INHIBITION OF HEMATOPOIETIC STEM CELLS BY SYNGENEIC LYMPHOCYTES

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The indirect (through the lymphocytes) action of antilymphocytic globulin (ALG) on the ability of bone marrow cells to form hematopoietic colonies in lethally irradiated (830 rad) mice was investigated. Lymphocytes treated in vitro with ALG have their functional state modified and, if injected together with intact bone marrow cells, acquire the property of inhibiting the formation of hematopoietic foci. The clone-forming ability of bone marrow cells became inhibited when they were treated with supernatant obtained after incubation of syngeneic thymocytes with ALG in vitro. The mechanism of inhibition of hematopoietic stem cells by treatment with ALG is probably a combination of the direct effect on the lymphocytes and of the action of humoral "factor" secreted by them into the medium after treatment with ALG.

Investigations have shown that contact with nonsyngeneic lymphocytes inhibits the function of hematopoietic stem cells by abolishing or sharply reducing their ability to form hematopoietic colonies in the spleens of lethally irradiated mice [5-7].

Treatment of transplanted hematopoietic cells with antilymphocytic serum (ALS) in vitro also inhibits the formation of hematopoietic foci in lethally irradiated mice [1, 3, 4, 8]. The mechanism of this effect of ALS can be explained either by direct action on the stem cells (a change of differential potential) or by indirect action.

The object of this investigation was to study the indirect action (through lymphocytes) of ALS on the ability of hematopoietic stem cells to form foci of hematopoiesis in irradiated recipients.

EXPERIMENTAL METHOD

The donor and recipient animals were (CBA × C57BL) F_1 hybrid mice weighing 22-24 g.

Antilymphocytic globulin (ALG) was isolated from ALS [10] obtained by immunizing rabbits with thymocytes from CBA mice [3]. The method of cloning hematopoietic cells in vivo in lethally irradiated mice [9] was used. The recipients were irradiated in a dose of 830 rad and 4 h later a mixture of bone marrow and lymph gland or thymus cells was injected intravenously into them. Before transplantation in this way the lymphocytes or thymocytes were treated in vitro with ALG (5 mg/ml) or phytohemagglutinin (PHA; Wellcome, England) for 30 min at 37°C, after which the cells were washed three times and incubated with an equal number of bone marrow cells for 30 min at 37°C. After incubation, the cells were washed off, and a mixture consisting of 0.5×10^5 bone marrow cells and 0.5×10^5 lymphocytes or thymocytes, suspended in 0.5 ml medium No. 199, was injected into each recipient.

In another series of experiments the thymocytes were incubated with ALG, washed off, and the cell residue was treated with 1.5 ml medium No. 199. After incubation for 30 min at 37°C the cell suspension was centrifuged. The supernatant was added to intact bone marrow cells and incubated for 30 min at 37°C. The cells were washed off with medium three times and injected intravenously into irradiated recipients.

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TABLE 1. Inhibition of Stem Cells by Lymphocytes or Thymocytes Treated in vitro with ALG or PHA (M ± m)

Cells transplanted	ALG			PHA «Wellcome»		
	types of cells treated	No. of mice	No. of foci per spleen	types of cells treated	No. of mice	No. of foci per spleen
BM* + lymphocytes	BM	18	0.2±0.1	BM	11	3.0±0.4
BM + thymocytes	BM	16	0.2±0.1	BM	7	2.3±1.1[2]
BM + lymphocytes	Lymphocytes	20	2.3±0.3	Lymphocytes	6	13.0±1.4
BM + thymocytes	Thymocytes	15	2.4±0.4	Thymocytes	11	19.3±1.1[2]
BM + lymphocytes	Med. No. 199 Lymphocytes	18	11.4±0.5	Med. No. 199 Lymphocytes	11	11.9±0.6
BM + thymocytes	Med. No. 199 Thymocytes	11	13.9±0.6	Med. No. 199 Thymocytes	14	19.9±0.7[2]
Irradiation control (formation of endogenous foci)		20	0	—	16	0,1±0,01

* BM) bone marrow cells.

†For transplantation into recipients, 1×10^5 bone marrow cells and 1×10^5 lymphocytes or thymocytes were used. In all other cases 0.5×10^5 cells of each were injected.

Nine days after transplantation the animals were killed, the spleens were removed and fixed in alcohol–formol, and macroscopically visible colonies on the surface of the organ were counted. Statistical analysis of the results was carried out with the use of Student's criterion.

EXPERIMENTAL RESULTS

Nine days after irradiation the number of endocolonies in the spleens of the mice (irradiation control) averaged 0.1. Intravenous transplantation of a suspension consisting of 0.5×10^5 bone marrow cells and an equal number of lymphocytes or thymocytes led to the formation, on the average, of 11.4 or 13.9 colonies respectively in the spleen. Injection of bone marrow cells mixed with lymphocytes or thymocytes previously treated in vitro with ALG led to a marked decrease in the ability of the bone marrow cells to induce hematopoietic colony formation, and the mean number of the colonies was 2.3 or 2.4 respectively (Table 1).

When the bone marrow cells for transplantation were treated directly with ALG in vitro the stem cells lost nearly all their ability to form hematopoietic foci. The number of foci was 0.2 per spleen. These results agree fully with those of earlier investigations showing that treatment of hematopoietic cells with ALS in vitro inhibits the formation of hematopoietic foci [1, 3].

