EFFECT OF LOW-MOLECULAR-WEIGHT LEUKOCYTE DIALYSATE ON ENDOGENOUS COLONY FORMATION IN MICE

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Transfer factor (low-molecular-weight dialysate of leukocyte extract) has attracted the attention of research workers as a substance with a marked stimulating action on the immune system [6]. It is considered to affect the T cell component of immunity [7, 9, 11], but as yet the precise biochemical structure and the concrete mechanisms of its action have not been discovered. The regulatory role of T lymphocytes in differentiation and maturation of hematopoietic stem cells has now been established [2-4]. The study of the effect of transfer factor on the hematopoietic precursors of immunocompetent cells is therefore interesting.

The object of this investigation was to study the effect of low-molecular-weight dialysate obtained from leukocytes of healthy blood donors (LD) on colony-forming stem units (CFU) in the mouse spleen.

EXPERIMENTAL METHOD

LD were obtained from the blood of healthy donors. To 1 volume of blood 3 volumes of 0.87% ammonium chloride solution were added and the mixture was incubated for 20 min at room temperature. After hemolysis the nucleated cells were sedimented by centrifugation (800g, 20 min, 4°C). The residue was washed twice with 0.87% ammonium chloride solution with centrifugation under the same conditions. Lysis of the leukocyte mixture was carried out with sterile distilled water by freezing and thawing three times followed by homogenization in the cold. After centrifugation (20,000g, 30 min, 4°C) the lysate was treated by ultrafiltration through a PM-10 filter (from Amicon) [10]. The LD had a molecular weight of below 10,000 daltons. To obtain 1 ml of LD 4.5×10^8 cells were used. The lyophilized preparation was kept at 4°C.

Adult male (CBA × C57BL/6)F_1 mice were sublethally irradiated with ^{60}Co γ-rays on an EGO-2 apparatus with a dose rate of 200 to 250 R/min. During irradiation the mice were kept in groups of 20-30 in plastic cages with perforated walls. LD in a dose corresponding to the amount of the lysate obtained from 10^7 nucleated blood cells (leukocyte extract) was injected intravenously twice: in the experiments of series I 24 h before and 3 h after irradiation, in the experiments of series II 3 and 24 h after irradiation. Some of the mice in the experiments of both series I and series II received heated (56°C, 60 min) LD, for in experiments to study transfer of cutaneous delayed-type hypersensitivity, the same procedure caused inactivation of transfer factor [8]. All the mice were killed on the 9th day after irradiation, the spleens were removed, and placed in Bouin's solution for 30 min, after which the number of colonies was counted. Spleens for histological analysis were embedded in paraffin wax and a series of sections was cut to a thickness of 5-7 μ. The sections were stained with hematoxylin–eosin and the number of erythroid, granuloid, megakaryocytic, undifferentiated, and mixed colonies was counted. The experimental results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

As Table 1 shows, injection of native LD in the experiments of series I caused a statistically significant increase in the number of colonies of CFU in the spleen ($P < 0.01$) com-

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TABLE 1. Effect of LD on Number and Histological Type of Endogenous Colonies of CFU in Spleen of Sublethally Irradiated Mice

Series of experiments	Group of animals	LD	Mean number of CFU in spleen	Type of hematopoietic CFU			E/G
				erythroid (E)	granuloid (G)	megakaryocytic	
I	1	Native	10,97±1,52 (36)	14,8±3,1 (11)	1,4±0,4 (11)	0,7±0,3 (11)	10,5
	2	Heated	8,42±1,5 (12)	8,5±1,3 (10)	1,0±0,3 (10)	0,4±0,3 (10)	8,5
	3	—	5,08±0,84 (13)	4,7±0,7 (10)	2,3±0,4 (10)	1,0±0,5 (10)	2,0
II	1	Native	10,41±0,64 (49)	9,3±1,0 (8)	0,4±0,2 (8)	0,4±0,4 (8)	23,3
	2	Heated	6,26±0,7 (19)	5,6±0,9 (5)	0,6±0,4 (5)	1,0±0,1 (5)	9,2
	3	—	6,0±0,6 (15)	4,4±0,5 (5)	2,0±0,8 (5)	—	2,2

Legend. Number of animals given in parentheses.

pared with the background, whereas injection of heated LD was not followed by any statistically significant increase in the number of CFU in the spleen of the experimental mice. It is interesting to note that native LD not only increased the number of colonies in the spleen but also caused changes in their cell composition: the number of colonies of erythroid type was increased ($P < 0.01$). It is remarkable that heated LD also increased the number of erythroid colonies compared with the background, and although the increase was smaller, it was statistically significant ($P < 0.05$). To rule out any possible protective action of LD on the hematopoietic tissue of the mice during irradiation, in the experiments of series II LD was injected only after irradiation. The results of the experiments of series II were identical, and native LD caused a greater increase in the number of colonies of erythroid type. It can be tentatively suggested that LD increases the rate of migration of hematopoietic stem cells from the bone marrow into the spleen and affects differentiation of stem cells indirectly through T lymphocytes. T lymphocytes activated by antigen have been shown to change the direction of differentiation of syngeneic stem cells toward granulopoiesis [4]. In the present experiments the increase in the number of erythroid colonies in the spleen after administration of LD could also have been due to interaction between a particular subpopulation of T lymphocytes and stem cells. The possibility cannot be ruled out that the stimulating effect of LD on T-cell immunity is associated with blocking of glucocorticoid synthesis in the adrenal cortex by the low-molecular-weight substance and the development of endogenous hypocorticism, during which the manifestations of T-cell immunity are intensified and the direction of differentiation of stem cells is changed toward erythropoiesis [1, 5].

LITERATURE CITED

1. G. I. Bezin, B. B. Moroz, R. V. Petrov, et al., Probl. Gematol., No. 12, 21 (1977).
2. Yu. I. Zimin and M. I. Fedotova, in: Current Problems in Radiation Medicine and Radiobiology [in Russian], Moscow (1975), pp. 40-41.
3. V. A. Kozlov and I. G. Tsyrlova, Byull. Eksp. Biol. Med., No. 9, 1102 (1976).
4. R. V. Petrov, R. M. Khaitov, V. M. Man'ko, et al., Control and Regulation of the Immune Response [in Russian], Leningrad (1981).
5. V. F. Semenov, in: Early Manifestations of Tissue Incompatibility [in Russian], Moscow (1976), p. 81.
6. P. H. Klesius, in: Immune Regulators in Transfer Factor, New York (1979), pp. 43-54.
7. B. H. Littman and I. R. David, in: Transfer Factor: Basic Properties and Clinical Applications, New York (1976), pp. 35-39.
8. M. R. Salaman, I. L. Sargent, and V. S. Slijivik, in: Transfer Factor: Basic Properties and Clinical Applications, New York (1976), pp. 661-662.
9. I. L. Sargent, M. R. Salaman, and V. Valdimarssion, in: Immune Regulators in Transfer Factor, New York (1979), pp. 129-136.
10. G. E. Thomas, J. M. Rodrigues, and C. L. Cooke, in: Immune Regulators in Transfer Factor, New York (1979), pp. 517-534.
11. F. Waxman, N. Condoulis, and P. Baram, in: Immune Regulators in Transfer Factor, New York (1979), p. 75-83.