ADHESION OF HEMATOPOIETIC CELLS AND LYMPHOCYTES TO STROMAL MECHANOCYTES IN VITRO

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Adhesion of guinea pig bone marrow and spleen cells to stromal mechanocytes of medullary, splenic, and thymic origin, to peritoneal exudate fibroblasts, and also to macrophages was studied in monolayer cultures. Primary cultures and subcultures of fibroblasts were able to bind hematopoietic cells and lymphocytes, and this property was independent of the origin of the mechanocytes. Hematopoietic cells were much less adherent to macrophages. Adhesion of cells to mechanocytes is determined by the number of adhesion sites present on the surface of the fibroblasts. There are significantly more of these sites for adhesion of myeloid cells on the surface of stromal mechanocytes than for adhesion of lymphocytes.

KEY WORDS: cultures of stromal mechanocytes; colonies of fibroblasts; adhesion of hematopoietic cells and lymphocytes.

Hematopoietic cells and precursors of lymphocytes repopulate hematopoietic organs in which they find a microenvironment which favors their proliferation and specific differentiation [2]. A principal role in the creation of the microenvironment is played by stromal mechanocytes, clonal colonies of which can be obtained by primary explanation; during passage of the colonies diploid strains of stromal fibroblasts appear [1]. The object of this investigation was a comparative analysis of adhesion of hematopoietic cells and lymphocytes in vitro to mechanocytes (fibroblasts) obtained from bone marrow, spleen, thymus, and peritoneal exudate, and also to macrophages.

EXPERIMENTAL METHOD

Primary monolayer cultures of bone marrow, spleen, thymus, and peritoneal exudate cells from guinea pigs and subcultures of fibroblasts were obtained as described previously [3], using 60-mm glass Petri dishes. Culture was carried out in the presence of HEPES buffer in an atmosphere containing 5% CO_2 in air and saturated with water vapor. Allogeneic bone marrow cells (4×10^6-4×10^7 per dish) or spleen cells (1×10^7-1×10^8 per dish) were added to 7-9-day primary cultures and 1-7-day subcultures of migrating fibroblasts. The suspensions to be added were first freed from cells adherent to the surface of the glass [4]. After combined culture for 21-23 h the dishes were washed with medium No. 199 and fixed with formalin vapor, calcium-formol, and methyl or ethyl alcohol. They were then stained with Sudan black, with peroxidase, by Gomori's method for alkaline phosphatase, or with azure-eosin by Romanowsky's method. The number of fibroblasts forming colonies and the number of hematopoietic cells or lymphocytes associated with fibroblasts and macrophages, and also the number adherent to the glass were counted.

EXPERIMENTAL RESULTS

The primary 7-9-day cultures consisted of small discrete colonies of fibroblasts virtually free from macrophages. On transplantation of bone marrow cells it could be clearly seen that adhesion of the hematopoietic cells to glass and to macrophages was on an extremely small scale. No hematopoietic cells were adherent to 84% of the macrophages, and not more than one or two cells to the other 16%. Conversely, binding of hematopoietic cells to the fibroblasts was definite in character. Among the hematopoietic cells adherent to mechanocytes most were morphologically distinguishable granulocytes, i.e., cells giving a positive reaction

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TABLE 1. Adhesion of Medullary Hematopoietic Cells and Splenic Lymphocytes to Fibroblast Colonies in Primary Monolayer Culture

