ADHESIVE PROPERTIES OF HEMATOPOIETIC AND LYMPHOID CELLS FORMING FIBROBLAST COLONIES IN MONOLAYER CULTURES

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Cells of the bone marrow and thymus of guinea pigs and rabbits, forming colonies of fibroblasts in monolayer cultures, have well-marked adhesive properties: when cultivated in medium without serum 80-95% of the cells of this category adhere to the slide in 90 min; most of them adhere to the slide during the first 30 min. Mainly precursors of histiocytes remain in the suspension.

Colonies of fibroblasts are formed in monolayer cultures of hematopoietic and lymphoid cells [1]. The ability to transfer the specific properties of the microenvironment for myeloid and lymphoid tissue during free transplantation of fibroblasts obtained by repeated subculture from cultures of bone marrow and spleen and the ability of precursors of colonies of fibroblasts to maintain themselves suggest that colony-forming cells are stromal precursor cells. The small number of these elements among the cells of hematopoietic and lymphoid tissues prevents the direct study of their properties. It is therefore interesting to study some characteristics of colony-forming cells which could prove useful for the identification and isolation of stromal precursors from an original cell population.

The results of an investigation of the adhesive properties of precursors for fibroblast colonies are described below.

EXPERIMENTAL METHOD

A suspension of bone marrow and thymus cells of guinea pigs (weighing 150-200 g) or thymus cells from rabbits weighing about 3 kg was prepared as described previously [2]. The resulting suspension was centrifuged, the residue was resuspended in fresh medium and filtered through a Kapron filter, and cultures of 1×10^7-5×10^7 cells were grown in 250-ml flasks. After adhesion for 48 h (0-48 h, Table 1) the cultures were placed at once in medium containing serum (80% medium No. 199 and 20% serum); embryonic calf serum was used for guinea pig cells and homologous inactivated serum for rabbit cells. To study the adhesive properties of the precursors of the fibroblast colonies the cell suspension was grown initially for 30 min (0-30 min). After gently stirring the suspension, the nonadherent cells were transferred to other flasks for the next 30 min (30-60 min), and this procedure was again repeated (60-90 min). Cells remaining in suspension after adhesion for 90 min were poured off. In another group of cultures a cell suspension was grown initially for 90 min (0-90 min), and the nonadherent cells were then poured off or transferred to other flasks, in which they were kept without a change of medium until 48 h (90 min-48 h). In one of the experiments (Table 1c) the suspension of cells nonadherent after 90 min was centrifuged, the residue was transferred to Leighton's tubes with cover slips, and the cultures on the glass were fixed after 72 h. To verify preservation of colony-forming ability of the cells nonadherent after 90 min, a suspension of bone marrow cells was explanted initially into siliconized flasks (Table 1a), from which they were transferred 90 min later into ordinary flasks (Table 1b) in which they were left until the medium was changed.

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TABLE 1. Adhesiveness of Bone Marrow and Thymus Cells Forming Colonies of Fibroblasts in Monolayer Cultures

Type of explanted cell suspension	Time from moment of explantation to removal of nonadherent cells	No. of explanted cells ($\cdot 10^7$)	Number of colonies in flasks	Efficiency of cloning (in %)
Guinea pig bone marrow	0—48 h	1	403, 462	100,0
	0—90 min	1	356, 424	90,2
	0—30 »	1	387, 435	95,0
	30—60 »	—	31, 56	10,1
	60—90 »	—	4, 4	1,0
Ditto	0—48 h	1	551, 597	100,0
	0—90 min	1	484	84,3
	90 min — 48 h	—	76	13,2
	0—90 min (a)	1	0 0	0
	90 min — 48 h (b)	—	569, 587	100,7
	0—90 min (c)	0,5	262, 266 270, 287 289	95,8
Guinea pig thymus	0—48 h	5	40, 53	100,0
	0—90 min	5	43, 47	96,8
	0—30 »	5	44, 53	104,3
	30—60 »	—	0, 5	5,4
	60—90 »	—	1, 4	5,4
Rabbit thymus	0—48 h	2	117, 173	100,0
	0—30 min	2	100, 131	79,0
	30—60 »	—	9, 14	7,9
	60—90 »	—	0, 0	0

Legend: —) number of cells not determined; a) cell suspension explanted primarily into siliconized flasks; b) suspensions of nonadherent cells from cultures (a) transferred to flasks; c) cell suspension explanted primarily into 100-ml flasks; suspension of cells nonadherent after 90 min poured off, removed by centrifugation, and transferred to Leighton's tubes with coverslips.

