FORMATION OF COLONIES OF FIBROBLASTS IN CULTURES OF CIRCULATING BLOOD CELLS

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Colonies of fibroblasts are formed in monolayer cultures of circulating blood cells of guinea pigs, indicating that categories of cells which are precursors of fibroblasts exist among the blood leukocytes. Their concentration is not less than 2.5 per 10^5 leukocytes.

Following explantation of circulating blood cells, growth of fibroblasts forming collagen fibers is observed [2-5]. The same result is found in cultures of bone marrow and spleen [1, 9, 10]. Previous investigations [6-8] have shown that if the concentration of explanted cells is not too high, growth of fibroblasts in monolayer cultures of guinea pig bone marrow and spleen takes place in the form of discrete colonies, and the evidence obtained suggests that these are clones.

Formation of colonies of fibroblasts in cultures of circulating blood cells from guinea pigs is described below.

EXPERIMENTAL METHOD

Blood obtained from guinea pigs by cardiac puncture was used for cultivation. Heparinized blood, mixed with gelatin solution, was allowed to stand at room temperature for 40-60 min. After sedimentation of the erythrocytes, the supernatant layer, rich in leukocytes, was transferred to a test tube and centrifuged for 10 min at 1000 rpm. The residue was resuspended in medium No. 199 and cultivated in 100-ml glass flasks or plastic dishes 50 mm in diameter. Parallel cultures were carried out on cover slips in Leighton's tubes. Between 2×10^6 and 3×10^6 cells in 1.5 ml medium was added to each Leighton's tube, between 8×10^6 and 15×10^6 cells in 12 ml medium to each flask, and between 1×10^6 and 4×10^6 cells in 6 ml medium to each dish.

Besides medium No. 199, the culture medium contained 20% bovine or calf serum and 50 units each of penicillin and streptomycin per ml. The medium was changed once 24 h after explantation, and the duration of cultivation did not exceed 12 days. Cultures on cover slips were fixed at successive intervals (from 1 to 12 days) for morphological investigations, while the flasks and dishes were fixed on the 12th day so that the number of colonies could be counted. Fixation was carried out with 96° alcohol or formalin, and the material was stained with hematoxylin or by Gomori's method for reticulin fibers.

The proliferative pool in foci of fibroblast-like cells was also determined in autoradiographs of the cultures on cover slips. Thymidine-H^3 was added to the medium of these cultures in a concentration of 1 μCi/ml at various stages of cultivation.

EXPERIMENTAL RESULTS

The culture morphology passed through a series of successive changes. During the first few hours after explantation, all blood cells were found among cells adherent to the glass or plastic. During the next 24 h, however, most of the cells degenerated. The cells which remained intact had the morphology of his-

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TABLE 1. Colony-Forming Activity of Blood Cells

Vessel	Number of explanted cells	Number of colonies	Effectiveness of colony formation	
			per 10^5 cells	mean
Glass flasks	$15.5 \cdot 10^6$	198	1.3	
	$8.8 \cdot 10^6$	175	2.0	
	$14.5 \cdot 10^6$	303	2.0	1.7
	$11.0 \cdot 10^6$	166	1.5	
Plastic dishes	$3.6 \cdot 10^6$	64	1.7	
	$3.5 \cdot 10^6$	68	2.0	
Plastic dishes	$3.5 \cdot 10^6$	104	3.0	2.5
	$3.5 \cdot 10^6$	108	3.0	
	$3.5 \cdot 10^6$	103	3.0	
	$3.5 \cdot 10^6$	66	2.0	

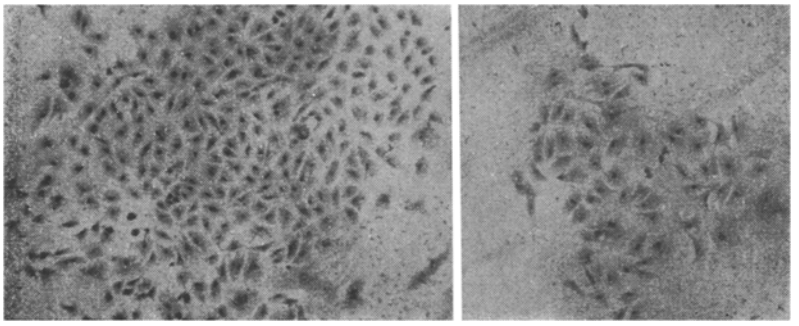

Fig. 1. Cultures of circulating blood cells. Colonies of fibroblasts (10 days), hematoxylin, 10 ×.

tiocytes. Sometimes the first colonies, consisting of a few fibroblast-like cells, appeared only 24 h after the beginning of cultivation. On the fourth day these colonies were considerably increased in both number and size. No other cell forms were present in the culture at this time, and only discrete colonies of fibroblasts remained. On the 10th-12th day of cultivation, large colonies of fibroblasts could be seen with the unaided eye, and each one consisted of several hundred cells. After further cultivation the number of colonies did not increase, but they continued to increase in size. The colonies consisted of typical fibroblasts, forming collagen fibers and containing tonofibrils in their cytoplasm. Sometimes the colonies differed in the packing density of their cells (Fig. 1). As a rule they were round in shape, with smaller cells in the center, where they were more densely packed.

Cells in the colony possessed high mitotic activity, and this was responsible for the rapid growth of the colonies. Following addition of thymidine-H^3 to the 4-day cultures, at 24 h all cells forming the colony had incorporated label. In 7-day colonies, more than 80% of cells were labeled at 24 h.

The number of colonies formed in the cultures was a linear function of the number of explanted blood leukocytes. The effectiveness of colony formation per 10^5 explanted leukocytes was 1.7 and 2.5, respectively, for cultures in glass flasks and plastic dishes (Table 1). The distribution of the number of foci in the cultures varied from the mean value only slightly in the same experiment.

Judging from the linear distribution of the number of colonies in relation to the number of explanted cells, by the dynamics of development of the colonies, by their morphology, and also by the fact that similar colonies, growing in monolayer cultures of spleen and bone marrow are clones, it may be considered that the colonies of fibroblasts in cultures of circulating blood leukocytes were also cell clones. In this case they were formed from a constant number of precursor cells. In special experiments, the number of colonies growing in cultures following the taking of blood by a single and by five repeated punctures of the heart was compared. In this case no difference was found between the number of colonies.

Precursors of fibroblasts are thus present in the circulating blood of guinea pigs. They number not less than 2.5 per 10^5 nucleated cells.

Since five repeated punctures of the heart did not increase the number of foci growing in the cultures compared with that obtained after a single puncture, it is evident that the presence of precursor cells for colonies of fibroblasts was not the result of a surge in their number during the taking of blood. These cells are the only ones in the population of blood cells. A similar conclusion was reached by Stirling et al., who cultivated blood cells in a diffusion chamber [11].

Hence, besides the two categories of precursor cells already known to be present in blood (immunocompetent cells and hematopoietic stem cells), yet another category of precursor cells can be counted among the circulating blood leukocytes, in this case forming colonies (probably clones) of fibroblasts. The method as developed enables the number of these precursor cells in the blood to be determined.

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