FRACTIONATION OF MOUSE BONE MARROW CELLS BY PREPARATIVE ELECTROPHORESIS

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Separation of bone marrow cells into fractions rich in cells of a particular type is essential for a number of investigations in modern hematology (when studying biochemical, immunologic, and morpholigic characteristics of hematopoietic cells).

The following methods are mainly used nowadays to fractionate cells: centrifugation in albumin gradients [10], spontaneous sedimentation [6], centrifugal elutration [5], electronic cell sorting based on data of fluorescence or light scattering [11].

A comparatively new method is fractionating cells by means of an apparatus for electrophoresis in a free flow of buffer, where the cells are separated in an electric field on the basis of differences in their surface charge [4]. In such an instrument, a buffer with low ionic strength moves at constant velocity within the narrow space in a vertical chamber between the anterior and posterior walls from above downward. The side walls of the fractionating chamber consist of ion-exchange membranes, to which a high-voltage direct current is applied. Uniformity of movement of the buffer is achieved by means of a peristaltic pump. The cell suspension to be fractionated is introduced through a hole in the anterior wall of the chamber. The cells move downward with the buffer and, depending on their surface charge, they deviate in the electric field. Several holes are provided in the floor of the chamber through which the cells pass into Teflon tubes, and from them into separate test tubes.

An advantage of cellular electrophoresis over other methods of fractionation is the short time taken to separate the cells and the possibility of obtaining large quantities of individual cell fractions. Attempts have been made by this method to detect heterogeneity of bone-marrow precursor cells [3, 12] and of human bone marrow and blood cells under normal conditions and in leukemia [1, 2, 8].

This paper gives data on fractionation of bone marrow cells of intact mice by electrophoresis in a free flow of buffer and the results of studies of the distribution of stem cells among the fractions.

EXPERIMENTAL METHOD

Bone marrow from C57BL, AKR, (CBA × C57BL)F_1 hybrid, and noninbred mice was used as the test material. Bone marrow cells were obtained from the femoral bones, which were flushed out with buffer. To prevent the cells from aggregating [7], syringes and metal needles were not used (as is usual) when obtaining the cells and the cell suspension was not centrifuged. The bone marrow was flushed from the medullary cavity and cell suspensions obtained by means of a Pasteur pipet. All these operations were carried out at a temperature of 1-3°C. To remove any "sticky" cells the suspension was filtered through cotton or incubated in columns packed with cotton (37°C, 20 min). As a result a suspension was obtained with a cell concentration of 2×10^7-3×10^7/ml. Triethanolamine buffer of the following composition was used as the cell fractionation buffer: 0.18 M triethanolamine, 0.27 Mglycine, and 0.005 M potassium acetate. The osmolarity of the buffer was 0.31 osmole and its electrical conductivity 9×10^{-4} Ω^{-1}, cm, pH 7.4.

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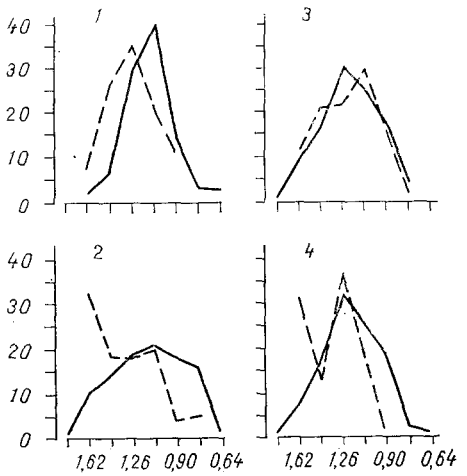

Fig. 1. Distribution of bone marrow cells (continuous line) and of CFUs (broken line) among fractions. Abscissa, fractions with electrophoretic mobility ($\times 10^{-4}$ $cm^2 \cdot V^{-1} \cdot sec^{-1}$), ordinate, distribution of cells among fractions (in %). 1, 2, 3, 4) Nos. of experiments.

The cells were fractionated on an Elphor VAR-5 instrument (from Bender und Hobein, West Germany), with a field intensity of 55-100 V/cm, the cells were kept in the buffer for 107-200 sec, and the cell suspension for fractionation was applied at the rate of 3.6 ml/h.

