EFFECT OF POLYINOSINIC-POLYCYTIDYLIC ACID ON THE NUMBER OF COLONY-FORMING CELLS IN THE HEMATOPOIETIC ORGANS OF GUINEA PIGS

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An increase in the number of cells forming discrete colonies in monolayer cultures of myelokaryocytes (colony-forming cells) was observed in guinea pigs 8-24 h after receiving an intraperitoneal injection of polyinosinic-polycytidylic acid in a dose of 300 μg/kg. The number of colony-forming cells in the spleen was reduced at these same times after injection of the compound. After 3 days the number of colony-forming cells in the spleen increased, but in the bone marrow it decreased. These values returned to normal 14 days after injection of the compound. The changes observed are evidently explainable on the grounds that the compound has a marked effect on the fraction of colony-forming cells in hematopoietic organs that are stromal elements of the stem-cell type.

KEY WORDS: polyinosinic-polycytidylic acid; colony-forming cells; diameter of colonies.

A close connection has now been demonstrated between cells forming colonies in monolayer cultures of hematopoietic tissues that are essentially stromal elements of the stem-cell type, and the process of hematopoiesis both in experimental animals exposed to various factors such as irradiation or administration of vinblastin [1, 5-7, 10, 11], and clinically in patients with lymphogranulomatosis [2, 8].

It therefore seemed appropriate to study the reaction of colony-forming stromal cells to injection of polyinosinic-polycytidylic acid (polyI-polyC), a substance increasing production of colony-stimulating factor [14, 15] with a significant effect on the population of hematopoietic stem cells in mice, which could possibly explain the radioprotective effect of this substance.

EXPERIMENTAL METHOD

Experiments were carried out on 25 guinea pigs of both sexes weighing 300-350 g. The polyI-polyC (Calbiochem, USA) was prepared by mixing solutions containing 500 μg/ml polyI and polyC in 0.14 M NaCl and 0.014 M sodium citrate, and then incubating the mixture of polynucleotides at 56°C for 2 h. The polyI-polyC was injected intraperitoneally in a dose of 300 μg in 0.2 ml physiological saline. The animals were killed after 8 h and 1, 3, and 14 days, the spleen and femur were removed, and a sterile suspension of splenokaryocytes and myelokaryocytes was prepared. The suspensions were explanted into 250-ml Povitskaya flasks, at the rate of $10 \cdot 10^6$-$15 \cdot 10^6$ cells to each flask, and grown in medium No. 199 with the addition of bovine serum at 37°C for 12 days, after which the cultures were fixed with absolute alcohol, stained with an aqueous solution of azure-eosin by Romanovsky's method, and the colonies in each flask were counted with the MBS-1 microscope. In parallel experiments with explanation of the cells, the number of karyocytes was counted in the spleen and the tibial marrow of the animals. Altogether two series of experiments were carried out, in which 95 cultures were studied; the longitudinal and transverse diameters of 2900 colonies were measured in order to analyze the distribution of the colonies by size.

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TABLE 1. Efficiency of Colony-Formation by Hematopoietic Tissue Cells of Guinea Pigs at Various Times after Injection of PolyI-PolyC (M ± m)

Hematopoietic organ	Series of expts.	Times of investigation				
		control	8 h	1 day	3 days	14 days
Bone marrow	I	8,8±0,4	36±6,0*	40±1,3*	0,7±0,1*	12±0,8*
	II	8,8±0,8	—	11,8±0,7*	5,5±0,8*	—
Spleen	I	32±6,8	21±3,6	16±3,3*	37±0,9	32±4,6
	II	8,9±0,7	—	3,8±0,6*	7,6±0,6	—

* Difference from control significant ($P < 0.05$).

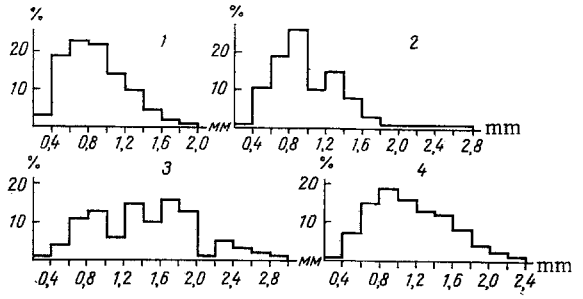

Fig. 1. Distribution of colonies by diameter in cultures of spleen cells explanted at different times after injection of polyI-polyC. 1) Intact animals: mean diameter (MD) of colonies 0.94 mm, mean area (MA) 0.69 mm^2; 2) 1 day after injection of preparation: MD 1.14 mm, MA 1.02 mm^2; 3) 3 days after injection of preparation: MD 1.52 mm, MA 1.82 mm^2; 4) 14 days after injection of preparation: MD 1.28 mm, MA 1.29 mm^2.

