ROLE OF PRODUCTS OF MACROPHAGAL ORIGIN IN THE REGULATION OF MONOCYTOPOIESIS

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Many investigators in the last decade have been drawn to the study of problems connected with regulation of the production of blood cells, including cells of the mononuclear phagocytic system. The study of the role of macrophages in the production and secretion of certain substances controlling hematopoiesis also has commenced: colony-stimulating factor [5], prostaglandin E, which inhibits granulocytopoiesis in vitro [7], and a factor stimulating monocytopoiesis [11].

The object of the present investigation was to study the effect of products secreted by mouse peritoneal macrophages (PM) on the proliferative and colony-forming activity of mouse bone marrow cells (BMC).

EXPERIMENTAL METHOD

BALB/c mice weighing 20 g were used. PM were obtained and cultured by the method described by the writers previously [2]. Three-day cultures of PM were incubated for 18 h at 37°C in the presence of latex microspheres (diameter 0.77 μ) in the proportion of 50 particles to 1 PM in a volume of 0.5 ml of Hanks' solution. After centrifugation in the cold at 600g for 10 min the incubation medium was passed through a millipore filter with pore diameter 0.25 μ and the filtrate was collected. To obtain a parallel control filter, a PM monolayer was incubated for the same 18 h in Hanks' solution without latex. To test monocytosis-inducing activity, the filtrates were injected in a volume of 0.15 ml intravenously into mice. The standard level of monocytosis-inducing activity of the filtrate was taken to be its ability to increase the absolute number of monocytes in the blood after 2 days by 4-6 times. The control filtrate caused an increase in monocytosis after the same 48 h by only 1.5-2 times. BMC were isolated by the method of Meerpohl et al. [9] with certain modifications, giving an average yield of $2 \cdot 10^7$ viable nucleated BMC from one mouse. The fraction of BMC adherent to glass (A-BMC), including promonocytes, monocytes, and macrophages, was separated from the nonadherent fraction (NA-BMC) by incubating the BMC suspension ($1 \cdot 10^7$ cells/ml) in Leighton's tubes for 4 h, after which the NA-BMC were transferred to siliconized test tubes.

To assess the effect of the filtrate on granulomonocytic precursor cells, a monolayer agar system was used as in the method of Bradley et al. [4] with slight modifications [1]. The cells were cultured in Leighton's tubes. Colonies (aggregates of 40 cells of more) and clusters (aggregates of 3 to 40 cells) were counted on the 5th day of culture. "Endotoxin serum" was used as the standard stimulator of colony formation (SCF).

To study the effect of the filtrates on the proliferative activity of BMC, filtrate, control filtrate, or physiological saline was added to the adherent and nonadherent BMC fractions in a volume of 0.1 ml to 1 ml medium No. 199 for 30 min at 37°C. Proliferative activity was assessed on the basis of ^{3}H-thymidine incorporation [3].

EXPERIMENTAL RESULTS

During culture of BMC in monolayer agar cultures in the presence of SCF, regular formation of colonies and clusters was observed. Removal of A-BMC had no significant effect on the number of colonies and clusters formed (Table 1). Single large, separately lying cells, with a foamy cytoplasm, giving a positive reaction for

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TABLE 1. Effect of Secretion Products of Mouse Macrophages (filtrate and control filtrate) on Accumulation of Colonies, Clusters, and Immature Macrophages in Agar Cultures of Mouse BMC and NA-BMC depending on the Presence of SCF in the Medium (M ± m)

Cells	Addition to culture	In presence of SCF			In absence of SCF		
		COL	CL	IM	COL	CL	IM
BMC	0,1 ml Hanks' solution	83,6±7,14	168,2±41,42	10,6±3,13	—	69,3±2,70	3,2±0,92
NA-BMC		106,6±8,15	129,2±12,60	10,0±1,61	—	77,0±3,56	4,1±0,65
BMC	0,1 ml control filtrate	44,6±2,49	102,6±7,30	40,0±5,80	—	50,7±5,43	13,7±0,98
NA-BMC		56,2±6,54	120,1±11,82	40,2±3,18	—	53,9±2,10	4,0±0,43
BMC	0,1 ml filtrate	21,3±1,53	35,5±2,49	274,0±36,00	—	40,3±5,98	100,2±8,71
NA-BMC		19,3±2,11	50,2±6,13	200,9±19,88	—	47,7±6,42	45,9±3,21

Legend. Mean values obtained by counting colonies (COL), clusters (CL), and immature macrophages (IM) and calculated per 10^5 myelokaryocytes in five independent series of experiments are given.

peroxidase, and with a comparatively small, eccentric nucleus, also were observed in the cultures. By their morphological criteria these cells were classed as "early" or immature macrophages [6, 9]. In the absence of SCF colony formation was not observed, the number of clusters was reduced by half, and only single immature macrophages were seen (Table 1).

