LITERATURE CITED

1. V. M. Barabanov, Dokl. Akad. Nauk SSSR, 234, 195 (1977).
2. V. M. Barabanov and D. B. Nikolova-Kitova, Byull. Éksp. Biol. Med., No. 8, 209 (1978).
3. M. S. Mitskevich, Glands of Internal Secretion in the Embryonic Development of Birds and Mammals [in Russian], Moscow (1957).
4. N. V. Éngel'gardt, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 165.
5. R. Ferrand, H. G. E. Pearse, J. Polak, et al., Histochemstry, 38, 133 (1974).
6. P. Leleux and C. Robin, in: In Vitro Methods in Reproductive Cell Biology, Edited by E. Diczfalusy, Stockholm (1971), p. 168.
7. S. J. Mikami, T. Hashikawa, and D. S. Farner, Z. Zellforsch., 138, 299 (1973).
8. H. Rahn, J. Morph., 64, 483 (1939).
9. G. Sainte-Marie, J. Histochem. Cytochem., 10, 250 (1962).
10. A. Tixier-Vidal, Arch. Anat. Micr. Morph. Exp., 43, 163 (1954).
11. K. G. Wingstrand, The Structure and Development of Avian Pituitary, Lund (1951).
12. J. Zwaan, "Immunological analysis of the eye lens during development," Thesis, Amsterdam (1963).

# EFFECT OF PRODUCTS OF DESTRUCTION OF TISSUE MACROPHAGES ON HEMATOPOIETIC STEM CELLS

L. I. Privalova, B. G. Yushkov, and B. A. Katsnel'son

UDC 612.419.015.349

Products of destruction of mouse peritoneal macrophages (MDP), obtained aseptically by freezing and thawing the cells three times, when injected intraperitoneally into syngeneic mice cause an increase in the number of splenic colony-forming units ($CFU_S$) in the hematopoietic tissue of the bone marrow and spleen, revealed by Till and McCulloch's method. This increase is a true increase, for it was shown that the fraction of transplanted stem cells adsorbed by recipient's spleen in donor mice receiving MDP was relatively smaller than in the control. Besides an increase in the total number of splenic colonies a decrease was found in the number of erythroid colonies relative to the number of colonies of granulocytic and monocytic type. One possible mechanism of the effect of MDP on the number of $CFU_S$ may be modification of the hematopoiesis-inducing microenvironment, as is indicated by the increase in the number of colonies in mice into which normal hematopoietic tissue was transplanted after preliminary repeated injection of MDP. Other possible mechanisms of the observed effects also are examined, allowing for the fact that no direct effect of MDP on the stem cell could be found in experiments with preincubation of the bone marrow tissue with MDP before its injection into lethally irradiated mice.

KEY WORDS: products of destruction of macrophages; hematopoietic stem cells.

The cellular phase of self-cleansing of the lungs from inhaled particles is a self-regulated process, controlled by the quantity of macrophage destruction products (MDP) formed [4, 5]. Besides causing local activation and attracting phagocytic cells and mobilizing them from the depots, MDP also promote granulocytopoiesis and monocytopoiesis [3], functions which can be regarded as a manifestation of the general principles of regulation of hematopoiesis, in which an essential role is ascribed to destruction products of blood cells [2, 7]. However, little is known of the action of such products on the initial stages of hematopoiesis. Accordingly, special attention must be paid to the study of the effect of MDP on hematopoietic stem cells.

---

Department of Work Hygiene, Institute of Work Hygiene and Occupational Diseases, and Department of Pathological Physiology, Sverdlovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 345-347, April, 1979. Original article submitted April 20, 1978.

