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PHAGOCYTIC ACTIVITY OF STROMAL HEMATOPOIETIC TISSUE PRECURSOR CELLS

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Phagocytic cells were separated from a cell suspension with the aid of iron powder in a magnetic field. Clonogenic stromal precursor cells of hematopoietic tissue which do not belong to the histiocyte–macrophage group but to the mechanocyte group have high phagocytic activity. After treatment of a suspension of bone marrow cells with iron fewer than 1% of stromal precursor cells are left behind, and during monolayer culture they give rise to colonies of fibroblasts. In suspensions of spleen and peritoneal exudate cells about 10% of clonogenic precursors of fibroblasts remain. KEY WORDS: bone marrow; stroma; phagocytosis.

On explanation in monolayer cultures of hematopoietic tissue cells the stromal precursor cells give rise to colonies consisting of clones of fibroblasts [4]; the content of stromal precursors in the cell population can be judged from the number of colonies. Stromal cells maintain themselves independently of hematopoietic cells and under conditions of chimerism of the whole hematopoietic system or part of it they differ in origin from the hematopoietic cells [1-3]. To understand the functions of the hematopoietic and stromal components of hematopoietic tissue and the relations between them it is important to have detailed information on the stromal precursor cells. In particular, investigation of the phagocytic activity of these cells is an interesting problem.

In the investigation described below the ability of stromal precursor cells of hematopoietic tissue, exhibiting clonogenic properties in monolayer culture, to carry out phagocytosis in vitro was studied.

EXPERIMENTAL METHOD

Guinea pigs weighing 180-300 g were the donors of bone marrow, spleen, and peritoneal exudate cells. Cell suspensions were prepared by the method described earlier [2, 5] at 37°C in medium 199 plus 2% embryonic calf serum plus 15 mM Hepes. To obtain peritoneal exudate, 24 h before the experiment the animals were given an intraperitoneal injection of 20 ml nutrient broth. The method of Lundgren et al. [7] was used to remove phagocytes from the cell suspension. To 10 ml of the cell suspension in a concentration of $1 \cdot 10^7$ to $3 \cdot 10^7$ cells/ml, in a smooth-bottomed vessel treated with antifoam silane (to prevent adhesion of the cells), 0.4 g of a sterile powder of iron carbonyl was added. The mixture was incubated for 30 min at 37°C, with gentle stirring every 5 min. After careful and thorough resuspension the particles of iron and the cells phagocytosing them were removed with a powerful magnet, placed near the bottom of the vessel. The remaining cells were resuspended in fresh medium after centrifugation. The same volume of the original cell suspension for use as the control was treated in the same way except for addition of the iron powder. After counting, the cells of the suspension were explanted into 100-ml flasks with the addition of $2 \cdot 10^7$ bone marrow cells, irradiated in a dose of 4000 R, as the feeder. The cultures were grown on medium 199 with 10% embryonic calf serum and the gaseous phase consisted of air with 5% CO_2. After 10-12 days the cultures were fixed with ethanol and

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TABLE 1. Effect of Iron Treatment on Number of Clonogenic Precursors of Fibroblasts in Suspensions of Hematopoietic Cells (M ± m)

Source of cells	No. of expts.	Treatment with iron	No. of cells remaining in suspension after incubation, %	No. of CFU-F per 10^5 cells	No. of CFU-F remaining in suspension after iron treatment, %*
Bone marrow	4	—	83±2	3,72±0,65	
		+	52±8	0,02±0,02	0,5±0,3
		—	80±2	1,90±0,68	
Spleen	5	+	36±6	0,20±0,14	8±4
Peritoneal exudate	5	—	90±5	8,4±2,5	
		+	5±1	12,8±2,6	12±5

* Ratio of number of CFU-F in iron-treated suspension to number of CFU-F in control incubated suspension.

TABLE 2. Effect of Combined Culture of Cell Suspensions Treated with Iron and Untreated Suspensions on Cloning Efficiency of Hematopoietic Cells.

Source of cells	No. of series of flasks	Treatment with iron	No. of cells explanted into flask, × 10^6	No. of colonies in flask (M ± m)	Expected No. of colonies in flask with mix. of treated and control cells*
Bone marrow	1	—	2	111±15	
	2	+	2	0	
	3	—	1	54±16	111:2+0:2=56
		+	1		
Spleen	1	—	6	39±5	
	2	+	6	2±0,4	
	3	—	3		
		+	3	21±5	39:2+2:2=21
	1	—	1	22±5	
	2	+	1	94±1	
Peritoneal exudate	3	—	0.5		
				70±1	22:2+94:2=58
		+	0.5		
	4	—	1	114±7	22+94=116
		+	1		

* The expected number of colonies is equal to the sum of the foci of fibroblasts growing from the corresponding number of explanted cells, treated or untreated with iron, when cultured separately.

stained with azure-eosin; the number of colonies of fibroblasts containing at least 50 cells was counted and on the basis of these results the number of colony-forming units (CFU-F) per 10^5 explanted cells and in the whole cell population was determined.

EXPERIMENTAL RESULTS

In the control suspension of bone marrow cells (Table 1) 80-86% of the original number of cells which retained their usual cloning efficiency (2.7-5.5 CFU-F/10^5 cells) still remained after incubation. After removal of the phagocytic cells in the suspension about 50% of the cells still remained, but their cloning efficiency was sharply reduced (0-0.07 CFU-F/10^5 cells), i.e., 0.5% of clonogenic precursors of fibroblasts remained in the suspension. In the control and experimental suspensions of spleen cells about the same number of cells remained after incubation (80% and about 40% respectively), but the cloning efficiency did not fall to the same degree after removal of the phagocytic cells as in the case of bone marrow (1.9 in the control, 0.2 CFU-F/10^5 cells in the experiment). The total number of precursors of colonies in the experimental suspension was about 8% of the control. After incubation about 90% of cells remained in the control suspension of peritoneal exudate cells, whereas in the experimental series only 5% of the cells were not phagocytic. The concentration of clonogenic precursors of fibroblasts did not fall after treatment with iron (8.4 ± 2.5 in the control, 12.8 ± 2.6 CFU-F/10^5 cells in the experiment), but if calculated relative to all cells incubated with iron, after removal of the

phagocytic cells only 12% of the original number of CFU-F still remained. Treatment of lymphoid tissue cells with iron [8] is known to remove A-cells of the highly adhesive population, the cells of which have well marked phagocytic activity, from lymphoid suspensions. After such treatment the efficiency of fibroblast colony formation by bone marrow, spleen, and peritoneal exudate cells fell sharply.

The decrease in the number of CFU-F after iron treatment could be the result of physical removal of CFU-F from the suspension or the result of the action of factors appearing in the medium after such treatment and inhibiting growth of fibroblast colonies. The second hypothesis, however, was disproved by the results of the experiments with combined culture of control and iron-treated cell suspensions (Table 2). It therefore follows from these findings that by treatment with iron powder in a magnetic field the overwhelming majority of stromal precursor cells was removed; this is evidence of their phagocytic activity. In suspensions of spleen and peritoneal exudate cells about 10% of CFU-F remained; the highest proportion of stromal precursor cells was removed by this method from the suspension of bone marrow cells, in which fewer than 1% of CFU-F remained. These differences show that under the conditions described the CFU-F of bone marrow are evidently practically all capable of phagocytosis, whereas in the spleen and peritoneal exudate a small proportion of these cells does not exhibit phagocytic activity.

Clonogenic stromal precursor cells of hematopoietic tissue, which belong to the mechanocyte and not the histiocyte-macrophage group [6], are thus characterized by high phagocytic activity.

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