INTERACTION BETWEEN CELLS TRANSPLANTED INTO SYNGENEIC AND ALLOGENEIC RECIPIENTS DURING THE IMMUNE RESPONSE TO HETEROLOGOUS RED CELLS

S. S. Gambarov and I. N. Golovistikov

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Interaction between bone marrow cells and cells from the thymus or lymph glands of CBA and C57BL/6 mice was investigated during the production of plaque-forming cells synthesizing antibodies against sheep's red cells after transplantation of the cells into lethally irradiated syngeneic recipients or (CBA × C57BL/6)F_1 hybrids. It was shown that the manifestation of allogeneic inhibition of the hematopoietic stem cells may be caused by depression of the immune response through interaction between cells transplanted into genetically foreign recipients.

It is now generally accepted that the development of the immune response to certain antigens requires interaction at least between cells of bone-marrow (B-cells) and of thymus (T-cells) origin [2, 5, 8].

The effects of interaction between bone marrow and thymus (or lymph gland) cells during the immune response to sheep's red cells and under conditions providing for manifestation of the allogeneic inhibition phenomenon were investigated. In the writers' view the use of a model of cell interaction is a promising method of studying the mechanisms of allogeneic inhibition at the level of the cells participating in immunogenesis.

EXPERIMENTAL METHOD

Male CBA, C57BL/6 and (CBA × C57BL/6)F_1 hybrid mice obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, were used. Interaction between the cells was investigated on a model of the production of plaque-forming cells (PFCs) synthesizing antibodies against sheep's red cells in culture in vivo. A mixture of bone marrow and thymus (or lymph gland) cells from syngeneic ($F_1 \rightarrow F_1$, CBA → CBA, C57BL/6 → C57BL/6) or allogeneic intact donors (CBA → F_1, C57BL/6 → F_1) was injected intravenously into the recipient mice 24 h after lethal irradiation. Simultaneously with transplantation of the cells into the recipients they received an intravenous injection of $2 \cdot 10^8$ sheep's red cells. Irradiated control recipients received injections of either bone marrow or thymus (or lymph gland) cells mixed with sheep's red cells. On the eighth day after transplantation of the cells and immunization the number of PFCs in the recipients' spleen was determined by Jerne's method [4].

Besides counting the number of PFCs, in some of the experiments the number of colony-forming units was determined in the spleen of the irradiated recipients by the method of Till and McCulloch [10] after transplantation of 100,000-500,000 bone marrow cells from syngeneic (C57BL/6 → C57BL/6, CBA → CBA) and allogeneic donors (C57BL/6 → F_1, CBA → F_1).

The recipients were irradiated with CE137 γ-rays on the "Stebel 3A" apparatus (dose rate 900 R/min) in a dose of LD 100/13, namely 900, 765, and 900 R for CBA, C57BL/6 and F_1 hybrid mice respectively. The methods of preparing the cell suspensions were described previously [1]. The numerical results were subjected to statistical analysis with calculation of the arithmetic mean M, the error of the mean (m), and the confidence interval (IP) for P = 0.01.

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TABLE 1. Number of Antibody-Forming Cells in Spleen of Lethally Irradiated Syngeneic and Semisyngeneic Recipients after Transplantation of Bone Marrow Mixed with Thymus or Lymph Gland Cells

Donor	No. of cells injected (millions)			Recipient	No. of mice	No. of antibody-forming cells (M ± I_p)
	bone marrow	thymus	lymph glands			
(CBA×C57BL/6)F_1	10	—	—	(CBA×C57BL/6)F_1	22	101,4±15,4
	—	20	—		18	38,8±9,1
	—	40	—		23	111,0±20,9
	—	—	1		15	79,6±16,5
	—	—	10		24	1604,4±209,8
	10	20	—		15	921,1±166,4
	10	40	—		23	1657,0±266,2
	10	—	1		17	1463,3±284,8
	10	—	10		24	17472,9±2061,0
CBA	10	—	—	CBA	19	109,2±21,9
	—	20	—		22	39,7±6,4
	10	20	—		25	1240,0±209,5
	10	—	—	CBA×C57BL/6)F_1	18	97,5±22,1
	—	20	—		16	37,0±13,9
	10	20	—		23	1075,0±206,5
C57BL/6	10	—	—	C57BL/6	18	129,3±22,6
	—	20	—		18	40,9±8,2
	10	20	—		27	1181,3±117,9
	10	—	—	CBA×C57BL/6)F_1	15	35,5±11,4
	—	20	—		17	28,8±9,2
	10	20	—		26	125,0±23,2

