HUMORAL NATURE OF THE COLONY-STIMULATING ACTION OF BONE MARROW CELLS ON STROMAL COLONY FORMATION IN BONE MARROW CULTURE

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Bone marrow contains stromal stem cells (CFU-f) which belong to an osteogenic cell line, histogenetically independent of cells of the hematopoietic series [4, 6]. In bone marrow cell cultures CFU-f form colonies or clones of fibroblasts (CFU-f colonies), which differ from macrophages and endothelial cells both morphologically and with the respect to cell markers [2, 9]. The clonal nature of CFU-f colonies has been proved with respect to chromosomal markers in mixed bone marrow cell cultures under conditions ruling out colony formation from stromal aggregates [2, 7]. CFU-f are readily adherent cells [5], so that the test for CFU-f colonies can be undertaken in cultures of adhesive bone marrow cells (ABMC), which have been freed from most hematopoietic cells. The use of such cultures has shown that for CFU-f-colony formation the presence of serum growth factors (including PDGF) alone is insufficient, and additional treatment of the nonadhesive bone marrow cells (evidently megakaryocytes) or blood platelets is required [3].

This paper gives data on the effect of colony-stimulating cells on proliferation of CFU-f and their early cell progeny in cultures. It was shown that the action of colony-stimulating cells is transmitted trhough the culture medium.

EXPERIMENTAL METHOD

None marrow was extracted from the femora of CBA mice and guinea pigs, and cell aggregates were broken down mechanically (mouse and guinea pig cells) or by trypsinization (mouse cells), washed, filtered through a Kapron filter [8], and explanted into plastic flasks (area of bottom 25 cm^2) or into plates (area of well 4.5 cm^2). The cells were cultured in medium α-MEM with 20% fetal calf serum in a CO_2 incubator at 37°C. To obtain cultures of ABMC the medium with unattached cells was removed after 1 h and the cultures were thoroughly washed and and the medium replaced by fresh. A plastic cylinder with a millipore (HA) filter glued beneath it was placed into each well, so that the filter was 2 mm away from the bottom of the well. Guinea pig bone marrow cells, irradiated in a dose of 60 Gy (^{60}Co) were added as feeder cells to some of the cultures [8]. Feeder cells were added to the cultures in the wells immediately after a change of medium, by introducing them either inside the cylinder or into the medium below the filter, so that they came into direct contact with the explained cells. Feeder cells were added to the cultures in the flasks 24 h after a change of medium. Before this, the position of the single spread out fibroblasts in the cultures was noted under an inverse microscope. CFU-c colonies (consisting of at least 50 fibroblasts) and also foci (consisting of a smaller number of fibroblasts) and single fibroblasts were counted in 24-h and 8-12-day cultures, fixed with 10% formalin and stained by Giemsa's method. The efficiency of colony formation (ECF-f) was expressed as the number of colonies per 10^4 explanted bone marrow cells.

EXPERIMENTAL RESULTS

In the presence of 10^7 feeder bone marrow cells, CFU-f in cultures of ABMC in flasks with an area of 25 cm^2 was 14.6 ± 4.1 for cells of trypsinized, and 1.7 ± 0.2 for cells of mechanically disaggregated CBA mouse bone marrow [3]. In this investigation, 10^5 trypsinized or $5 \cdot 10^5$ mechanically disaggregated mouse cells were explained into the flasks. Single spreading fibroblasts were distinguishable in flasks with living and stained cultures of

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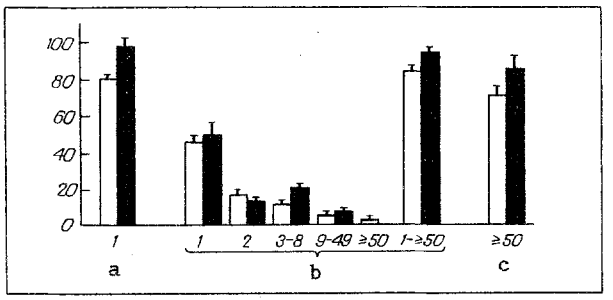

Fig. 1. Effect of irradiated feeder cells on size of fibroblast clones. Altogether $5 \cdot 10^5$ cells of mechanically disaggregated (unshaded columns) or $5 \cdot 10^4$ cells of trypsinized (black columns) bone marrow were explanted for each culture. Some cultures were fixed after 24 h (a). The rest grew for 10 days without feeder (b) or in the presence of irradiated feeder cells (c – 10^7 guinea pig bone marrow cells per culture). Abscissa, single fibroblasts (1) are indicated, number of fibroblasts in composition of focus (2, 3-8, and 9-49, respectively) in colony (≧50). (I – ≧50) total number of single fibroblasts, foci, and colonies; ordinate, number of single fibroblasts or number of foci in colony.