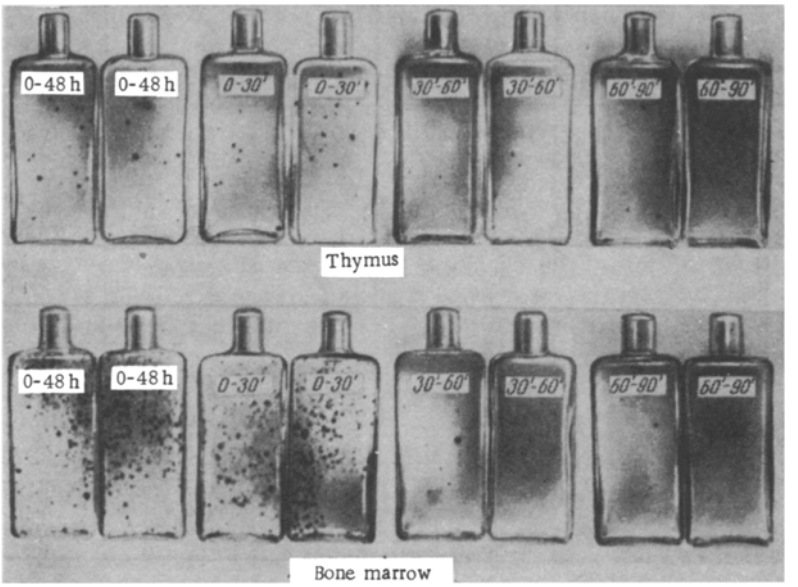

Fig. 1. Colonies of fibroblasts in 12-day monolayer cultures of bone marrow and thymus cells of adult guinea pigs after adhesion of original cell suspensions for various times. Duration of experiment marked on flasks. Explanation in text.

Cultures with cell adhesion times of between 30 and 90 min were kept throughout this period in flasks with medium No. 199. After removal of the nonadherent cells medium containing serum was poured into these flasks and the suspensions with a cell adhesion time of more than 90 min were grown in the same medium. To each flask 2×10^7 cells of homologous bone marrow, irradiated in a does of 4000 R, were added as a feeder simultaneously with the culture medium containing serum. After 48 h the medium of all the cultures was changed, and on the 12th day they were fixed with alcohol and stained by Giemsa's method, after which the colonies were counted.

EXPERIMENTAL RESULTS

If the efficiency of cloning of cultures with a cell adhesion time of 48 h was taken as 100%, during the first 90 min of cultivation 80-95% of the precursors of the fibroblast colonies contained in the bone marrow and thymus were adherent (Table 1); the overwhelming majority of them adhered to the glass during the first 30 min (Fig. 1). During this period, judging from cultures on coverslips, some precursors of histiocytes adhered also. Later a very few precursors of fibroblast colonies remained in the suspension, although keeping the nonadherent cells for 90 min in medium without serum did not affect their colony-forming ability, as was shown by cultivation in siliconized flasks (Table 1a, b). Cultures on coverslips grown from the suspension obtained after adhesion of bone marrow for 90 min in flasks (Table 1c) consisted of many histiocytes, which are usually present in primary bone marrow cultures. In flasks in which this suspension was incubated initially the efficiency of cloning was 96% of the control; i.e., the overwhelming majority of precursors of the fibroblast colonies had been removed from the suspension. In fact, hardly any fibroblasts were adherent to the glass in cultures after preliminary adhesion. The bone marrow and thymus cells which give rise to colonies of fibroblasts thus have the property of adhering rapidly (during the first 30-90 min) to glass.

LITERATURE CITED

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