 0007-4888/79/8704-0352$07.50 

TABLE 1. Effect of Macrophage Destruction Products of Number of $CFU_S$ in Bone Marrow and Spleen of CBA Mice (M ± m)

| Index | Control | Experiment | P |
|---|---|---|---|
| No. of $CFU_S$ in femoral bone marrow | 895,4±281,2 | 2827,4±616,4 | <0,01 |
| E/G | 4,6±1,4 | 1,4±0,3 | <0,05 |
| No. of $CFU_S$ in spleen | 2520,0±362,9 | 7585,5±1221,0 | <0,001 |
| E/G | 3,4±1,1 | 1,8±0,3 | >0,05 |
| "Distribution factor" | 0,61±0,13 | 0,15±0,004 | <0,001 |
| No. of colonies in spleen of recipients previously receiving physiological saline or MDP, after injection of $10^5$ bone marrow cells of intact donors | 7,3±1,0 | 29,9±0,6 | <0,001 |
| E/G | 2,90±0,52 | 0,59±0,05 | <0,001 |
| No. of colonies in recipients' spleen after injection of $10^5$ bone marrow cells of intact donors, preincubated with MDP | 7,0±1,0 | 8,3±1,2 | >0,05 |

Note. E/G denotes ratio between erythroid and granulocytic colonies.

## EXPERIMENTAL METHOD

Experiments were carried out on CBA and BALB/c mice. MDP were obtained by freezing and thawing peritoneal macrophages three times in physiological saline [3]. The number of splenic colony-forming units ($CFU_S$) in the bone marrow and spleen was determined by the method of Till and McCulloch [11], for which purpose a suspension of bone-marrow ($10^5$ karyocytes) and spleen ($10^6$ karyocytes) cells from mice which had received three intraperitoneal injections of physiological saline (control) or of MDP in a dose equivalent to $3 \times 10^7$ destroyed cells was injected in medium No. 199 into lethally irradiated (1000 rad) syngeneic animals. The spleen was removed from the recipient mice 8 days later, fixed in Bouin's fluid, and the number of macroscopically visible colonies was counted. The cell types of the colonies were determined histologically.

To assess the affinity of $CFU_S$ for the spleen, the "distribution factor" (f-fraction) was determined by the method described previously [10]. To study the direct effect of MDP on CFUs, a suspension of mouse bone marrow cells, before transplantation into irradiated recipients, was incubated in medium No. 199 with MDP in a concentration equivalent to $3 \times 10^7$ cells/ml at 37°C for 45 min. In a special series of experiments, mice receiving the same dose of MDP by intraperitoneal injection 72 and 48 h before irradiation were used as recipients of normal bone marrow.

## EXPERIMENTAL RESULTS

Injection of syngeneic MDP into CBA mice led to an increase in the number of $CFU_S$ both in the bone marrow and in the spleen (Table 1). This reaction of the $CFU_S$ to MDP cannot be explained by the particular features of this line of mice, for the number of $CFU_S$ in the bone marrow of BALB/c mice receiving MDP increased to 52.0 ± 3.1 per $10^5$ karyocytes compared with 30.4 ± 8.5 in the control ($P < 0.05$). As Table 1 shows, under the influence of MDP a relative increase in the ability of the $CFU_S$ to differentiate in the erythroid direction also was observed and was accompanied by increased differentiation into cells of the granulocytic—monocytic series (a decrease in the E/G ratio). However, the absolute number of colonies of both types in the spleen, and also of mixed (mainly granulocytic) colonies increased under the influence of MDP, whereas the number of megakaryocytic colonies was the same as in the control.

By the method of Till and McCulloch it is not possible to determine all $CFU_S$ in transplanted hematopoietic tissue, but only those which settle in the recipient's spleen. The results described above might therefore reflect only an increase in this "f-fraction" of cells, while the total number of $CFU_S$ injected into the recipients was unchanged. However, as Table 1 shows, the relative content of this fraction ("distributiom factor") not only did not increase in response to the action of MDP on the donor, but decreased substantially. Consequently, the increase in the number of colonies formed in the recipient's spleen was connected with a true increase in the number of $CFU_S$ in the injected donor's hematopoietic tissue.