Addition of filtrate at the beginning of incubation of the BMC suspension in the presence of SCF led to a significant decrease in the number of colonies and clusters, which was no less marked in the NA-BMC fraction ($P < 0.05$). At the same time there was a marked increase in the number of immature macrophages: by 25.8 times in the BMC cultures and by 20.9 times in the NA-BMC cultures. Addition of the control filtrate to the system also led to a reduction in the number of colonies and clusters and an increase in the number of immature macrophages, but by a much lesser degree ($P < 0.05$, Table 1).

In the absence of SCF the number of clusters in BMC and NA-BMC cultures fell a little under the influence of the filtrate but the number of immature macrophages increased significantly ($P < 0.05$). The action of the control filtrate under the same conditions was to produce smaller changes.

Incubation of A-BMC in the presence of filtrate led to elevation of the level of ^{3}H-thymidine incorporation, whereas the control filtrate did not change the activity of ^{3}H-thymidine incorporation by the same cells. In the NA-BMC fraction the filtrate caused considerable depression of proliferative activity. The control filtrate inhibited ^{3}H-thymidine incorporation by the NA-BMC fraction, but by a lesser degree (Fig. 1).

The results described above are evidence that during phagocytosis of latex microspheres in a monolayer culture of PM secretory products capable of inducing monocytosis accumulate, at approximately the same times (18-20 h) as the similar factor accumulates in the blood serum of mice after intraperitoneal injection of latex [11]. Accumulation of similar products, but to a much lesser degree, was observed in control PM cultures without latex. The secretion of these products can perhaps be induced not only by phagocytosis, but also by the processes of adhesion of PM to the glass and their spreading over it, which have common features with phagocytosis [10].

To establish the precise points of application of the monocytosis-inducing activity of the macrophagal products their effect on pools of mouse bone marrow precursor cells was analyzed. A statistically significant decrease in the number of colonies and clusters in agar cultures of BMC in the presence of macrophagal products indicated their inhibitory action on the population of colony-forming cells. This conclusion is in agreement with data showing a decrease in the intensity of ^{3}H-thymidine incorporation by the NA-BMC fraction, which includes colony-forming cells. At the same time, macrophagal products under analogous conditions increased the intensity of incorporation of ^{3}H-thymidine by the A-BMC fraction, i.e., mainly by promonocytes. Intensive accumulation of unusual cells, identified by their morphological criteria as immature macrophages, was observed in agar cultures of BMC in the presence of macrophagal products [6, 8].

Comparison of these findings suggests that the monocytosis-inducing action of macrophagal products is based on their stimulation of proliferation of promonocytes and also, perhaps, acceleration of their differentiation into macrophages. The accumulation of immature macrophages, discovered in agar cultures of NA-BMC in the presence of filtrate may be evidence that PM secretion products can influence differentiation of even less mature precursor cells: monoblasts, promonoblasts, and also, possibly, subpopulations of colony-forming cells.

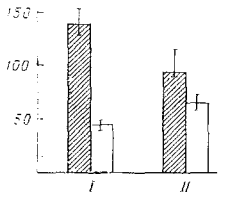

Fig. 1. Effect of secretion products of mouse macrophages (filtrate and control filtrate) on level of incorporation of ^{3}H-thymidine in cultures of mouse A-BMC (shaded columns) and NA-BMC (unshaded columns). I) Incubation with addition of filtrate; II) incubation with addition of control filtrate. Level of incorporation of ^{3}H-thymidine in control cultures (without addition of filtrates) taken as 100.

The results indicate the possible existence of a special mechanism of regulation of monocytopoiesis with the participation of products of macrophagal origin, the secretion of which is intensified during phagocytosis. Macrophagal products with monocytosis-inducing activity are responsible for regulatory feedback between peripheral effector cells and precursor cells of monocytopoiesis.

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