ROLE OF BREAKDOWN PRODUCTS OF TISSUE MACROPHAGES IN THE REGULATION OF GRANULOCYTOPOIESIS

L. I. Privalova, A. V. Osipenko, and V. N. Frash

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After intraperitoneal injection of breakdown products of rat peritoneal macrophages (BPM) into recipient rats leukocytosis was observed in the peripheral blood with an increase in mature forms of neutrophils and monocytes in the bone marrow and with an accompanying improvement in the oxygen supply of the bone marrow cells (as shown polarographically). Similar changes in the bone marrow were obtained after intraperitoneal injection of cytotoxic quartz dust particles. If mouse BPM were injected intraperitoneally into mice, the formation of granulocytic colonies in the spleen was sharply stimulated in syngenetic mice receiving a suspension of bone marrow or spleen cells taken from these donors by intravenous injection after lethal x-ray irradiation. The results are discussed in the light of the possible role of broken-down tissue macrophages in the formation of colony-stimulating factor and in the autoregulation of phagocytic reactions.

KEY WORDS: macrophages; neutrophils; monocytes; colony-forming units; regulation of hematopoiesis.

The role of breakdown products of erythrocytes and neutrophils in the regulation of systems of erythropoiesis and leukopoiesis is well known [2, 5]. On the other hand, it has been shown that the blood monocytes and the macrophages produce a factor which promotes the formation of granulocytic colonies by bone marrow cells in vitro [8] and, it is supposed, is identical with colony-stimulating factor (CSF). CSF has been isolated from various tissues and biological products, and its ability to induce leukocytosis has been demonstrated by experiments in vivo [10]. According to one hypothesis, CSF of macrophagal origin is a mediator in the physiological mechanism of the positive feedback which plays an important role in the autoregulation of the formation of granulocytes and monocytes from their common precursor in the bone marrow [11]. However, these experiments in vitro did not show whether the formation or liberation of this macrophagal CSF is connected with destruction of the macrophages or with activity of the living cells. Investigations to confirm the stimulating activity of macrophagal breakdown products on leukopoiesis in vivo could not be found in the accessible literature.

The object of this investigation was to study the role of destruction of tissue macrophages in the regulation of granulocytopoiesis.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-250 g and on CBA mice weighing 20-25 g. Peritoneal macrophages were obtained from the exudate 45 h after intraperitoneal injection of sterile mineral oil into rats or mice. Macrophages accounted for 82-86% of the cells obtained. After repeated washing the cells were broken up by frequent freezing and thawing in physiological saline.

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TABLE 1. Energy Metabolism and Cytological Indices of Bone Marrow Cells from Rats after Triple Intraperitoneal Injection of BPM, Quartz, and Titanium Dioxide (M ± m)

Index	Physiological saline	BPM	Quartz	Titanium dioxide
Endogenous respiration rate, μatom O_2/min/million	3,2±0,3	3,14±0,38	3,72±0,84	2,95±0,2
Critical pO_2 level, mm Hg	38,3±3,8	27,8±3,2*	28,4±2,1*	29,4±4,5
Proportion of mature forms of neutrophils, %	62,0±4,3	77,0±5,0*	82,5±4,7*	67,0±2,5
Content of monocytes, % †	0,8±0,1	2,1±0,4*	1,3±0,1*	1,3±0,2*

*$P < 0.05$.

†After counting 1000 bone marrow cells in the film.

The breakdown products of the macrophages (BPM) were injected intraperitoneally into rats and mice in doses corresponding to $15 \cdot 10^7$ cells /100 g body weight; the control animals received injections of physiological saline. The rats were killed 24 h after the 3rd daily injection and the cell composition of their femoral marrow was studied and the rate of the endogenous respiration and the "critical" level of the partial pressure of oxygen-limiting respiration (in a cell with closed platinum electrodes on the Lp-60 polarograph [7], with isologous serum as incubation medium) were determined. The same investigations were repeated in a parallel series on rats receiving 30 mg finely dispersed quartz or titanium dioxide powder in physiological saline by intraperitoneal injection daily for 3 days and killed 24 h after the third injection. In a separate group of rats changes in the leukocyte count in the peripheral blood were studied for 10 days after a single injection of BPM or of physiological saline.

