ACTION OF A POLYSACCHARIDE FROM Salmonella typhi ON HEMATOPOIETIC STEM CELLS

M. S. Gordeeva, A. V. Izvekova, and M. A. Tumanyan

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The dynamics of the number of colony-forming units (CFUs) in the spleen and bone marrow of unirradiated F_1(CBA × C57BL) mice was studied after intraperitoneal injection of polysaccharide (PC) from Salmonella typhi. The method of exogenous colony formation was used. After a single injection of PC the number of CFUs in the bone marrow was increased by 2-2.5 times and in the spleen by 3 times. Repeated (6-9 times) injections of PC were no more effective than a single injection. PC evidently acts as an inducer which, by inducing proliferation of hematopoietic stem cells, maintains this process automatically for a certain period of time.

Key words: stem cells; polysaccharide from Salmonella typhi; foci of Till and McCulloch.

Bacterial preparations, drugs, heterologous and homologous cells, blood loss, irradiation, and other factors are known to give rise to an increase in the number of endogenous colonies in irradiated mice [2, 5, 8]. It has been shown by the method of exogenous colony formation that typhoid endotoxin accelerates proliferation of colony-forming (stem) cells in unirradiated mice [6]. The problem of whether the injected antigen acts directly on the stem cells or indirectly, by promoting the liberation of a stimulating factor, was studied in vitro by McNeil [7]. Few investigations into the mechanisms of action of the various factors of stem cells have been published. Meanwhile much information would be particularly important for the elucidation of the dynamics and mechanisms of regulation of proliferation of hematopoietic stem cells.

TABLE 1. Number of CFUs in Bone Marrow and Spleen of F_1(CBA × C57BL) Mice after Single and Repeated Injections of PC (M±m)

Time after injection of PC into donors (in days)	Number of injections of PC into donors	Bone marrow		Spleen	
		Number of recipient animals	Number of CFUs per 10^5 bone marrow cells	Number of recipient animals	Number of CFUs per 10^6 spleen cells
1	1	36	16,6± 1,8	30	24,9± 1,6
7	1	30	18,0± 1,2	38	42,7± 2,7
10	1	32	22,3± 2,4	36	43,2± 3,2
18	1	26	12,3± 1,4	28	30,8± 2,6
30	1	23	7,2± 0,9	25	18,8± 1,9
Control (intact) donors	0	56	8,2± 1,2	49	13,2± 1,4
1	6	28	22± 1,8	27	56± 3,1
1	9	24	17,3± 1,6	30	44± 2,6
Control (intact) donors	0	36	9,2± 1,0	42	14,4± 0,8

Legend. Number of endogenous colonies in each experiment not more than 0.5%.

Laboratory of Radiation Immunology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 3, pp. 66-68, March, 1975. Original article submitted December 26, 1973.

On the basis of the observations [2] that injection of a polysaccharide (PC) from Salmonella typhi before irradiation increased the number of endogenous hematopoietic colonies and increased the chances of survival of the irradiated mice, in the present investigation the dynamics of the population of colony-forming units (CFUs) was studied in the bone marrow and spleen of unirradiated mice after a single and repeated injections of the PC, using the method of exogenous colony formation [9].

EXPERIMENTAL METHOD

F_1(CBA×C57BL) mice aged 2.5-4.5 months were used. In the experiments with a single injection the intact donor animals received 50 μg PC from S. typhi, isolated from the S. Ty_2 somatic antigen [1], intraperitoneally; in the experiments with repeated (6 or 9 times) injections, 25 μg PC was injected daily (50 μg at the first injection). The animals were killed 1, 7, 10, 18, and 30 days after the single injection and 1 day after the end of the courses of 6 and 9 injections of PC and a suspension of cells from the bone marrow and spleen was prepared in medium No. 199. The cells were injected intravenously into syngeneic recipients 1-2 h after irradiation with γ rays in a dose of 850 rad. The sex of the animals was taken into account during transplantation.

In each experiment 4 groups of recipients were used: the mice of group 1 each received 10^5 bone marrow cells from donors killed at various intervals (shown above) after the injection of PC; group 2 (control) each received 10^5 bone marrow cells from intact donors; group 3 each received 10^6 spleen cells from the same donors as were used for group 1; group 4 (control) each received 10^6 spleen cells from intact donors.

The recipients were killed 9 days after irradiation and injection of the cells, their spleens were placed for 1-2 h in Bouin's mixture, after which the colonies were counted. In each experiment the number of endogenous colonies averaged not more than 0.5 per spleen. In some cases the spleens were subjected to histological analysis after fixation by Zenker's method and sections were stained with azure-eosin. The experiments were repeated twice. From 23 to 50 animals (recipients) were used in each group. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The following dynamics of the number of CFUs in the bone marrow and spleen of the unirradiated donors was revealed after a single injection of PC. The number of CFUs in the bone marrow was doubled 24 h after the injection of PC (Table 1); by the 7th-10th day it reached its maximum, after which it fell (18th day) to regain the control level by the 30th day. The number of CFUs in the spleen reached a maximum on the 7th-10th day, when it was more than 3 times the level of CFUs in the spleen of the control animals, and it returned to normal more slowly than in the bone marrow.

After repeated injections of PC the number of CFUs changed in a similar manner. For instance, after 6 and 9 injections of PC the number of CFUs in the bone marrow was twice or three times greater than the number in the bone marrow of the control animals (Table 1). The number of CFUs in the spleen after repeated injections of PC was 3 to 4 times higher than normal.

The histological analysis showed that erythroid and mixed (myeloid-erythroid) colonies were the predominant types after single and repeated injections of PC.

A single injection of PC in nontoxic doses for mice thus led to a rapid increase in the number of hematopoietic stem cells in the bone marrow and spleen. Analysis of data in the literature [3, 4, 6] and of the writers' own results shows that different factors, in certain doses, stimulate the proliferation of hematopoietic stem cells. The increase in the number of stem cells was largely proportional to the strength of the stimulus used, for when different doses of the same agents were used definite correlation was found between the times of appearance of the colonies and the intensity of their formation.

In the present experiments repeated injections of PC were not more effective than a single injection, i.e., repeated injections led to hardly any increase in the number of hematopoietic stem cells. For instance, investigation of the bone marrow and spleen 24 h after the end of courses of 6 and 9 injections, i.e., on the 7th and 10th days after the 1st injection of PC, showed considerable similarity with the distribution of CFUs at the same periods after a single injection of PC. The results indicate that, under the experimental conditions used, PC acted as an inducer which, by stimulating proliferation and differentiation of the stem cells, enabled these processes over a certain period of time to maintain themselves automatically, for further injections of PC had virtually no effect on the numbers of stem cells in the bone marrow and spleen. Evidently this stimulation is affected by PC not through its direct action on the stem cells but through the activa-

tion of a mechanism controlling the cell system and maintaining it in equilibrium.

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