Characterisation of normal and Pathological Growth of Human Bone Marrow Cells by Means of a New System Cell Assay

by

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The disturbance in growth of hemopoietic cells in human leukemia takes place at the stem cell level. In order to characterize the leukemic cell populations attention has been focused on first steps of the development of the hemopoietic system.

The stem cell level can be understood as a compartment of cell populations which are distinguished by their capacity of growth and differentation. Normally, the system is in a steady state. Little, however, is known about the precise relationship between the cells within this compartment (Lajtha 75). The variety of these cells complicates considerably a detailed study. Therefore attempts were made to cope with this problem by using isolated cell classes (Moore 72, Quesenberry 74).

Besides cell density (Leif 70) and cell volume (Miller 67) cell surface specific parameters are used for cell separation procedures since it has been shown that the cell surface is involved in various cell functions as maturation (Lichtmann 72), antigenicity (Sandford 67), malignant transformation or metastasizing (Abercombie 62). The negative electric surface charge of mammallian cells is one of the cell surface specific parameters which can be used to discriminate between functionally defined cells (Ruhenstrodt-Bauer 61). The preparative continuous free flow cell electrophoresis enables additionally the functional testing after the separation (Hannig 61). The successful application of this method on mammallian cell mixtures has been shown by several investigators (Hannig 69, Zeiller 72a, 72b, Schubert 73, v. Boehmer 74). It is the purpose of this presentation to show that preparative cell electrophoresis combined with the diffusion chamber assay contributes to further characterisation of the human stem cell compartment.

For the isolation of human bone marrow cells the electrophoretic cell separator FF5 (Bender and Hobein, München) was used. The separation conditions were described elsewhere (Schubert 73, Zeiller 75). After separation the single fractions were tested for their capacity of growth and differentiation by the conventional diffusion chamber method (Benestad 70, Boyum 72). NMRI mice were used as hosts. In order to stimulate erythropoiesis the animals were kept at 0.5 atm.

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immediately after chamber implantation (Schopow 75, Walther 76). Each chamber was filled with 750,000 nucleated cells. Harvesting was done on day nine.

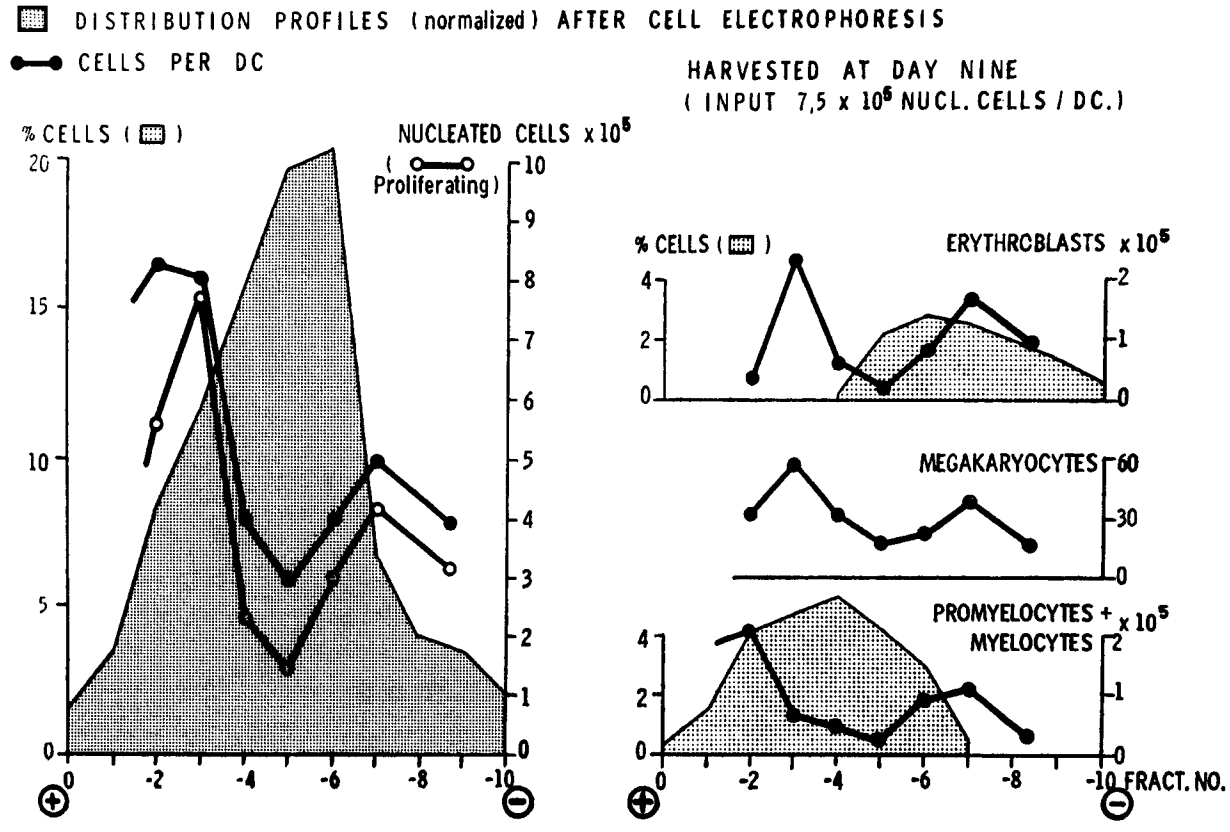

Fig. 1: For explanation see text.