TABLE 1. Colony-Stimulating Activity of Irradiated Bone Marrow Cells Separated from CFU-f by Millipore Filter

Concentration of irradiated bone marrow cells to 1 ml of medium	Quantity of medium per well, ml	ECF-f[2]	
		in wells without filter/in wells with filter	average
10^6 /ml	1,5	2,3/0,2	
	2,0	2,3/0,7	
	3,0	2,7/1,0	2,4/0,6
$3 \cdot 10^6$ /ml	1,5	5,3/1,2	
	2,0	4,0/1,0	
	3,0	3,0/2,0	4,1/1,4
10^7/ml	1,5	—/1,2	
	2,0	2,7/0,3	
	3,0	0,3/0	1,5/0,5
Without irradiated bone marrow cells	1,5	0/0	
	2,0	0/0	
	3,0	0/0	

Note. [1]$3 \cdot 10^4$ Trypsinized bone marrow cells were explanted into each well, 4.5 cm^2 in area, to obtain ABMC cultures. [2]CFU-f colonies were counted on 8th-10th day and ECF-f determined per 10^4 explanted bone marrow cells (means value for 3-4 wells).

ABMC without feeder cells after 24 h, but they amounted to only 0.02% of the number of mechanically disaggregated and 0.2% of the number of trypsinized bone marrow cells. Isolated fibroblasts remained in the 10-day cultures and, in addition, foci and colonies of fibroblasts also were found (Fig. 1). Their total number agreed well with the number of single fibroblasts of the 24-h cultures, and it is therefore natural to conclude that foci of 2, 3-8, and 16-32 cells and colonies of fibroblasts were formed on account of 1, 2-3, 4-5, and over 6 divisions, respectively, of single original fibroblasts (Fig. 1). In fact, some foci occupied the same position in the flasks as the single fibroblasts observed in living 24-h cultures. ECF-f in ABMC cultures without feeder cells was 0.02 and 0.4. Only colonies of fibroblasts were found in cultures to which feeder cells had been added, on the 10th day. Their number corresponded to the number of single fibroblasts in 24-h cultures and many colonies were located on the site of the fibroblasts observed previously. ECF-f in cultures with feeder cells was 1.6 and 20, and the colonies consisted of a much larger number of fibroblasts than colonies in cultures without feeder cells.

Thus all clonogenic stromal cells in 24-h cultures have the morphology of spreading fibroblasts, but without additional colony-stimulating action, 90% of them will not divide or will pass through 1 to 3 cell divisions, and only 1.5% will form CFU-f colonies. In bone marrow 90% of the CFU-f are outside the proliferative pool [1], and it seems likely that the low ECF-f in ABMC cultures in the absence of feeder cells is connected with the fact that additional colony-stimulating action is required to bring the CFU-f out of the period 0 of the cell cycle.

To obtain ABMC cultures in plates, $(3\text{-}5) \cdot 10^4$ trypsinized mouse bone marrow cells per well were explanted. Bone marrow feeder cells were added in a dose of $5 \cdot 10^6$-$3 \cdot 10^7$ per well, and they were either introduced directly on to the layer of adhesive bone marrow cells or above the millipore filter. The colony-stimulating action was manifested in both cases, and when CFU-f and feeder cells were separted by the filter, ECF-f depended not only on the number of feeder cells, but also on the volume of medium in the well (Table 1). Thus the colony-stimulating action of the feeder cells was connected with the secretion of substances diffusing through the millipore filter, and it depended on the concentration of these substances

created in the culture medium. It was shown previously [3] that colony-stimulating activity of bone marrow feeder cells cannot replace serum growth factors, especially PDGF, and that the formation of CFU-colonies is made possible by their combined action on bone marrow stromal stem cells. The fact that the colony-stimulating action of feeder cells is transmitted through the culture medium makes it possible to identify substances activating resting CFU-f.

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ROLE OF FIBRONECTIN IN THE PATHOGENESIS OF MENINGOCOCCAL INFECTION

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Among the factors determining resistance of the nasopharyngeal epithelium (the portals of entry for meningococcal and certain other infections) to colonization by Gram-negative flora, much attention has been paid to the fibronectin layer covering these cells – this is a high-molecular-weight glycoprotein which performs widely different functions in the body [9, 11]. Gram-negative bacteria as a whole are known to differ from Gram-positive in that they bind only weakly with fibronectin and, according to some authorities, the weak colonization of the nasopharyngeal epithelium by the Gram-negative flora under normal conditions can be explained by blocking of the cell receptors for these bacteria by fibronectin [10], although there is evidence [8] that fibronectin promotes adhesion and colonization of certain Gram-negative bacteria also [7, 10], and for that reason in each concrete case, when the role of fibronectin in the pathogenesis of a particular infection is being evaluated, a special investigation is required.

In this investigation, in experiments in vitro we studied binding of various strains of Neisseria meningitidis with fibronectin and the effect of cell surface structures [pili; bacterial adhesins) on the character of this interaction, with a view to explaining the role of fibronectin in adhesion of the pathogen to cells of the host's nasopharyngeal epithelium.

EXPERIMENTAL METHOD

Experiments were carried out with 20 strains of N. meningitidis, whose characteristics are given in Table 1. Meningococcal pili were detected by electron microscopy of negatively stained preparations by the method in [6]. Freeze-dried strains were cultured for 18 h on Hottinger's agar with the addition of 20% horse serum at 37°C in an atmosphere with 5% CO_2, and then transferred into 30 ml of semisynthetic medium [1], pH 7.4 (in some experiments the pH of the medium was adjusted to 6.6) and cultured for 8-10 h at 37°C with continuous mixing.

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