DEPRESSION OF FUNCTION OF STEM CELLS OF HEMATOPOIETIC TISSUE BY PHYTOHEMAGGLUTININ AND ANTILYMPHOCYTIC SERUM

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Mouse spleen cells were treated in vitro with antisplenic serum or phytohemagglutinin and transplanted into lethally irradiated syngeneic or semisyngeneic (F_1) mice. Both agents reduced the number of foci of hematopoiesis formed in the recipients' spleen on the ninth day.

Antilymphocytic sera (ALS) can induce blast-transformation in short-living cultures of lymphocytes [9-11, 15], and in this respect their action is not inferior to that of phytohemagglutinin (PHA) [9, 11]. It has also been shown that both PHA and ALS have a marked inhibitory action on the transplantation and humoral immunity response [4, 13, 14]. A previous investigation showed that treatment of mouse spleen cells (intended for transplantation) with antisplenic serum in vitro leads to inhibition of the formation of exogenous hematopoietic foci in the spleen of a lethally irradiated recipient [1]. The identical effect of these two factors on the immunologic reactivity of lymphoid cells suggested that the mechanism of action of ALS is evidently not entirely explained by its specific (cytotoxic) action [1, 9, 12].

The object of the present investigation was to compare the action of antisplenic serum (ASS) and of PHA on the stem cells of hematopoietic tissue following its transplantation into irradiated mice.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice and on CBA $\times$ C57BL$_1$ F_1 hybrids. Antiserum was obtained in rabbits by immunization with two injections of a suspension of CBA mouse spleen cells. The cytotoxic titer of the serum, estimated by the usual method, was 1:256. To study the comparative action of ASS and PHA on the stem cells, the method of cloning of hematopoietic cells in a lethally irradiated recipient, as developed by Till and McCulloch, was followed. Animals were irradiated with γ rays in a dose of 830 rad. Each recipient, 4 h after irradiation, received an intravenous injection of 0.5 ml of a suspension consisting of 2×10^6-2.5×10^6 spleen cells from intact donors. The cells for transplantation (in a concentration of 20×10^6 cells) were treated in vitro with ASS in different dilutions or with PHA for 30 min at 37°, and then carefully washed with Hanks's solution, before injection. PHA (Wellcome, England) was used. The contents of the flask were diluted in 5 ml medium No. 199 or with Hanks's solution. The animals were sacrificed 9 days after transplantation, and the spleens were removed and fixed in alcohol–formol. Macroscopically visible foci on the surface of the organ were counted after fixation.

EXPERIMENTAL RESULTS

Antilymphocytic sera possess a marked depressive action on the stem cells of hematopoietic tissue. This conclusion is based on the writers' previous findings [1], data in the literature [8], and the experiments described in this paper.

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TABLE 1. Inhibition of Stem Cells of Hematopoietic Tissue after Treatment with ASS in vitro

Exptl. No.	Donor	Number of transplanted spleen cells	Dose of anti-CBA ASS (ml/ml cell suspension)	Recipient irradiated in dose of 830 rad	No. of animals	Number of foci in spleen	
						$M \pm m$	% of formation of foci
1	CBA mice	$2,5 \cdot 10^6$	0,001 Hanks's solution	(CBA × C57BL) F_1 hybrids	15	1,8±0,4	15
		$2,5 \cdot 10^6$			7	11,8±1,7	100
2	The same	$2,5 \cdot 10^6$	0,1	(CBA × C57BL) F_1 hybrids	10	1,1±0,4	7
		$2,5 \cdot 10^6$	0,001		12	2,4±0,7	16
		$2,5 \cdot 10^6$	Hanks's solution		14	14,7±1,1	100
	—	—			17	0,3±0,03	2
3	CBA mice	$2,5 \cdot 10^6$	0,001	CBA mice	7	1,3±0,4	11
		$2,5 \cdot 10^6$	0,0001		8	5,7±1,0	33
		$2,5 \cdot 10^6$	Hanks's solution		9	15,3±1,3	100

TABLE 2. Inhibition of Stem Cells of Hematopoietic Tissue after Treatment with PHA in vitro

