ELECTROPHORETIC DISTRIBUTION PROFILES OF BONE MARROW STEM CELLS

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The preparative electrophoretic separation technique has found successful application in the separation of hemopoietic cells. Within human bone marrow cells – in mice we received similar datas – different distribution profiles could be observed. Granulopoiesis (fig. 1) shows a decreasing electrophoretic mobility (EPM) correlated with increasing maturation whereby the myeloblasts possess the highest and the segments the lowest EPM. Fraction 0 is characterized by the mass of erythrocytes. No differences exist between normal and leukemic granulopoiesis concerning the EPM. Antibody producing cells as normal plasma cells and lymphoid cells in M. WALDENSTRÖM show a low EPM.

The lymphoid cell series have intensively been investigated by ZEILLER et al. (1) by means of this method. Two subpopulations of lymphocytes in lymphoid tissues of rodents can be distinguished. The lymphocytes with high EPM mediated the graft versus host reactivity whereas the lymphocytes of low EPM provided antibody forming cells. Both cell classes have been classified as T- and B-cells, respectively. In cell cooperation experiments these cell functions have been confirmed (2).

Undergoing the total cell distribution of murine bone marrow the TILL and McCULLOCH-technique (3) for the demonstration of Colony Forming Units (CFU) different stem cell properties can be detected (fig. 2) (4). Each electrophoretically separated fraction was i. v. injected into lethally irradiated 8–12 weeks old mice (F_1 hybrids: DBA/2J/Bom x C3H/Tif/Bom). Isogenic grafts and hosts were used. Most of the colonies were found in the region of relatively higher EPM. As the CFU obtained of all fractions gave rise to differentiated cells and to new colonies in regrafting experiments the criteria of pluripotent stem cells were fulfilled.

According to the electrophoretic mobility different properties of the CFUs achieved could be demonstrated.

1) The colonies originated from bone marrow cells of high EPM showed another cellular composition than those ones of low EPM. The first ones are characterized by mostly erythropoietic cells while the other ones are more frequent mixed colonies of two or three hemopoietic cell systems.

2) Pretreatment of donor mice with lethal doses of vinblastine 24 or 48 hours before cell separation resulted in a complete loss of colonies derived of bone marrow cells of high EPM.
3) Reelectrophoresis of pooled colonies derived of either the slow or the fast region showed colony growth after the second electrophoresis *only* with cells of high EPM. Apparently the CFU of low EPM have transformed into CFU of high EPM after colony growth.

These results with rodent bone marrow have encouraged us to investigate human bone marrow stem cells with the preparative cell electrophoresis. A modified agar-colony-technique as developed by BRADLEY and METCALF (5) and the millipore chamber system were used as test for stem cell properties.

