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INACTIVATION OF STEM CELLS BY ALLOGENEIC LYMPHOCYTES: COMPETITION BETWEEN T_1 AND T_2 SUBPOPULATIONS

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Transplantation of bone marrow cells mixed with allogeneic T lymphocytes into irradiated recipients is accompanied by inactivation of stem cells of the graft. Lymphocytes from lymph nodes of T mice possess greater inactivating power than T lymphocytes from the spleen. In the case of the combined action of T lymphocytes from the spleen and T lymphocytes from the lymph nodes, inactivation of the stem cells was slight in degree or absent altogether.
KEY WORDS: T lymphocytes; stem cells.

Combined transplantation of genetically foreign cells of hematopoietic tissues into irradiated recipients is known to be followed by inactivation of the stem colony-forming units (CFU) of the grafts by the allogeneic lymphocytes of the mixture [2]. It has been shown that the chief, it not the exclusive inactivating role in these processes is played by T lymphocytes [1].

The object of this investigation was to continue the study of the inactivating power of T lymphocytes and, in particular, of their subpopulations with affinity for the spleen (T_1) and for the lymph nodes (T_2) of the recipients and also the interaction between these cells in the processes of CFU inactivation.

EXPERIMENTAL METHOD

Experiments were carried out on CBA, C57BL/6J (C57BL), and (CBA × C57BL/6)F_1 mice. The donors of thymus cells were CBA mice aged 4-7 weeks and the donors of bone marrow cells were C57BL mice aged 2-5 months. The recipients were CBA and (CBA × C57BL)F_1 mice aged 2-5 months irradiated in a dose of 850-900 rad 24 h before transplantation of cell suspensions. The dose rate was 170-149 rad/min. To obtain T mice, $3 \cdot 10^7$-$5 \cdot 10^7$ syngeneic thymus cells were injected into lethally irradiated CBA mice and, 7 days later, the spleen (Spl) and inguinal, popliteal, mesenteric, and submandibular lymph nodes (LN) were removed for the preparation of cell suspensions. The cells obtained were mixed in various proportions with bone marrow cells of C57BL mice and the cell mixtures were transplanted into lethally irradiated (CBA × C57BL)F_1 mice. Animals receiving bone marrow cells of C57BL mice only served as the control. In all the experiments cell suspensions were injected intravenously into the irradiated mice. The methods used to prepare the cells were described previously [3]. The recipients were killed 7-8 days after transplantation, their spleens were removed and fixed in Bouin's solution, after which the number of macroscopically visible colonies was counted. The decrease in the number of colonies in the experimental group of animals compared with the controls was expressed as the index of CFU inactivation [3].

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TABLE 1. Comparative Inactivating Power of Spl and LN Lymphocytes from CBA T Mice

LN : BM or Spl : BM ratio	Number of CFU after transplantation					
	BM	BM + LN	CFU inactivation index, %	BM	BM + Spl	CFU inactivation index, %
1 : 2	11,5±0,71 (121)	8,0±0,55 (113)	30,4	13,1±1,19 (24)	10,0±0,99 (52)	23,7
1 : 1	8,1±0,70 (98)	5,4±0,59 (87)	33,3	9,8±0,96 (60)	6,9±1,0 (54)	29,6
2,5 : 1	9,7±0,86 (95)	4,9±0,63 (79)	49,5	11,7±0,97 (76)	7,6±0,68 (79)	35,0
5 : 1	10,2±0,84 (78)	2,6±0,43 (76)	74,5	10,3±1,07 (53)	5,4±0,82 (56)	47,6
10 : 1	11,6±0,99 (36)	4,2±1,07 (30)	63,8	13,0±1,39 (34)	7,2±1,18 (34)	44,6

Legend: 1) In all experiments $2 \cdot 10^5$ bone marrow (BM) cells of C57BL mice mixed with Spl or LN cells from CBA T mice were transplanted into lethally irradiated (CBA × C57BL)F_1 mice. 2) Here and in Table 2 number of recipients shown in parentheses.

TABLE 2. Competition between Spl and LN T Lymphocytes during Inactivation of Allogeneic Stem Cells

Cells transplanted into lethally irradiated (CBA × C57BL)F_1 mice	Number of CFU	CFU inactivation index, %
BM C57BL 2×10^5	14,2±1,54 (34)	—
BM C57BL 2×10^5 + T LN CBA 10^5	7,4±1,0 (31)	47,9
BM C57BL 2×10^5 + T Spl CBA 5×10^5	8,5±0,97 (40)	40,1
BM C57BL 2×10^5 + T LN CBA 10^5 + T Spl CBA 5×10^5	14,6±1,7 (37)	No inactivation present
BM C57BL 2×10^5	16,0±2,2 (21)	—
BM C57BL 2×10^5 + T LN CBA 5×10^5	5,9±0,95 (14)	63,1
BM C57BL 2×10^5 + T Spl CBA 10^5	10,2±1,53 (23)	36,2
BM C57BL 2×10^5 + T LN CBA 5×10^5 + T Spl CBA 10^5	8,9±1,6 (18)	44,4