Meanwhile, on combined incubation of MDP and bone marrow cells before their transplantation into irradiated recipient mice the number of colonies developing in the spleen did not change, evidence of absence of any direct effect of MDP on the polypotent stem cell. It is interesting to note that MDP evidently has no direct effect likewise on the committed stem cells. For instance, Bolde (University of California, Los Angeles) showed in experiments with agar sandwich cultures of bone marrow that addition of MDP (which he obtained by the method described above) to the feeder layer of these cultures caused no change in the number of colonies formed (personal communication).

After injection of intact bone marrow into recipients previously receiving MDP by intraperitoneal injection, a sharp increase was observed in the number of colonies formed in the spleen, with a considerable decrease in the E/G ratio (Table 1). This increase was not connected with preservation of their own stem cells in recipients receiving the MDP before irradiation, for, just as in all the previous experiments, the number of endogenous colonies in the irradiated mice not receiving donors' bone marrow on average did not exceed 0.2.

A possible explanation of the true increase in the number of $CFU_S$ in the hematopoietic tissue under the influence of products with no direct stimulating action on stem cells could be an increase in the proliferative activity of the stem cells as a result of a change in the hematopoiesis-inducing microenvironment. The results of the last of the experiments described above indicate that MDP can change this microenvironment so that the stroma of the hematopoietic organ can accomodate a larger number of polypotent stem cells or lead to their more rapid proliferation up to the formation of distinguishable colonies. If, however, MDP was injected into animals that received no $CFU_S$ from outside (i.e., into donor mice), the analogous change in their hematopoiesis-inducing microenvironment ought evidently to lead to an analogous increase in the proliferative activity of the endogenous $CFU_S$.

Another stimulus to proliferation of $CFU_S$ in these mice may be the action of the well-known mechanisms of short-range regulation in response to the increase in the liberation of granulocytes and monocytes into the blood stream, with their more rapid maturation in the bone marrow demonstrated previously [3] and which, in turn, may lead to the more rapid migration of cells from it [9]. At the time chosen for taking hematopoietic tissue from these animals for transplantation, it may be that an overshoot has developed, and this was reflected in the increased number of $CFU_S$.

It must also be noted that under the influence of MDP the ratio changed between colonies of different types found in the recipients' spleen. Although changes of this sort have been observed by other workers under certain conditions [1, 6], in the case now being examined this change was qualitatively adequate to enable the animal to increase its production of granulocytes and monocytes, and it is therefore particularly interesting. It was shown previously that under the influence of erythrocyte destruction products the number of $CFU_S$ increases without any corresponding shift in the E/G ratio, or even with a tendency for it to change in the opposite direction [8]. Special investigations are required to elucidate the mechanisms of this shift, which is evidently self-regulatory in character.

## LITERATURE CITED


1. M. S. Gordeeva, A. V. Izvekova, and M. A. Tumanyan, Byull. Éksp. Biol. Med., No. 3, 66 (1975).
2. V. Menkin, The Dynamics of Inflammation [in Russian], Moscow (1948), pp. 102-107.
3. L. I. Privalova, A. V. Osipenko, and V. N. Frash, Byull. Éksp. Biol. Med., No. 12, 1480 (1976).
4. L. I. Privalova, B. A. Katsnel'son, and A. V. Osipenko, Byull. Éksp. Biol. Med., No. 3, 342 (1977).
5. L. I. Privalova and B. A. Katsnel'son, in: Occupational Diseases of Dust Etiology [in Russian], No. 4, Moscow (1977), pp. 85-95.
6. T. A. Prigozhina et al., Patol. Fiziol., No. 5, 66 (1976).
7. Ya. G. Uzhanskii, Physiological Mechanisms of Regeneration of the Blood [in Russian], Moscow (1968).
8. Ya. G. Uzhanskii et al., Byull. Éksp. Biol. Med., No. 8, 143 (1977).
9. G. Meuret, J. Bammert, and U. Gessner, Blut, 33, 389 (1976).
10. G. Siminovitch, E. A. McCulloch, and J. E. Till, J. Cell. Physiol., 62, 327 (1963).
11. J. E. Till and E. A. McCulloch, Radiat. Res., 14, 213 (1961).