EXPERIMENTAL RESULTS

The results of the study of the number of colony-forming cells in the hematopoietic organs of the guinea pigs at different times after injection of polyI-polyC are given in Table 1. For instance, 8 h after injection the number of colony-forming cells in the bone marrow was increased fourfold, and it remained high after 24 h (4.5 times higher than in the control). The number of these cells then fell, and after 3 days it was several times smaller than initially. Later the number returned to normal, although 14 days after injection of the compound the number of colony-forming cells was significantly higher than in the intact animals. The changes in this index in the spleen were opposite in character, for in the experiments of series I the number of colony-forming cells was lowest 8 and 24 h after injection of the compound (65 and 50% respectively compared with the control) and highest after 3 days (117%). The direction of the changes was the same in the experiments of series II; the smaller scale of the quantitative changes was evidently due to the quality of the preparation or to individual differences between the guinea pigs.

It is important to note that the changes in the number of spleen and bone marrow cells were small and that the calculated absolute number of colony-forming cells in the spleen and tibial marrow did not differ significantly from the relative number of these cells as determined experimentally.

PolyI-polyC caused a significant increase in size of the colonies (Fig. 1) formed in cultures of splenokaryocytes, and this increase was particularly marked 3 days after injection of the compound (when the mean area of the colonies was 3 times greater than in cultures from intact animals). Meanwhile deformation of the histograms was observed (some flattening with the appearance of colonies of large size), which was observable 1 and, in particular, 3 days after administration of the compound. After 14 days the histogram differed very little from the control. Probit transformation of the data on the distribution of the colonies by diameter showed that, despite substantial shifts of this index after injection of the compound, the distribution of the colonies remained normal at all times of the investigation.

Administration of polyI-polyC thus caused substantial shifts in the population of stromal cells of the hematopoietic organs of the guinea pigs. Work in recent years has shown that it is the survival of the stromal cells, creating a "microenvironment," that determines the success of heterotopic transplantation of bone marrow, thymus, lymph gland, and spleen cells [3, 4, 9]; stromal cells participate in the restoration of hematopoiesis after irradiation in doses causing aplasia of the hematopoietic tissues [13].

The stimulation of proliferation of the stromal hematopoietic cells by polyI-polyC must be considered in conjunction with the earlier evidence obtained on the stimulant effect of the compound on the fraction of hematopoietic stem cells. Considering the close connection between colony-forming cells and hematopoiesis, expressed as the wave-like change in the number of cells in the hematopoietic organs in response to the action of stimuli acting on the blood system [1, 5, 7], it can be postulated that a change in the proliferative activity of the stromal cells (the microenvironment) possibly induces increased proliferation of the stem fraction of the bone marrow cells. Under these circumstances, as analysis of integral curves of the distribution of the colonies by size shows, despite the increased proliferative activity, the population of colony-forming cells continues to remain homogeneous, indicating its definite stability.

Although the mechanism of action of polyI-polyC on the organism is not known, clearly different polyanions are capable of inducing lymphocytosis in the period immediately after administration by mobilizing lymphocytes from the spleen and lymph glands, [12, 18]. Stromal stem cells may possibly migrate from the spleen in a similar way and accumulate in the bone marrow; this could explain the decrease in their number in the spleen and its increase in the bone marrow during the first few hours after injection of polyI-polyC. A factor stimulating colony growth [14, 15] and also interferon [16], capable of inhibiting cell growth [17], have been found to appear in the serum of mice receiving injections of polyI-polyC.

It can therefore be postulated that changes observed in the number of stromal stem cells in the spleen in the later periods constitute a response to the decrease in the number of splenokaryocytes caused by injection of the polyanion and by the action of interferon induced by it.

The direct cause of the observed increase in size of the colonies and in the number of stromal stem cells may be the colony-stimulating factor, the concentration of which in the serum reaches a maximum on the 2nd day after injection of polyI-polyC [14].

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