EFFECT OF POLY-A : U, DEXTRAN SULFATE, AND YEAST RNA ON THE COLONY-FORMING ABILITY OF BONE MARROW IN IRRADIATED MICE

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Poly-A : U, dextran sulfate, and yeast RNA were shown to increase the number of endogenous colonies (CFU) in the spleen of sublethally irradiated (525 R) mice, evidently as a result of their mitogenic action on proliferating CFU. The substances had no effect on the number of exogenous colonies when injected at the same time as transplantation of syngeneic bone marrow cells from intact donors. Dextran sulfate increased the number of endogenous colonies by 2.7 times in the spleens of unevenly irradiated mice mainly through migration of CFU from protected areas of bone marrow. The poly-A : U complex and yeast RNA were ineffective under these experimental conditions. The possibility cannot be ruled out that one of the essential factors in the mechanism of the adjuvant activity of dextran sulfate is its ability to increase the powers of migration of hematopoietic stem cells.

KEY WORDS: polyanions; migration; stem cells.

Analysis of recently published experimental data directly or indirectly concerned with problems of immunostimulation reveals increasing interest in adjuvants of polyanion nature. This interest is based on results showing the undoubted stimulating effect of natural and artificial RNA-polynucleotides and other polyanions on immunogenesis [3, 6, 9, 10]. This in no way rules out the possibility [2, 7, 8] that the target cells for these substances may be different types of immunocompetent cells. Meanwhile the mechanism of the adjuvant action of substances of polyanion nature may incorporate their direct effect not only on immunocytes, but also on the processes of migration and circulation of stem cells forming the pool of immunocompetent cells.

The object of this investigation was to study the effect of some polyanions on the colony-forming ability of the bone marrow in sublethally, lethally, and unevenly irradiated (CBA × C47BL) F_1 mice.

EXPERIMENTAL METHOD

The following substances were used: 1) sodium salt of dextran sulfate, with a molecular weight of 500,000 (from Ferak, Berlin). The product was dissolved in physiological saline immediately before injection, in a dose of 500 μg per mouse; 2) the potassium salt of polyadenylic (poly-A) and the sodium salt of polyuridylic acid (poly-U) (from the Special Design and Technological Bureau for Biologically Active Substances, Novosibirsk). To form the poly-A : U complex, solutions of the acids were mixed in equal volumes and kept in a refrigerator (4°C) for 30 min. Dose 200 μg per mouse; 3) a commercial preparation of yeast RNA (mol. wt. 550,000), dose 500 μg per mouse.

(CBA × C57BL) F_1 mice weighing 20-22 g were irradiated on the RUM-3 apparatus (tube voltage 160 kV, current 20 mA, dose rate 75 rd/min, focal length 50 cm). All the preparations were injected intraperitoneally 2 h after irradiation. In the experiments of series I the mice were irradiated in a dose of 525 rd. The number of endogenous colonies was counted on the 9th day after irradiation. In the experiments of series II the effect of the substances on migration of CSU from bone marrow was studied by the method of Petrov and Khaitov [4]. Mice were irradiated in a dose of 850 rd with one leg screened (6 mm Pb + 1 mm Al). On the 9th day after ir-

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TABLE 1. Effect of Polyanions on Endogenous Colony Formation in Spleen of Sublethally Irradiated Mice

Test substance	Number of animals	Number of colonies per spleen (M ± m)	P
Control	38	15,4±1,8	—
Poly-A : U	30	24,2±1,7	<0,01
Dextran sulfate	38	24,3±1,6	<0,01
RNA	38	27,5±2,3	<0,01

TABLE 2. Effect of Polyanions on Migration of CFU from Bone Marrow after Uneven Irradiation

Test substance	Number of animals	Number of colonies per spleen (M ± m)	P
Control	36	18,4±3,1	—
Poly-A : U	38	15,8±2,6	>0,05
Dextran sulfate	36	50,6±4,8	<0,001
RNA	27	13,5±1,6	>0,05

radiation the number of macroscopic colonies on the surface of the spleen was counted. In the experiments of series III the mice were irradiated in a dose of 850 rd, and 2 h later syngeneic bone marrow cells were transplanted intravenously into them (1×10^5 cells per mouse) from intact donors. The test substances were injected intraperitoneally at the same time. The number of exogenous colonies was counted on the 9th day after irradiation. The results were subjected to statistical analysis [1] by calculation of the arithmetic mean (M) and standard error of the mean (m).

EXPERIMENTAL RESULTS

The results of the experiments of series I are given in Table 1. They show that injection of all the test substances 2 h after irradiation led to a significant increase in the number of endogenous colonies in the spleen of the animals of the experimental groups compared with the control. These results indicate that under the experimental conditions used an increase in endocolonization took place under the influence of the test substances by similar mechanisms, namely through their mitogenic action on the proliferating pool of stem cells and (or) intensification of the powers of migration of the CFU.

To test this last hypothesis, and bearing in mind results [3] indicating that injection of preparations active against CFU migration immediately after irradiation should be reflected in the number of colonies in the spleen, the experiments of series II were carried out (Table 2). The results showed that an increase in the number of endogenous colonies on the 9th day after irradiation was observed only in the groups of animals receiving dextran sulfate (an increase of 2.7 times). The substances poly-A : U and RNA were ineffective under these experimental conditions. None of the substances tested had any effect on the number of exogenous colonies in the spleen of the lethally irradiated mice when injected 2 h after irradiation, simultaneously with bone marrow cells (21.1 ± 4.3 in the control, from 25.5 ± 2.5 to 30.0 ± 2.1 in the experimental groups).

The following conclusions can be drawn from these results. An increase in the number of endogenous colonies after sublethal irradiation (525 rd) obtained under the influence of polyanions took place as a result of their mitogenic action on the proliferating CFU, though this does not rule out some effect of these substances on the stroma of the spleen also. The fact that the substances had no effect on the number of exogenous colonies can evidently be explained by the action of the large dose of irradiation (850 rd) on the microenvironment of the exogenous stem cells.

Dextran sulfate stimulated migration of the hematopoietic stem cells from protective areas of bone marrow mainly through the intensification of CFU migration; however, the possibility cannot be ruled out that the pool of stem cells in the protected area was increased, for we know [5] that stem cells leave the screened bone marrow for the circulation as a result both of migration and of proliferation. One of the essential factors in the mechanism of the adjuvant action of dextran sulfate is evidently its ability to intensify migration and proliferation of stem cells.

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