Exptl., No.	Donor	Number of transplanted spleen cells	Dose of PHA (in ml/ml cell suspension)	Recipient irradiated in dose of 830 rad	No. of animals	Number of foci in spleen		P
						$M \pm m$	% of formation of foci	
1	CBA mice	$2 \cdot 10^6$	0,1	(CBA×C57-BL) F_1 hybrids)	12	1,0±0,4	10	
		$2 \cdot 10^6$	0,01		9	2,3±0,6	23	>0,05
		$2 \cdot 10^6$	0,001		7	3,8±1,2	38	>0,1
		$2 \cdot 10^6$	Hanks's solution		9	10±1,1	100	0,002
	—	—	—		8	0,1±0,05	1	
2	CBA mice	$2,5 \cdot 10^6$	0,1	CBA mice	3	4±1	19,4	
		$2,5 \cdot 10^6$	0,01		5	6,2±1,1	30	>0,1
		$2,5 \cdot 10^6$	0,001		3	14,3±2,8	70	<0,01
		$2,5 \cdot 10^6$	Hanks's solution		5	20,6±3,1	100	<0,01
	—	—	—		2	0	0	
3	(CBA×C57-BL) F_1 hybrid	$2 \cdot 10^6$	0,1	(CBA×C57-BL) F_1 hybrids)	15	1,2±0,2	9,2	0,02
		$2 \cdot 10^6$	0,01		14	3,0±0,6	23	<0,001
		$2 \cdot 10^6$	0,001		12	11,2±0,8	87	>0,1
		$2 \cdot 10^6$	Hanks's solution		13	13±1,2	100	
	—	—	—	—	16	0,3±0,2	2,3	

Transplantation of 2.5×10^6 spleen cells into an irradiated (830 rad) syngeneic or semisyngeneic recipient led to the formation of a mean number of 15 foci of hematopoiesis in its spleen. If the transplanted cells were preliminarily treated with heterologous ASS, the number of foci formed was substantially fewer (Table 1). The difference between the number of foci in recipients receiving untreated cells and recipients of the experimental groups was statistically significant.

In the experiments of series II, the spleen cells were preliminarily treated in vitro before transplantation with PHA in different concentrations under the same conditions as those when ASS was used. The results indicate a marked inhibitory action of PHA on the formation of foci (Table 2).

Other workers [3, 5] have shown conclusively that colonies of hematopoietic cells formed under these conditions are the progenies of a hematopoietic stem cell. With the dose of irradiation selected, this could only be a donor's stem cell, because the number of endogenous foci formed was not more than 2% of the number of foci in the control mice (receiving injections of untreated cells; Tables 1 and 2). Consequently, both PHA and ASS act on stem cells, modify their normal function, and prevent the formation of foci in the spleen.

When the results of exposure to these two factors are compared, the following fact must be noted: neither ASS nor PHA, in the concentrations used, is toxic to spleen cells in vitro. The cytotoxic action of ASS begins to be apparent only in concentrations of 0.1-0.001 ml/ml cell suspension, and only in the presence of complement. At the same time, in the present experiments ASS was inactivated by heating for 30 min at 56°, and no complement was added during treatment of the cells. The intensity of the two factors used diminished as their concentration was reduced. Finally, incubation for 30 min (followed by rinsing) was sufficient to inhibit stem cell function. In addition, both agents are able to induce blast-transformation of lymphoid cells. However, whereas the action of PHA is mainly confined to blast-transformation, with respect to ASS the possibility of a cytotoxic effect in the recipient's body cannot be completely ruled out, as a result of the bringing together of cells which have adsorbed ASS and the host's complement.

Experiments were carried out to test the effect of injecting PHA into donors of the hematopoietic tissue on the stem cells. The model designed by Till and McCulloch was used as the criterion. It is difficult to say what the action of PHA can be, because some workers [6] have found that the number of clone-forming units was slightly reduced, while according to others [7], it was increased.

On the basis of data showing that PHA depresses part of the stem cell genome, Chertkov and Fridenshtein [2] conclude that there are several possible ways of differentiation of the stem cell depending on the inducer. So far as the present experiments are concerned, it could be suggested that following injection of a stem cell treated with PHA into a semisyngeneic donor, having encountered high concentrations of foreign antigen, the stem cell may perhaps "select" the path of lymphoid differentiation. However, the results obtained do not confirm this possibility, for both with semisyngeneic and syngeneic combinations of donor and recipient, the inhibition of stem cell function was virtually identical (Table 2).

The experimental results thus indicate that PHA has a marked action on the stem cells of hematopoietic tissue. Most probably inhibition of the formation of hematopoietic foci is the result of a change in the differentiation potential of the stem cell under the influence of PHA.

Comparative analysis shows that the results of exposure to both factors (ASS and PHA) can be regarded as identical. This suggests that ASS also exerts its effect on stem cells through its property of inducing blast-transformation, although it is impossible at the present time to rule out categorically any other explanation for the mechanism of action of ASS on stem cells.

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