The number of colony-forming units (CFU) in the bone marrow and spleen was determined by the usual method. For this purpose a suspension of bone marrow (10^5) or spleen (10^6) cells from mice receiving 3 injections of BPM was injected into lethally irradiated (1000 rad) syngenetic mice. The spleens were removed 8 days later from the recipient mice, fixed in Bouin's fluid, and the number of colonies in them was counted. The number of endogenous colonies did not exceed 0.2 per spleen. The cell types of the colonies were determined histologically.

EXPERIMENTAL RESULTS

Injection of BPM did not affect the rate of endogenous respiration of bone marrow cells (Table 1) or the total number of cells (46.0 ± 4.2 after BPM compared with 50.0 ± 5.0 million/100 g body weight). Meanwhile, to judge from the fall in the "critical" pO_2 level limiting cell respiration, injection of BPM led to a significant improvement in the oxygen supply to the bone marrow cells. A differential count of the neutrophils showed an increase in the proportions of stab cells and polymorphs; the number of monocytes also was considerably increased (Table 1).

As Table 1 shows, intraperitoneal injection of quartz dust caused changes in energy metabolism and in the cytological picture in the bone marrow similar to those produced by the action of exogenous BPM, whereas the corresponding injection of titanium dioxide dust resulted only in an increase in the number of monocytes. Both types of dust induced active migration of macrophages to the site of injection, but whereas quartz particles are well known for their high cytotoxicity, titanium dioxide particles cause only slight injury to macrophages both in vitro [9] and in vivo [1]. With an increase in the number of macrophages destroyed, the role of neutrophils in the phagocytosis of dust particles is also known to be increased [4]. Finally, it has been stated that higher concentrations of CSF are required to stimulate granulocytopoiesis than monocytopoiesis [6]. The changes discovered after injection of quartz can therefore be associated with its particularly well marked injurious action on macrophages, i.e., with endogenous formation of BPM.

The dynamics of the mean neutrophil and monocyte counts in the peripheral blood of rats after a single injection of BPM is shown in Fig. 1. The increase in the number of neutrophils during the first day after injection can be assumed to be mainly redistributive in character, whereas the neutrophilic leukocytosis observed 48 h after injection of BPM and later was due to the stimulation of neutrophil formation in the bone marrow, as mentioned above. The monocyte count in the peripheral blood also was considerably increased at this time. Similar changes in the composition of the blood also were observed after triple injection of BPM. Similar changes in neutrophilopoiesis were observed previously in response to the action of breakdown products of

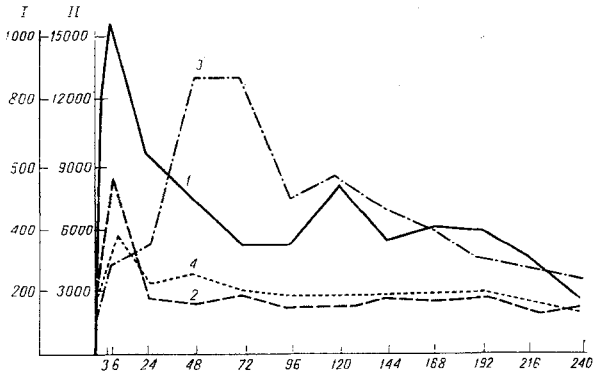

Fig. 1. Effect of breakdown products of macrophages (BPM) on neutrophil and monocyte counts in rat peripheral blood: 1) neutrophils after injection of BPM; 2) the same after injection of physiological saline (control); 3) monocytes after injection of BPM; 4) the same after injection of physiological saline (control). Differences between experimental and control series statistically significant ($P < 0.05$) for neutrophils at all times of testing except the last; for monocytes from 24 h until 144 h inclusive. Abscissa, time after single injection of BPM; ordinate: I) number of monocytes in 1 μl blood, II) number of neutrophils in 1 μl blood.