Stem cells were identified by the method in [9]. Cells of the fraction obtained were injected in a dose of 3×10^4-5×10^4 cells into the cadual vein of (CBA × C57BL)F_1 hybrid mice irradiated in a dose of 1300 rads. On the 8th day the recipient's spleen was fixed in Bouin's solution, after which the number of colonies of hematopoietic stem cells (CFUs) was counted.

EXPERIMENTAL RESULTS

On filtration through cotton usually 7-13% of nucleated cells were lost from the original number of myelokaryocytes, compared with 55-75% after sedimentation in columns. The number of cell fractions obtained was dependent only a little on the duration of electrophoresis. Myelokaryocytes were found in 15-16 fractions. However, most of them (over 98%) were concentrated in 5-8 fractions. The number of cells in the extreme fractions was under 2% and this was usually insufficient for morphological and functional analysis. After fractionation the viability of the cells did not go below 97% and their yield was 60% of the original number in the suspension before fractionation.

Distribution curves of myelokaryocytes among fractions (Fig. 1) always had one peak. Morphological investigation revealed predominantly precursors of granulocytes in fractions with low electrophoretic mobility (EPM), and mainly precursors of erythrocytes in fractions with high EPM. These observations agree with results obtained by other workers for murine [3, 12] and human [8] bone marrow cells.

Determination of splenic colonies showed that CFUs were found in fractions with both high and low EPM. In experiments 1 and 2 most CFUs were located in the region of high EPM (Fig. 1). In experiment 3 most CFUs were found in fractions with low EPM, but in experiment 4 the curve of distribution of CFUs among fractions had two peaks: peak 1 in the region of high EPM (1.62×10^{-4} $cm^2 \cdot V^{-1} \cdot sec^{-1}$), peak 2 in the region of low EPM ($1.25 \times 10^{-4} \cdot cm^2 \cdot V^{-1} \cdot sec^{-1}$). This heterogeneous distribution of CFUs in individual experiments was observed by other workers also [3, 12]. In fractions with the largest number of CFUs the concentration of stem cells was 3-4 times greater than in the original bone-marrow suspension.

The nature of the CFUs with high and low EPM is not yet clear. Zeiller et al. [10] postulated on the basis of the results of experiments with elimination of actively proliferating CFUs by means of cytostatics before bone marrow transplantation into irradiated animals, and also of the results of experiments with retransplantation of cells of splenic

colonies after electrophoretic fractionation, that CFUs with low EPM are in the G_0 stage, and those with high EPM are in the mitotic cycle. Boll [3], who compared EPM of CFUs from normal and regenerating bone marrow, reached a directly opposite conclusion: CFUs in the mitotic cycle possessed lower EPM than CFUs in the G_0 stage.

The nature of CFUs with low and high EPM thus requires further investigation. It can be concluded from data in the literature and the results of the present investigation that fractionation of hematopoietic cells on the basis of differences in their surface charges is a promising method. By means of the electrophoresis method in conjunction with fractionation of cells on the basis of other parameters (volume, density, and so on) it will probably be possible to isolate and identify morphologically and functionally different types of stem cells in hematopoietic tissue.

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DISTRIBUTION BY RNA CONTENT OF CELLS ISOLATED FROM THE NORMAL AND ATHEROSCLEROTIC HUMAN AORTA

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Cells of the intima and media of blood vessel walls play a key role in the process of thickening of the intima and formation of the atherosclerotic plaque [3, 5, 6, 8]. So far the cell composition of the human vascular wall has been studied mainly by classicial microscopic methods [3]. Very little is yet known about the functional and metabolic properties of these cells. The writers have developed a method of obtaining human aortic cells in suspension, which has made it possible to study some of the properties of these cells by the use of the method of flow cytofluorometry. Unlike traditional microscopic methods, the method of flow cytofluorometry can be used to analyze rapidly and with great accuracy many cells and to detect differences between them in their physical, biochemical, and functional properties [2, 4]. The object of this investigation was to determine the heterogeneity of a cell population from the intima and media of the normal and atherosclerotic human aorta on the basis of a metabolic parameter such as the RNA content.

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