{x}}$) for P = 0.05.

EXPERIMENTAL RESULTS

After transplantation of 300,000 bone marrow cells from C57BL/6 mice 4 h after irradiation, the mean number of colonies formed in the spleen of the syngeneic recipients was 32.6±1.3 (n = 44). Under similar experimental conditions, 3.4 colonies were formed in the spleen of F_1(CBA × C57BL/6) hybrids by bone marrow cells from C57BL/6 mice. In the C57BL/6→F_1 model, therefore, the degree of allogeneic inhibition of the stem cells was about 90%, i.e., only about 10% of the transplanted allogeneic parental cells were capable of forming colonies in the spleen of the F_1 hybrids.

As the results in Table 1 show, injection of bone marrow 4-48 h after irradiation did not affect the degree of allogeneic inhibition of the stem cells. An increase in the number of colonies in the spleen of the F_1 hybrids was observed when bone marrow was transplanted 72 h and, in particular, 96 h after irradiation (by 2.2 and 6.4 times respectively). Transplantation of bone marrow even later after irradiation might possibly have abolished the allogeneic inhibition altogether. However, it would be difficult to confirm or refute this observation by the use of a model requiring lethal irradiation, because of the virtually complete death of all the recipients by the 13th day after irradiation.

Stimulation of colony formation by remote transplantation of bone marrow after irradiation took place only when the allogeneic cells were transferred to F_1 hybrids. In the syngeneic F_1→F_1 model the number of colonies in the recipients' spleen following transplantation of equal doses of bone marrow cells 4 and 96 h after irradiation was practically the same.

With the lethal doses of irradiation used the number of endogenous colonies in the recipients' spleen (the "irradiation control" group) was very small (0.46-1.2) and could not have significantly affected the experimental results.

The results indicate that allogeneic inhibition is due to some factors in the recipients which are inactivated starting from the 4th day after irradiation. In other words, allogeneic inhibition is the result of the active response of the recipient to the injected stem cells. This conclusion does not agree with the views of those workers who consider that the cause of allogeneic inhibition is absence of a syngeneic microenvironment [1, 6, 7]. Very probably it is the radioresistant lymphocytes (or macrophages) of the recipient that are responsible for allogeneic inhibition. Hence it follows that the lifespan of the nondividing radioresistant cells is about 4 days, for transplantation of bone marrow at this time after irradiation was most effective as regards abolishing allogeneic inhibition of the hematopoietic stem cells.

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