EXPERIMENTAL RESULTS

Inactivation of the stem cells of C57BL mice by Spl and LN lymphocytes of CBA T mice is shown in Table 1. Clearly the inactivating power of T lymphocytes from LN increased with an increase in the dose of killer cells until the ratio between killer cells and target cells was 5 : 1. A further increase in the number of lymphocytes in the mixture (ratio between the cells 10 : 1) did not cause any increase in the index of CFU inactivation. Basically the same result was obtained by transplantation of bone marrow cells of C57BL mice ($2 \cdot 10^5$) mixed with various doses of Spl lymphocytes from CBA T mice into irradiated F_1 mice. However, in the latter case, the indices of CFU inactivation when lymphocytes were used in doses of $1 \cdot 10^6$-$2 \cdot 10^6$ were 1.4-1.7 times smaller than when T lymphocytes from LN were used (Table 1). This could indicate that the population of T lymphocytes, especially Spl, was heterogeneous and consisted of at least two types of cells. Some of them inactivate proliferation and differentiation of allogeneic stem cells, whereas others suppress syngeneic T killer cells. With an increase in the number of lymphocytes in the mixture, the fraction of these cells in the population evidently rises and, at the same time, their suppressive action on the processes of CFU inactivation increases.

The LN and Spl lymphocytes from CBA T mice, incidentally, had weaker inactivating power than the cortisone-resistant T lymphocytes or lymphocytes from LN or Spl of intact mice of the same strain [1]. The low inactivating power of T lymphocytes fractionated on the basis of affinity for Spl (T_1 subpopulation) and for LN (T_2 subpopulation) can be explained by the necessity for their cooperative interaction in the inactivation of nonsyngeneic stem cells. In the absence of such interaction the suppressive activity of the lymphocytes could be manifested more strongly than their inactivating power. The necessity for cooperation of T cells of the "T–T" type in transplantation immunity is well known [5, 6]. In order to assess the necessity for interaction between the subpopulations of T lymphocytes in the processes of CFU inactivation, bone marrow cells ($2 \cdot 10^5$) from C57BL mice mixed with LN and Spl cells from CBA T mice were transplanted into lethally irradiated F_1 mice, varying the ratio, depending on the cells used, from 2 : 1 : 5 to 2 : 5 : 1. As Table 2 shows, T lymphocytes from Spl and LN inactivated 40-60% of the allogeneic CFU. In the case of combined transplantation of these cells the cooperative effect was absent. Furthermore, in these experiments inactivation of the stem cells by the allogeneic lymphocytes was abolished. When the ratio bone marrow cells : LN T lymphocytes : Spl T lymphocytes was 2 : 1 : 5, the abolition of inactivation was complete (90% inactivation was expected on

summation of the inactivation indices of the separately transplanted cells), and when the ratio between the cells was 2 : 5 : 1 the inactivation index was only 44.4%, instead of the expected 100%. These results show that the composition of the subpopulation of T lymphocytes used includes cells protecting the stem cells against the inactivating action of allogeneic T lymphocytes. It will also be evident that the population of splenic T lymphocytes contained more cells abolishing the inactivating action of the syngeneic killer cells than the population of T lymphocytes from LN. This fact can evidently explain the lower inactivating power of the Spl than of the LN T lymphocytes (Table 1).

The nature of the protector cells found in these experiments is unknown. The possibility cannot be ruled out that they are a subpopulation of lymphocytes intended to protect stem cells against the harmful action of various factors. Another possibility is that the experimental conditions chosen facilitate the accumulation of large numbers of the cells known as suppressor cells, with an important role in the regulation of immunity reactions [4].

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SYNTHESIS OF α-FETOPROTEIN AND ALBUMIN BY HUMAN EMBRYONIC HEPATOCYTES

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Population aspects of the production of specific secreted proteins (serum albumin and α-fetoprotein) were studied in cultures of hepatocytes from human embryos at 6-12 weeks of development. A method based on local hemolysis in gel using sheep's erythrocytes conjugated with antibodies against the proteins for testing was used. The overwhelming majority of hepatocytes were shown to synthesize both proteins.

KEY WORDS: human embryo; hepatocyte cultures; fetoprotein; serum albumin; local hemolysis in gel.

In mammalian development a strictly regular change in the composition of the blood serum is observed. In the early stages of development α-fetoprotein (α-FP) is predominant, whereas albumin and transferrin are present in very low concentrations. In the course of development the α-FP level falls from 3-5 to 10^{-6} mg/ml, whereas the concentrations of albumin and transferrin rise sharply [1]. All three proteins are synthesized by the liver [1]. However, it was not previously known whether there exists in the liver a "mosaic" of hepatocytes, each synthesizing one particular protein, or whether they are produced by the same cells. In particular, it was not known whether different populations of cells synthesizing embryonic and "adult" proteins exist in the

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