COLONY-FORMING PRECURSORS OF CIRCULATING BLOOD FIBROBLASTS

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Mechanocytes (fibroblasts and reticular cells) play an important role in the establishment of the hematopoietic microenvironment [1-3, 5-7]. In this connection the problem of whether their precursor cells circulate or not in the blood stream is particularly interesting. Evidence in support of the possibility of such circulation was given by the results of an investigation by Maximow (1928), who obtained blood cells in culture [9].

Some valid objections were subsequently raised: when blood was taken, fragments of connective tissue of the vessel wall may have contaminated it and acted as the source for growth of fibroblasts in plasma cultures [11, 12]. This objection was overcome when explantation of blood cells was carried out by the method of detection of clonogenic bone marrow stromal cells (CFCf) [4]. It has been shown [8] that the number of CFCf colonies is independent of the number of punctures used to obtain the blood but is a linear function of the number of explanted leukocytes, i.e., it follows that colony-forming precursors of fibroblasts do in fact circulate in the blood. To identify fibroblasts in blood cultures, their morphological features have hitherto been used [8, 9], or their weak pagocytic activity [9, 10], and absence of antigen of factor VIII [10]. With respect to these features, cells composing CFCf-colonies in blood cultures differ form macrophages and from endothelial cells.

Meanwhile the direct marker of fibroblasts, namely the presence of interstitial collagen (procollagen) in their cytoplasm has not been used to verify that cultural progenies of CFCf of blood are in fact fibroblasts.

In the investigation described below cells of CFCf-colonies were typed on the basis of the following markers: collagens of type I-III, type IV collagen, fibronectin, and Fc-receptors. The numbers of CFCf in venous blood flowing from different organs also was compared.

EXPERIMENTAL METHOD

Blood was obtained by puncture from the heart, inferior vena cava, and renal veins of noninbred guinea pigs weighing 150-250 g, using heparin (25 U/ml blood) as the anticoagulant. The blood, diluted with 4% gelatin solution in the medium, was allowed to stand for 25-30 min, after which the leukocyte were separated from erythrocyte-free plasma by centrifugation at 400 g. The cells thus obtained were washed with medium 199 and used for explanation. The complete culture medium consisted of α-MEM with 20% embryonic calf serum. Cells were cultured in a CO_2-incubator in plastic flasks with a base 25 cm^2 in area; the explanation density was (8-12) × 10^4 leukocytes/cm^2. The living cultures were studied in phase contrast for 10-16 days, after which the cultures were fixed with 10% neutral formalin in phosphate buffer with saponin. Indirect immunohistochemical tests were carried out with cells growing on the surface of the flasks for type IV collagen, using affinity-purified rabbit antibodies, and for fibronectin, using mouse monoclonal antibodies. In the second stage of the reactions, affinity-purified antirabbit and antimouse antibodies, labeled with peroxidase, were used. Activity and specificity of the rabbit antibodies were verified by indirect ELISA, using goat antirabbit antibodies, labeled with peroxidase, and also in immunohistochemical reactions with frozen sections of guinea pig kidney, using FITC-conjugated sheep's antirabbit antibodies.

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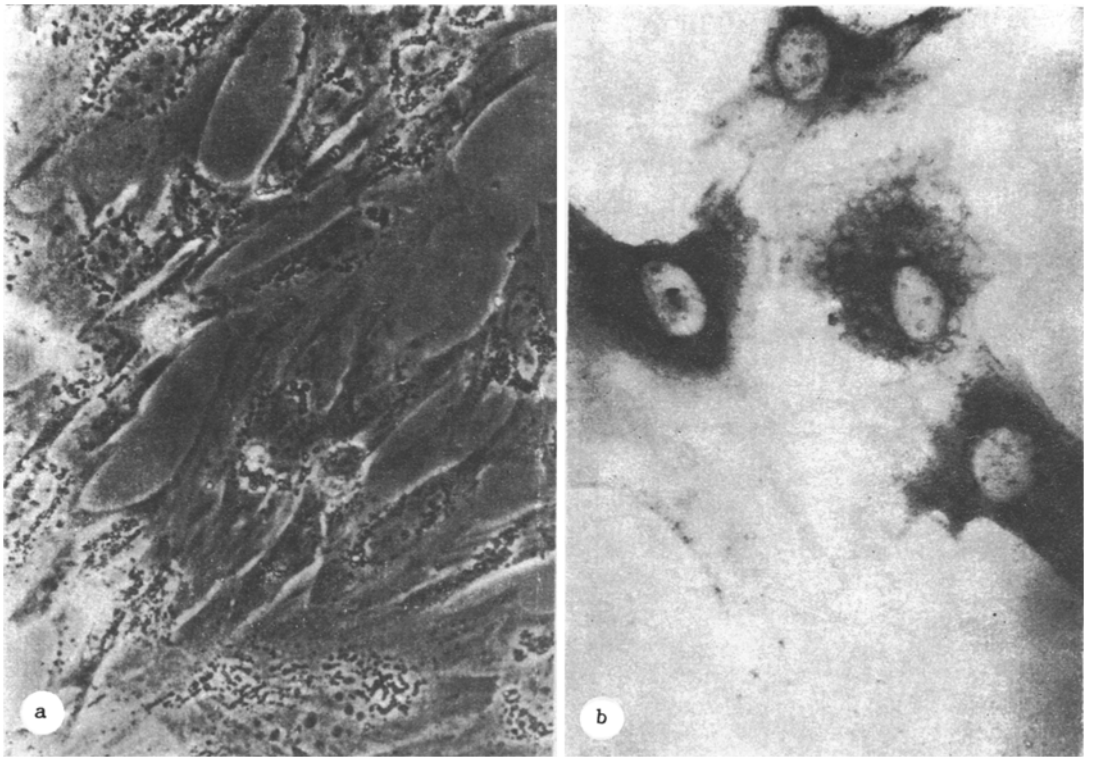