TABLE 2. Number of Colonies in Spleen of Lethally Irradicated Mice after Transplantation of Bone Marrow from Syngeneic and Allogeneic Donors

Donor	Recipient	No. of mice	No. of colonies per 100,000 cells (M ± m)
CBA	CBA	90	14,9±0,3
CBA	(CBA×C57BL/6) F_1	70	13,8±0,4
C57BL/6	C57BL/6	10	15,9±0,9
C57BL/6	(CBA×C57BL/6) F_1	80	1,2±0,1

EXPERIMENTAL RESULTS

After transplantation of bone marrow or thymus cells only a very few PFCs were formed in the spleen of lethally irradiated syngeneic recipients immunized with sheep's red cells (Table 1). Co-operative interaction between the bone marrow and thymus cells was revealed by the fact that when these cells were injected together the number of PFCs was 7 to 10 times greater than the expected number (the sum of the PFCs obtained by transplantation of the components of the mixture separately). Interaction between bone marrow and thymus cells during PFC production took place in all syngeneic donor–recipient combinations used. A qualitatively similar effect of interaction was found when lymph gland cells were used instead of thymocytes (the $F_1 \rightarrow F_1$ model).

Addition of lymph gland cells in a dose of 1 million to bone marrow cells (10 million) led to the same effect as addition of 40 million thymus cells.

An increase in the number of thymus or lymph gland cells in the interacting mixture (with a constant number of bone marrow cells) was accompanied by an increase in the number of PFCs in the recipients' spleen, in agreement with observations made by other workers [2, 5, 8].

After transplantation of a mixture of bone marrow cells and thymus cells from CBA mice the same number of PFCs was found in the spleen of lethally irradiated CBA and F_1 mice. A different picture was observed in the C57BL → F_1 combination. In that model the effect of interaction between thymus and bone

marrow cells was weak: The number of PFCs was only twice the expected total. In the syngeneic combination C57BL/6 → C57BL/6 the number of PFCs obtained by interaction between thymus and bone marrow cells was 10 times higher than in the C57BL/6 → F_1 model.

The number of PFCs formed is known to depend on the relative numbers of B- and T-cells [2, 5, 8]. The PFCs arise from the bone marrow cells [7] and the degree of cooperative interaction is a function of the number of colony-forming units [3]. With these facts in mind, the colony-forming activity of bone marrow cells transplanted into lethally irradiated syngeneic and semisyngeneic mice was investigated (Table 2).

Bone marrow stem cells of CBA mice underwent virtually no allogeneic inhibition after transplantation into irradiated F_1 hybrids. The number of PFCs formed in the spleen of the CBA and F_1 recipients after transplantation of thymus and bone marrow cells from CBA mice was the same (Table 1). In the C57BL/6 → F_1 model, in which the effects of interaction between thymus and bone marrow cells during production of PFCs were manifested only very slightly (Table 1), the degree of allogeneic inhibition of the bone marrow stem cells was 95% (Table 2).

It can be concluded from the results of these experiments that depression of the effects of interaction between cells participating in the immune response, when transplanted into lethally irradiated, genetically foreign recipients, is connected with manifestation of the phenomenon of allogeneic inhibition of hematopoietic stem cells. This interpretation of the experimental results becomes understandable if the fact is remembered that the precursors of the antibody-forming cells originate from the stem cells of the bone marrow [7].

It is postulated that suppression of the immune response during interaction between thymus and bone marrow cells after transplantation into a foreign organism may be due also to allogeneic inhibition of cells of thymus origin, and not the result of development of a graft versus host reaction [6, 9].

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