BONE MARROW CULTURE IN MEDIUM CONDITIONED BY CELLS OF LYMPHOID ORGANS

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Prolonged self-renewal of the main categories of hematopoietic cells, including stem cells, *in vitro* can be ensured by culturing suspensions of bone marrow cells [7], most of which carry immunohistochemical markers of fibroblast [5], grown beforehand. By cloning it is possible to estimate quantitatively the number of precursor cells, forming colonies of fibroblasts (CFC-F) [4], in mammalian hematopoietic organs. CFC-F is the ancestral cell of a histogenetic line of stromal fibroblasts, which perform the main functions of the microenvironment of hematopoietic organs. Meanwhile clonal proliferation of hematopoietic precursors *in vitro* [8] is possible even without preliminary formation of a stromal substrate, but in the presence of humoral colony-stimulating factors (CSF), the source of which may be thymus-dependent lymphocytes, stimulated by lectins [12]. Interaction between factors of the stomal microenvironment and factors of lymphocytic origin in the regulation of proliferation and differentiation of hematopoietic cells have not been defined.

This paper gives the results of experiments to culture mouse bone marrow in conditioned medium (CM) from short-term cultures of syngeneic spleen or thymus cells stimulated by concanavalin A (con A).

EXPERIMENTAL METHOD

Donors of bone marrow, spleen, and thymus were female CBA mice aged 2-4 months. Bone marrow cells were grown in monolayer cultures by the methods described previously [1, 4]. Cell suspensions in nutrient medium (NM), consisting of 85% of medium RPMI-1640 (from Serva, West Germany) and 15% of calf embryonic serum (from Flow Laboratories, Scotland), explanted into plastic Petri dishes (Flow Laboratories) or glass flasks (Bellco Glass, USA), with initial explantation density of $2 \cdot 10^5$ or $2.5 \cdot 10^5$ cells/cm^2 of cultural surface of the vessel. At different times of culture the number of viable and nucleated cells not adherent to the cultural surface (nonadherent cells, NAC) in NM was determined. In some cultures during or 24 h after explantation, various quantities of CM were added.

To obtain CM, 10^7 spleen or thymus cells were cultured in 2 ml of nutrient medium consisting of medium RPMI-1640, 5% of embryonic calf serum, 20 mM L-glutamine (Flow Laboratories), and $5 \cdot 10^{-5}$ M 2-mercaptoethanol (from Ferak, East Germany), in the presence of con A (Calbiochem, USA) in a dose of 5 μg/ml (CM—con A$^+$) or without the lectin (CM—con A$^-$). After 72 h the CM was freed from cells by centrifugation (1500 rpm for 15 min) and filtered through nitrocellulose filters (Millipore, USA) with a pore diameter of 0.3 μ, and kept at −20°C.

Cells forming colonies in the spleen of irradiated recipients (CFC-S) and precursor cells for granulocytic-macrophagal colonies in semisolid agar cultures (CFC-C) were determined by methods described previously [6, 14]. CM—con A$^-$ (CSF-CM) from cultures of spleen cells or nutrient medium from cultures of L-cells (CSF-L), generously provided by Yu. O. Kudryavtsev, were used as CSF for the CFC-C.

EXPERIMENTAL RESULTS

After culture of bone marrow for 12 days in NM colonies of fibroblasts formed on the surface of the cultural vessels [1, 4], and their number depended on the number of cells explanted and varied from experiment to experiment between 1.2 and $4.8 \cdot 10^5$ cells of the primary explant.

