STUDY OF THE COMBINED ACTION OF ANTILYMPHOCYTIC SERUM AND PHYTOHEMAGGLUTININ ON HEMATOPOIETIC TISSUE CELLS

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The effect of antilymphocytic serum (ALS) and phytohemagglutinin (PHA) on hematopoietic tissue cells was studied in vitro. ALS and PHA were used either separately or together. Treatment of the graft both with ALS and with PHA led to changes in the distribution of the cells among the recipient's organs. After treatment with both these agents summation of the redistribution effect took place and the inhibition of colony formation was intensified. It was accordingly postulated that separate cell populations react either to PHA or to ALS.

Treatment of transplantable cells in vitro both with phytohemagglutinin (PHA) and with antilymphocytic serum (ALS) inhibits the formation of hematopoietic foci and changes the character of distribution of the spleen cells in the body of the lethally irradiated mice [1, 2, 4]. Injection of PHA together with other preparations used in immunodepressive therapy and, in particular, with ALS leads to weakening of the reactions of transplantation immunity [3, 6, 12, 16]. Since this problem has not been fully elucidated, further investigations into the simultaneous action of ALS and PHA would evidently give useful results.

This paper describes an attempt to study the combined action in vitro of ALS and PHA on cells of transplanted hematopoietic tissue. Antisplenic serum (ASS) was used as the ALS.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 mice weighing 22-24 g. The recipients of the cells were irradiated in a dose of 830 rad. ASS was obtained by immunizing rabbits with spleen cells from CBA mice (titer of cytotoxins 1:256-1:512, in the leukoagglutination test 1:1024-1:2048). PHA (Wellcome) was used. The technique of labeling the spleen cells with Cr^{51} (Na_2CrO_4) was described previously [2]. The labeled cells were treated in vitro with ASS and PHA separately, simultaneously (ASS + PHA), or successively (ASS → PHA, PHA → ASS) for 30 min at 37°C. After exposure for 30 min and before treatment with the next agent the cells were washed with medium No. 199. In order to study the similar action on stem cells of hematopoietic tissue, the method of cloning hematopoietic cells in lethally irradiated mice in vivo was followed [17].

EXPERIMENTAL RESULTS

Most radioactivity 4 h after intravenous injection of $20 \cdot 10^6$ spleen cells, labeled with Cr^{51}, into irradiated (830 rad) mice had accumulated in the liver (34.6%), spleen (24.8%), lymph glands (18.0%), lungs (10.5%), and bone marrow (9.1%). The splenic index, or the ratio between the percentages of radioactivity in the liver and in the spleen, was 1.4 (Fig. 1, group 1).

Treatment of the spleen cells for 30 min with ASS or PHA in vitro changed the character of distribution of the cells, as shown by an increase in the radioactivity in the liver and its decrease in the spleen

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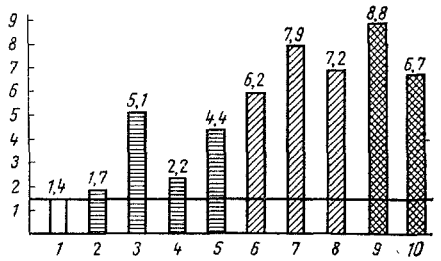

Fig. 1. Effect of ASS and PHA on distribution of transplanted hematopoietic cells (splenic index). Group 1) untreated cells, 46 mice used; 2) treatment with ASS in dilution 10^{-4}, 28 mice used; 3) treatment with ASS in dilution of 10^{-3}, 33 mice used; 4) PHA in dilution of 10^{-2}, 20 mice used; 5) treatment with PHA in dilution 10^{-1}, 35 mice used; 6) treatment with ASS in dilution of 10^{-3} simultaneously with PHA in dilution of 10^{-2}, 14 mice used; 7) treatment with ASS in dilution of 10^{-4} simultaneously with PHA in dilution of 10^{-1}, 14 mice used; 8) treatment with ASS in dilution of 10^{-3} simultaneously with PHA in dilution of 10^{-1}, 14 mice used; 9) treatment with ASS in dilution of 10^{-3} followed by PHA in dilution of 10^{-1}, 14 mice used; 10) treatment with PHA in dilution of 10^{-1} followed by ASS in dilution of 10^{-3}, 14 mice used. Each group contains results of several experiments. Abscissa, group No.; ordinate, splenic index (ratio between percentage of radioactivity in liver and percentage of radioactivity in spleen).