Treatment of the hematopoietic tissue for transplantation with PHA in vitro, just as with ALS, led to a decrease in the formation of hematopoietic foci [1], possibly because of the well-marked blast-transforming action. It was decided to study the effect of this agent under the experimental conditions used.

After intravenous injection of a mixture of intact bone marrow cells with lymphocytes or thymocytes previously treated in vitro with PHA in a dilution of 1 : 10 into irradiated recipients the number of colonies in the spleens of the lethally irradiated mice was virtually identical with the control (Table 1).

Although PHA evidently induces blast-transformation of the cells, it is thus evidently unable to modify lymphocytes so that they become "heterologous" (functionally) for the stem cells, whereas such an action is perfectly permissible for ALG.

However, this still left one other possibility of an indirect action on the stem cells through a humoral factor possibly secreted by the treated lymphocytes. To test this hypothesis the following experiments were carried out. Thymocytes treated with ALG were washed off and incubated in medium No. 199 for 30 min at 37°C, and the supernatant was used to treat bone marrow cells intended for transplantation. Treatment with the supernatant led to a marked decrease in the formation of hematopoietic foci (Table 2). The degree of the effect depended on the number of thymocytes used for treatment. The results suggest that the supernatant contains a certain "factor" inhibiting stem cells. This factor could be ALG eliminated from the surface of the thymocytes or a product of their activity, like the macrophagal migration inhibiting factor (MIF). The presence of eliminated ALG in sufficient concentration to inhibit stem cells is unlikely, but a special

TABLE 2. Effect of a Humoral Factor Secreted by Thymocytes Treated with ALG or PHA on Hematopoietic Stem Cells

No. of transplanted bone marrow cells	No. of thymocytes used for treatment and preparation of the "factor"	Agent used for treatment	Formation of foci in spleen	
			No. of animals	$M \pm m$
$0{,}5 \cdot 10^5$	$1 \cdot 10^7$	ALG	27	2,3±0,2
$0{,}5 \cdot 10^5$	$0{,}5 \cdot 10^5$	ALG	29	3,4±0.2
$0{,}5 \cdot 10^5$	$1 \cdot 10^3$	ALG	21	6,2±0,3
$0{,}5 \cdot 10^5$	$0{,}5 \cdot 10^5$	Med. No. 199	26	15,2±0,6
$1{,}0 \cdot 10^5$	$1{,}0 \cdot 10^5$	PHA	14	19,4±0,7
$1{,}0 \cdot 10^5$	$1{,}0 \cdot 10^5$	Med. No. 199	14	19,9±0,7
Bone marrow cells ($0{,}5 \cdot 10^5$) treated with ALG			10	0,5±0,2
Bone marrow cells ($1 \cdot 10^5$) treated with PHA			7	2,3±0,9
Irradiation control (formation of endogenous foci)			21	0,39±0,01

study is called for. In turn, the MIF is a product secreted by sensitized lymphocytes under the influence of a specific antigen. PHA, with both blast-transforming and antigenic activity, did not, however, induce the appearance of the "factor" (Table 2).

Unlike PHA, ALG thus modifies the function of lymphocytes so that they become capable of inhibiting syngeneic stem cells, as has also been demonstrated for allogeneic lymphocytes [5-7] or syngeneic lymphocytes [2] under the influence of foreign antigenic information. In the present experiments PHA, although it had antigenic activity, did not modify lymphocyte function.

The results of these experiments confirm that a humoral "factor" had the greatest effect on the stem cells. However, neither direct contact with the treated lymphocytes nor the "factor" present in the supernatant gave so complete an inhibitory effect on the stem cells as the direct action of ALG. Most probably the mechanism of inhibition of the stem cells under the influence of ALG is more complex and is a combination of direct action of ALG on them, possibly inducing a change in the pathways of differentiation (evidently the only mechanism for PHA) and indirect action through the treated lymphocytes (most probably on account of a certain "factor").

LITERATURE CITED

1. T. A. Golovanova and D. R. Kaulen, Byull. Éksperim. Biol. i Med., No. 11, 86 (1970).
2. I. N. Golovistikov, M. S. Blyakher, and Yu. V. Zykov, Dokl. Akad. Nauk SSSR, 204, No. 4, 980 (1972).
3. D. R. Kaulen and T. A. Golovanova, Vestn. Akad. Med. Nauk SSSR, No. 7, 33 (1970).
4. L. N. Lemeneva and I. L. Chertkov, Probl. Gematol., No. 4, 17 (1971).
5. R. V. Petrov, Zh. Vsesoyuzn. Khim. Obshch. im. Mendeleeva, 13, No. 4, 409 (1968).
6. R. V. Petrov, Uspekhi Sovr. Biol., 69, No. 2, 261 (1970).
7. R. V. Petrov and L. S. Seslavina, Dokl. Akad. Nauk SSSR, 175, No. 5, 202 (1967).
8. T. De Meester, N. Anderson, and C. Shaffer, J. Exp. Med., 127, 731 (1968).
9. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).
10. M. Zeppezauer and S. Brishammer, Biochim. Biophys. Acta, 94, 581 (1965).