Fig. 1. Cells of colonies in 12-day blood cultures. a) Phase contract. Objective 24×; b) indirect immunohistochemical reactions for type I collagen. Objective 40×.

Before fixation, some of the living cultures were tested for rosette formation with sheep's red blood cells (SRBC), conjugated with antierythrocytic antibodies. The efficiency of formation of fibroblast colonies (ECFf) was determined in cultures stained by the Giemsa method.

EXPERIMENTAL RESULTS

The number of adherent cells was less than 0.01% of the explanted nucleated blood cells; these cells were uniformly distributed over the surface of the culture flasks. After 48 h some of them acquired the shape of spread out fiboblasts, and later, colonies of fibroblasts were formed in their place. On the 10th-16th day the colonies contained between several tens and several hundreds of fibroblasts.

As regards the shape of the cells the colonies were of three types: consisting of grass-like fibroblasts, of polygonal fibroblasts, or of a mixture of the two kinds of cells, and this description applied to both large and small colonies (Fig. 1). In 10-16-day colonies all the fibroblasts gave an intense reaction for types I-III of collagen (Fig. 1b). This applied to all colonies regardless of their size and of the shape of the cells composing them. The fibroblasts did not form rosettes with erythrocytes. The reaction for type IV collagen in fibroblasts of the colonies also was positive and was stronger in cells composing small colonies, in which polygonal fibroblasts predominated.

Fibronectin was found on all fibroblasts regardless of their shape and of the size of their colonies. A concentration of CFCf was discovered in the blood of 15 donors, from whom blood was taken in parallel punctures from the heart, inferior vena cava, and renal veins. It amounted to (per 10^5 explanted leukocytes): 0.87 ± 0.22 for blood from the heart, 3.2 ± 0.6 for blood from the inferior vena cava, and 0.4 ± 0.2 for blood from the renal vein. No CFCf could be identified in some donors.

The results show that colony-forming cells detected in the blood by the CFCf-colonies method are in fact circulating precursor cells of fibroblasts, for their descendant cells contain interstitial collagen, a differential marker of fibroblasts. With respect to other marker features, such as the absence of Fc receptors and of antigen of factor VIII, these cells differ clearly from macrophages and endothelial cells. The CFCf-colonies formed by medullary CFCf are cell clones [1]. This conclusion is evidently valid also for CFCf-colonies of blood, although this requires direct confirmation.

Certain differences have been found between CFCf of blood and CFCf from bone marrow. In particular, the former evidently synthesize type IV collagen, which is observed during culture of certain other types of fibroblasts also.

The concentration of CFCf in blood obtained by cardiac puncture was about 10^{-5} among circulating leukocytes; meanwhile it was not the same in venous blood flowing from individual organs: it was far higher in the inferior vena cava than in the renal vein. Meanwhile, in about 25% of guinea pigs no CFCf could be found in the venous blood.

CFCf are essentially blood cells of a new type [1]. Their morphology and origin still remain to be established, and in particular, the organs from which they enter the blood stream and how they do so, and also the direction of their possible migration. Other open questions include the physiological functions of the CFCf of the blood, the factors regulating their number, and requirements of blood CFCf of growth-stimulating factors for proliferation in culture.

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