TABLE 2. Effect of BPM on Colony-Forming Activity of Mouse Bone Marrow and Spleen

Agents injected into donors	Number of donors	Bone marrow					Spleen				
		number of CFU per 10^5 cells (M ± m)	types of colonies, %				number of CFU per 10^6 cells (M ± m)	types of colonies, %			
			E	G	Me	Mi		E	G	Me	Mi
Physiological saline	7	7,3±1,0 (20)	70	15	10	5	7,0±0,7 (18)	70	20	5	5
BPM	7	21,1±2,1*	42	27	2	29	19,5±1,8*	41	24	3	32

Legend. 1) *$P < 0.001$. 2. One preparation disregarded after injection of spleen cells and two after injection of bone marrow of mice with BPM, in which confluent growth of (mainly granulocytic) colonies was observed. 3. Number of recipients in parentheses 4. E) Erythroid, G) granulocytic, Me) megakaryocytic, Mi) mixed colonies (almost entirely erythroid-granulocytic).

neutrophils [3]. An increase in the number of neutrophils and monocytes has also been described during the action of a factor isolated from human urine and possessing the properties of CSF in vitro [10].

The marked stimulation of colony formation in the spleen of lethally irradiated mice receiving an injection of bone marrow or spleen cells from mice treated with BPM also was revealed by macroscopic examination. As Table 2 shows, stimulation of growth predominantly of granulocytic and mixed erythroid-granulocytic colonies was observed under these circumstances.

The results thus agree with the hypothesis that disintegration of macrophages plays an important role in the formation of colony-stimulating factor. The mechanism of the positive feedback mentioned at the beginning of this paper is biologically advantageous in the event that death of macrophages, which protect the body against various corpuscular noxious agents, when mobilization of additional reserves of cells capable of phagocytosis (i.e., macrophages and neutrophils) becomes necessary, should act at the same time as a signal for an increase in their pool; i.e., for their migration into the blood stream to begin with, and for the increased formation of monocytes and neutrophils later.

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CHALONES OF THE LIVER

A. S. Loginov,* M. D. Speranskii,
L. I. Aruin, E. D. Matyushina, and
G. S. Magnitskii

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Injection of liver extract and blood serum of healthy intact mice and also of the blood serum from clinically healthy persons into CBA × C57BL hybrid mice sharply inhibits mitotic activity of hepatocytes in the liver regenerating after partial hepatectomy. Extracts of regenerating liver and blood serum of animals with a regenerating liver do not inhibit mitosis in hepatocytes. Blood serum from a patient with postnecrotic active cirrhosis of the liver not only did not inhibit mitoses in the hepatocytes but actually increased their number. It is suggested that the concentration of chalones is reduced in the cirrhotic liver.

KEY WORDS: chalones; regeneration of the liver; cirrhosis of the liver; blood serum.

Recent work has shown that cells contain, and probably produce, substances inhibiting mitotic activity in the same tissues. Bullough [4] has called these substances chalones. They have been shown to be tissue specific but not species specific.

By using liver extracts from adult intact animals some workers have induced inhibition of mitotic activity of hepatocytes in the regenerating liver [9-13]. Blood serum of adult intact animals has also been shown to have a chalone-like action. When injected into animals after partial hepatectomy, it inhibited the mitotic activity of the hepatocytes [3, 6, 8, 10].

The object of this investigation was to study the action of mouse liver extract and also of mouse and human blood sera on the regenerating liver.

EXPERIMENTAL METHOD

Liver extract was prepared by the method of Verly et al. [11]. The mice were decapitated and the livers removed and homogenized with water (in the ratio of 1:4) in a Potter's homogenizer. The resulting homogenate was centrifuged on the VAC-601 ultracentrifuge at 20,000 rpm for 30 min and the supernatant was drawn off and centrifuged again at 40,000 rpm for 100 min. All operations were carried out at 4°C.

To obtain serum, the blood was centrifuged at 3000 rpm for 10 min at 4°C.

*Corresponding Member, Academy of Medical Sciences of the USSR.

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