The results of the analysis of normal human bone marrow are shown in Fig. 1. The number of nucleated cells grown in the diffusion chamber are plotted against electrophoretic migration. Each point represents the mean value of up to six chamber contents. It is evident that two different stem cell populations based on their electrophoretic migration are existent with comparable growth capacity. The maxima are in fraction –3 and –6 repsectively. On the right side of Fig. 1 the morphological analysis of the chamber output into erythroblasts, megacaryocytes and immature granulocytes is shown. Again a bimodal distribution is also exhibited by the subpopulations. The cellular composition in both maxima is nearly identical. Besides the cell types described mature granulocytes, unknown blasts and macrophages have also been classified. No correlation exists between the cellular composition filled originally into the chambers (shaded areas Fig. 1) and that harvested on day nine. This gives evidence that the cells grown in the diffusion chambers are newly generated from stem cells which are not accessible to a precise morphological characterisation within the bone marrow cell mixture. The application of this method to human acute leukemia shows a striking difference of the growth behaviour in comparison to normal conditions as shown in Fig. 2 (The graphic presentation is in accordance to Fig. 1). On day nine only those cells caused progeny in the chambers which originated from the region of slow electrophoretic migration (fraction –5 to –10). The lack of cell growth in the fast migrating region

which contained originally the myeloblasts of the bone marrow can be due to very different growth kinetics or insufficient proliferation capacity. Both statements are not in accordance with observations made with unseparated peripheral human blood myeloblasts (Hoelzer 74).

If these results with bone marrow cells of human acute leukemia are specific must be proven in further experiments. With the presented experimental approach it might be possible to seize upon disturbances at the stem cell level and contribute to further insight into the pathogeneses of human leukemias.

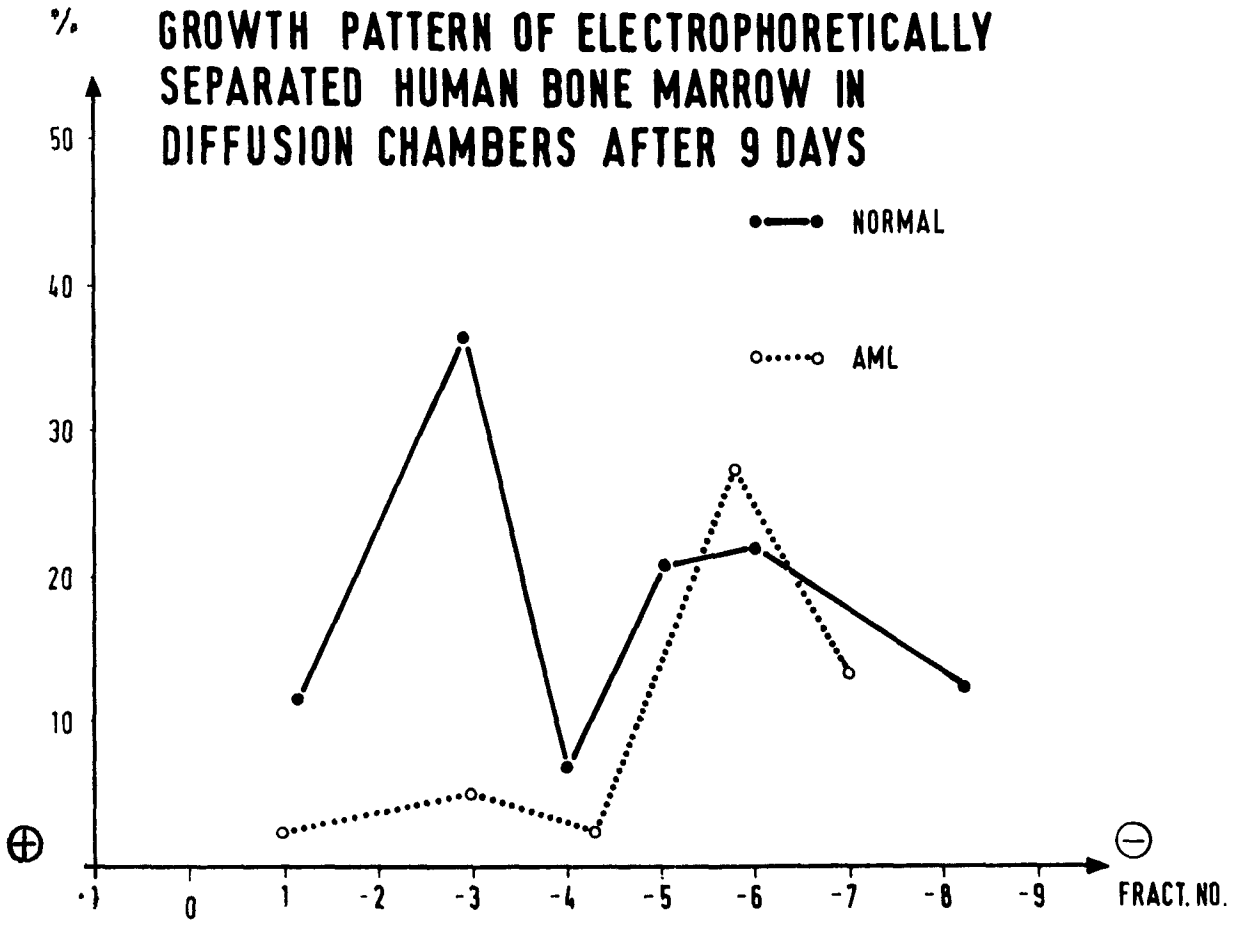

Fig. 2: Nucleated cells normalized grown in DC on day nine plotted against electrophoretic migration. (Myeloblasts subtracted).

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