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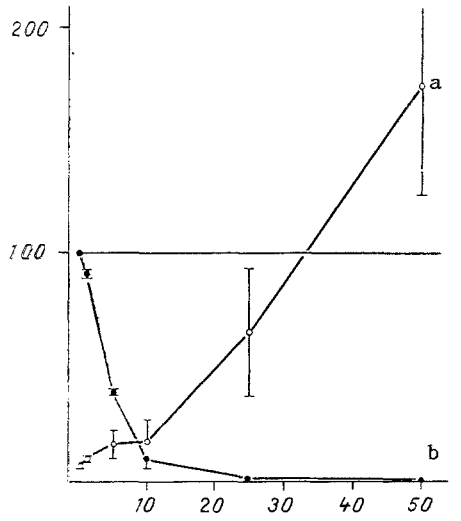

Fig. 1. Dependence of number of NAC and number of fibroblast colonies on dose of CM in bone marrow cultures. Abscissa, dose of CM (in %; whole volume of culture medium taken as 100%); ordinate, number of nucleated NAC and fibroblast colonies (in %; number of nucleated NAC in culture at time of explanation and number of fibroblast colonies in control cultures without CM taken as 100%). a) Nucleated NAC, b) fibroblast colonies.

Addition of 50% CM of splenic or thymic origin to the NM had a twofold effect: It completely suppressed growth of the fibroblast colonies and significantly increased the survival rate of the NAC (Table 1). Replacement of CM by balanced Hanks' salt solution, although it suppressed growth of colonies on stromal fibroblasts by more than 50%, but did not guarantee survival of NAC. Preliminary stimulation of the spleen cells with con A was essential for the production of an effective CM; meanwhile, addition of con A directly to bone marrow cultures did not lead to complete inhibition of column formation and had no significant effect on survival of NAC (Table 1).

The effect of CM definitely depended on its dose in the bone marrow cultures (Fig. 1). Growth of fibroblast colonies was completely absent in cultures containing from 25 to 50% of CM, and was significantly depressed after the addition of 5-10% of CM. Increased survival of bone-marrow NAC was obtained with CM in a dose of not less than 50%, possible evidence that CM contained two independent activities: one depressing growth of fibroblast colonies, the other increasing the survival rate of NAC.

In bone marrow cultures with CM the conditions for prolonged maintenance of hematopoiesis (Fig. 2) and self-renewal of categories of hematopoietic precursor cells such as CFC-S and CFC-C (Table 2) were not guaranteed. Granulocytes of different degrees of maturity were the dominant cells in films of NAC from the experimental cultures, and macrophages, cells of the lymphoid and erythroid series, and fat cells were less frequently found. The total number of cells adherent to the cultural surface was less than in the control, and virtually no fibroblasts were found among them. The low concentration of NAC in the control cultures did not allow them to be obtained in sufficient numbers for morphologic analysis and determination of the numbers of CFC-S and CFC-C.

One probable cause of multiplication of NAC and, in particular, of cells of the granulocytic series, in the first two weeks of culture could be the presence of CSF for granulocytic-macrophagal colonies [12] in medium conditioned by mitogenic-stimulated lymphocytes. Such CSF was present in the splenic CM which we used: according to averaged results of 7 experiments, after explantation of 10^5 bone marrow cells into agar cultures in the presence of CSF-L 51 ± 4 colonies were formed, compared with 88 ± 12 colonies in the precence of CSF-CM. Without the stromal substrate, guaranteeding self-renewal of hematopoietic stem cells in suspension-monolayer bone marrow cultures [7, 8], in the presence of CSF conditions may evidently be created for exhaustive differentiation of precursor cells.

TABLE 1. Number of NAC and Fibroblast Colonies in Bone Marrow Cultures with CM

Expt. No.	Composition of medium	Number of explanted cells ($\times 10^5$)	Number of NAC, per cent		Number of colonies of fibroblasts
			viable	nucleated	
1	100% NM	70	3±1	10±4	103±4
	50% NM +50% CM–con A^+	70	49±4	116±15	0
	50% NM +50% Hanks' solution	70	4±1	13±6	46±4
	50% NM +50% CM–con A^-	70	11±2	21±2	89±5
	100% NM + con A	70	4±2	17±8	46±8
2	100% NM	50	10±6	20±10	108±11
	50% NM+50% CM–con A^+ (thymus)	50	67±15	193±36	0

Legend. Number of viable and nucleated cells at time of explanation taken as 100% of NAC. Con A added to cultures in a dose of 5 μg/ml.