TABLE 1. Formation of Hematopoietic Foci in Spleen of Irradiated Recipients after Transplantation of Spleen Cells Treated in vitro with ASS, PHA, ASS + PHA, PHA → ASS, and ASS → PHA

Series	Type of treatment of transplanted cells	No. of animals tested	No. of foci per spleen (M ± m)
I	ASS (10^{-3})	23	1,8±0,5
	PHA (10^{-1})	10	3,0±0,6
	PHA (10^{-2})	14	7,9±1,0
	ASS (10^{-3})—ФГА (10^{-1})	10	1,5±0,5
	PHA (10^{-1})—ACC (10^{-3})	15	1,5±0,3
	PHA (10^{-2})+ACC (10^{-3})	16	2,6±0,6
	Medium No. 199	21	14,1±0,9
	No treatment	22	0,4±0,3
II	ASS (10^{-4})	15	17,8±0,9
	PHA (10^{-1})	16	1,1±0,2
	ASS (10^{-4})+ФГА (10^{-1})	10	1,3±0,5
	Medium No. 199	10	25,8±0,8
	No treatment	12	0,1±0,09
III	ASS (10^{-3})	6	13,2±1,6
	PHA (10^{-2})	8	20,4±0,9
	ASS (10^{-3})+PHA (10^{-2})	10	7,2±1,5
	Medium No. 199	3	19,3±3,4
	No treatment	7	0,4±0,3

(Fig. 1, groups 2-5). The maximal effect was observed after dilution of the PHA by 10 times, and of ASS by 1000 times. Higher dilutions of ASS (10^{-4}) and PHA (10^{-2}) caused virtually no change in the migration potential of the transplanted cells, as shown by the magnitude of the splenic index.

Incubation of the spleen cells with a mixture of ASS + PHA led to a sharper decrease in the migration of the injected cells into the lymphoid organs than after treatment of the graft with each preparation separately. As a result of this combined action the splenic index rose (7.2; Fig. 1, group 8) and it was very close to the sum of the indices obtained by separate treatment with these two agents (Fig. 1, groups 3 and 5). Even when suboptimal doses of ASS (10^{-4}) or PHA (10^{-2}) were used the combined effect still remained (Fig. 1,

groups 6 and 7). This "summation" of the redistribution effects was also observed when ASS and PHA were added consecutively. The combined effect was particularly well marked when PHA was given immediately after ASS (Fig. 1, group 9).

A parallel investigation was made of the action of ASS and PHA on the ability of the hematopoietic stem cells to induce the formation of hematopoietic foci in lethally irradiated mice (Table 1). Preliminary treatment of the spleen cells in vitro with ASS + PHA, ASS → PHA, and PHA → ASS, like separate treatment with these preparations, caused a decrease in the number of hemtopoietic foci formed. If only one agent was used in high concentration (Table 1, series I and II) the inhibitory action of the second on the hematopoietic stem cells was masked. However, when concentrations of PHA and ASS causing only slight depression of function of the stem cells were used, the combined administration of the agents led to a distinct synergism of their action (Table 1, series III). Nevertheless, the conclusion regarding sensitization of the stem cells by one of the agents relative to the other must be regarded as provisional, for several different combinations of their action must be studied before this problem is finally solved.

It has recently been shown that ASS and PHA lead to blast-transformation of lymphocytes [5, 8], depress the formation of transplantation and humoral immunity [5, 13, 14], and inhibit the function of hematopoietic stem cells [1,2]. Hence it might be supposed that ALS and PHA act on the same cells and, perhaps, on the same cellular structures.

The results of the experiments described above provide some answer to this question. Analysis of the distribution of radioactivity among the organs reveals a number of factors. Both ASS and PHA cause changes in the cell distribution, as reflected by an increase in the splenic index. However, the action of PHA is characterized by a decrease (by almost 10 times) in the migration of the cells into the lymph glands (from 18 to 1.7%) and by an increase in their migration into the lungs (from 10 to 16%). In turn, the ASS had a greater effect on migration of the cells into the bone marrow, for treatment of the transplanted cells with this antiserum caused a decrease in their migration into the bone marrow from 9.1 to 3.5%.

Although these results indicate some similarity between the action of ASS and PHA, at the same time they also point to certain differences. The view is held that the mechanism of action of ALS on lymphocytes is explained by its opsonizing activity. Cells loaded with foreign antibodies are held back chiefly in the liver, where they undergo phagocytosis by Kupffer cells and are eliminated from the body [10, 11]. In turn, PHA induces primarily blast-transformation of lymphocytes, chiefly cells of thymic [7] origin. It is also considered that cells have specific determinants for PHA which differ from those for antigen.

The results described above may be regarded as an indication that there are at least two different populations of lymphocytes and also, perhaps, of hematopoietic stem cells, one of which reacts actively to ASS and the other to PHA. Meanwhile the possibility of an indirect action of a factor of the M1F type on these cells cannot be ruled out, and further study of this problem is required.

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