Experiment No.	No. of transplanted cells	Origin of transplanted cells	Origin of fibroblasts	No. of cultures	No. of colonies counted	Mean ratio of number of adherent cells to number of fibroblasts (M ± m)
1	$3 \cdot 10^7$	Medullary	Medullary	2	20	6,6±0,8
			Splenic	1	11	5,2±0,8
			Thymic	2	23	5,9±0,4
2	$7 \cdot 10^6$	Same	Medullary	3	47	5,1±0,5
			Splenic	2	23	4,9±0,7
			Thymic	3	34	5,2±0,7
	$4 \cdot 10^7$	» »	»	1	11	5,2±0,9
3	$4 \cdot 10^6$	» »	Medullary	4	56	5,9±0,4
			Splenic	5	59	5,5±0,3
			Medullary	4	50	5,8±0,3
4	$5 \cdot 10^6$	» »	Splenic	4	48	4,9±0,3
			Thymic	3	32	5,4±0,5
			Peritoneal exudate	2	47	5,7±0,3
5	$3 \cdot 10^7$	Splenic	Medullary	3	32	0,31±0,05
6	$3 \cdot 10^7$	Same	Medullary	1	10	0,34±0,13
			Splenic	1	10	0,43±0,11
7	$1 \cdot 10^8$	» »	Medullary	2	24	0,36±0,05
			Splenic	2	22	0,39±0,06
	$1 \cdot 10^7$	» »	Medullary	2	22	0,27±0,04

for alkaline phosphatase and peroxidase and staining with Sudan black. The colonies of fibroblasts were heterogeneous as regards the number of adherent hematopoietic cells (from two to 18 per fibroblast). Hematopoietic cells were less adherent to large colonies with tightly packed fibroblasts than to small, loosely packed colonies.

The mean ratio between the number of adherent hematopoietic cells and the number of fibroblasts of medullary, splenic, thymic, and peritoneal origin was 5-6 : 1 (Table 1); the differences between the ratios for the different cells were not significant.

The mean ratio of the number of adherent myeloid cells to the number of fibroblasts did not change significantly with an increase in the number of transplanted bone marrow cells from 4×10^6 to 4×10^7 per dish. Since primary cultures of fibroblasts, similar in number and size of the colonies, were used in these experiments, the result means that the number of adherent myeloid cells was determined not by the number of cells in contact with fibroblasts, but by the limited number of adhesion sites present on the surface of the mechanocytes.

Splenic lymphocytes were much less adherent to the mechanocytes. Mean ratio of the number of adherent lymphocytes to the number of fibroblasts was 0.3-0.4 (Table 1), and was the same for fibroblasts of medullary and splenic origin and for cultures to which 1×10^7, 3×10^7, and 1×10^8 spleen cells were added. There are evidently significantly fewer adhesion sites for lymphocytes on the surface of the mechanocytes than adhesion sites for myeloid cells.

After addition of bone marrow cells to the cultures a bone marrow and thymus fibroblasts after the 2nd-4th passage, hematopoietic cells adhered to 76-84% of fibroblasts. Between 15 and 21% of fibroblasts were bound to one hematopoietic cell, 13-19% to two, and 9-13% to three. From 2.9 to 6.1% of fibroblasts were bound to 11 hematopoietic cells or more, and between 0.5 and 1.7% to 16 or more. On average each subcultured fibroblast had 3-3.5 hematopoietic cells adherent to it. Differences in the ability of fibroblasts subcultured from bone marrow and thymus to bind hematopoietic cells were not statistically significant.

If bone marrow cells which, during the previous 22 h, had not adhered to fibroblasts of other analogous cultures were added to subcultures of fibroblasts, the same number of myeloid cells adhered to the mechanocytes as when bone marrow cells not previously associated with fibroblasts were added. This also confirms that the number of cells bound to fibroblasts is determined, not by the number of myeloid cells capable of forming associations with mechanocytes, but by the limited ability of the mechanocytes to bind hematopoietic cells.

Neither in primary monolayer cultures nor in subcultures were any differences found between stromal mechanocytes of medullary, splenic, or thymic origin, and also peritoneal exudate fibroblasts as regards ability to bind hematopoietic and lymphoid cells. This nonselective adhesion to mechanocytes of different origin can be explained by the fact that morphologically identifiable myeloid and lymphoid cells (which mainly were adherent to fibroblasts in cultures) no longer require specific stromal mechanocytes for their development. It must evidently be earlier, morphologically indistinguishable precursors of hematopoiesis and lymphopoiesis, which specifically colonize the territories of the hematopoietic and lymphoid organs, that distinguish stromal mechanocytes of different origin. The problem of whether such cells adhere to stromal mechanocytes of different origin in the same way or differently will be a subject for later communications.

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