TABLE 2. Changes in Number of CFC-S and CFC-C in Bone Marrow Cultures with CM

Time of culture of bone marrow, days	Number of CFC-S	Number of CFC-C ($\times 10^{-5}$)	
		with CSF-L	with CSF-CM
0	9,8±1,5	48,8±5,0	47,2±3,3
14	1,1±0,4	0,4±0,3	7,9±1,2

Legend. Averaged results of one typical experiment are given. Number of CFC-S and CFC-C determined in a pool of cells from 6 cultures.

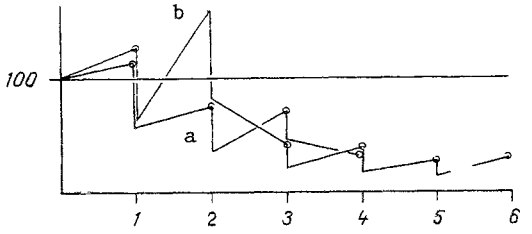

Fig. 2. Changes in number of nucleated NAC in bone marrow cultures with CM at different times of culture. Abscissa, time of culture (in weeks); ordinate, number of nucleated NAC (in %; number of nucleated NAC in culture at time of explanation taken as 100%). a, b) Two different experiments.

The reason for suppression of growth of the fibroblast colonies in the presence of CM is not yet known. Previously a similar phenomenon was described for culture of peritoneal exudate cells of guinea pigs [2] and human bone marrow cells [10]. In view of data in the literature [13], the cytotoxic activity of CM for CFC-F cannot yet be ruled out.

The data thus confirm the important role of thymus-dependent lymphocytes in the regulation of hematopoiesis [3], and the model described can be used to study factors regulating hematopoiesis and arising from cells of the lymphoid organs and the stromal microenvironment.

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ROLE OF THE LACRIMAL GLANDS IN WOUND HEALING

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Although the history of the study of wound healing goes back many centuries, the problem still reamins of the utmost medical, general biological, and social importance. Its urgency is determined by the fact that none of the many therapeutic substances suggested until now can be regarded as a sufficiently effective agent for stimulating healing. One of the most promising ways of investigation is the search for endogeneous biologically active compounds, together with a more penetrating analysis of the physiological mechanisms of healing.

We know that during strong nociceptive stimulation lacrimal gland function is intensified, with abundant tear production [1]. The biological importance of this reaction is not clear. We submit a hypothesis according to which a nociceptive stimulus, causing injury to an organism, acts at the same time as a signal for activation of healing processes.

On this basis excitation of the lacrimal glands under the influence of pain can be explained on the assumption that the lacrimal glands participate in the healing of injuries which always arise under the influence of nociceptive stimulation. The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar albino rats weighing 200-300 g (312 animals altogether). Each series consisted of control and experimental groups, each of 8-10 animals. A circular area of skin 23 or 17 mm in diameter was removed with the aid of a suitable stencil, in the dorsal region always at the same level. The time course of healing was studied by measuring the area of the wounds. In some series of experiments, this was accompanied by the photography of the wounds, followed by projection of the wound area on tracing paper (with a constant focal length), and weighing the cut-out areas. The results of planimetry and weighing did not differ significantly, and accordingly, data obtained only by planimetry will be described. The time of complete cicatrization and epithelization of the wound was taken as an indication that healing was complete. Only those animals whose wounds were uncomplicated were considered. The results of the measurements were analyzed by the Fisher—Student method, with a 95% level of significance of differences ($P \leqslant 0.05$).

The lacrimal glands were excited by nociceptive stimulation of the conjuctiva of both eyes at the boundary with the sclera with a thermocautery (70-80°C), in the form of a small local burn. To maintain the stimulation, the thermocautery was applied four times at intervals of 3 days.

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