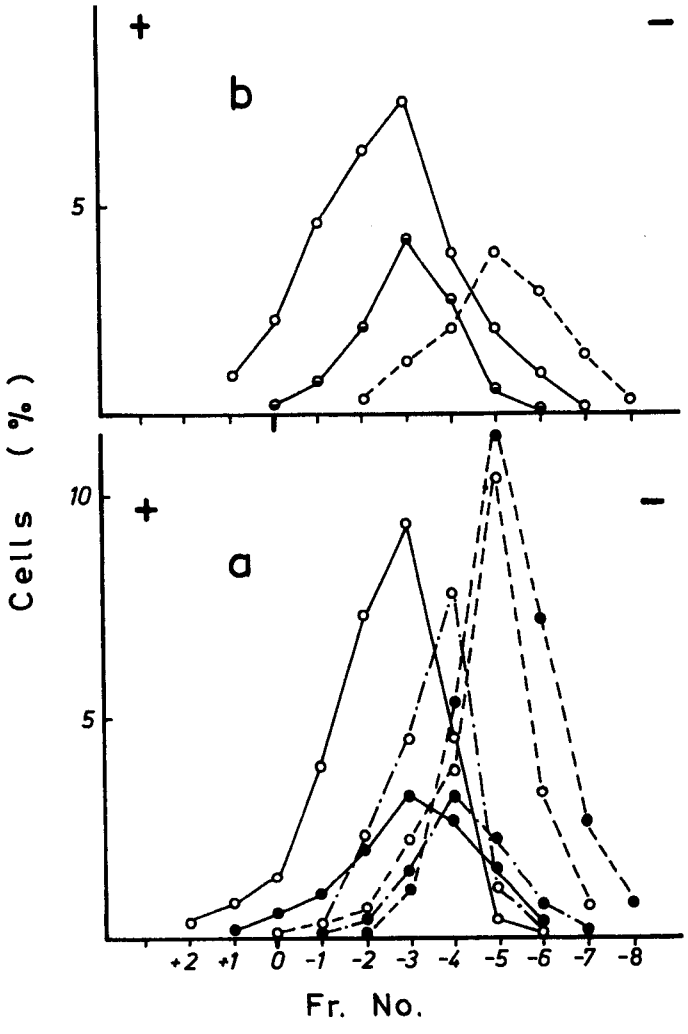

Fig. 1: Electrophoretic Distribution Profiles of Granulopoiesis.

a) —— promyelocytes and myelocytes
—.— metamyelocytes and rods
– – – segments
● normal, O chronic myeloid leukemia

b) acute myeloblastic leukemia
●—● myeloblasts
O—O promyelocytes and myelocytes
O– –O segments

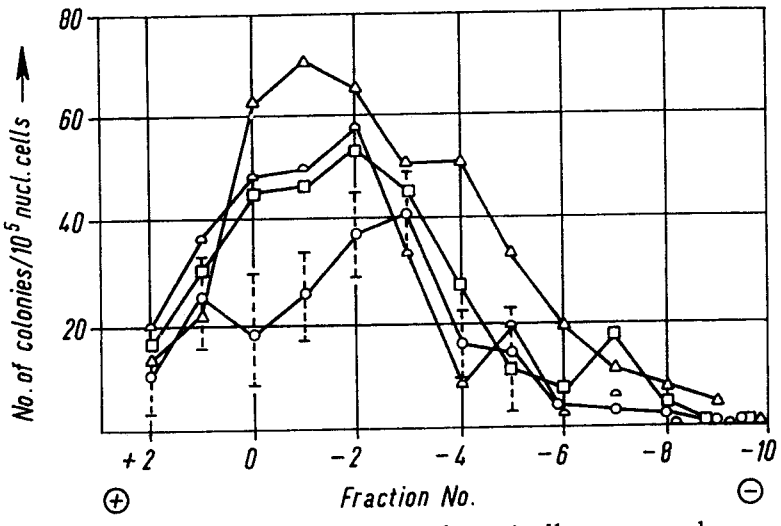

Fig. 2: Relative distribution of CFU in electrophoretically separated normal bone marrow. Four separate experiments are shown. Each point represents the mean of 10 animals. Vertical bars indicate 90 % confidence limits (4).

Patient	Region of EPM / Diagnosis	High	Medium	Low
		Number of colonies per 3 x 10^5 implanted nucleated BM cells		
		$\bar{X}$	$\bar{X}$	$\bar{X}$
S	Normal	160		7
Wi	Normal	127		12
We	Normal	63		35
K	Chronic grannlocytic leukemia	98		5
M	Acute leukemia (blast cells POX +++)	18	11	6
H	Acute leukemia (blast cells non specific esterase +++)	31	31	18
B	Acute leukemia (blast cells glycogen staining+++)	14		10
		73 ±		13 ±

Fig. 3: Colony Formation by Human Nucleated Bone Marrow Cells in Agar-gel after Separation in an Electric Field.

The agar in vitro system based on a CO_2-free medium buffered by carrier ampholytes. The optimum of colony growth was found at pH 6.7. The results obtained by this method were comparable to those reported in literature (6, 7).

After cell separation of human bone marrow the nucleated cells were devided into two fractions of low and high EPM respectively. The slow migrating fractions consisted predominantly of rods, segments, mature monocytes, normoblasts, plas-

ma cells and lymphoid cells, whereas the high EPM region showed mostly erythrocytes, myeloblasts, immature granulocytes and in addition lymphoid cells. In two cases a third region with medium EPM was distinguished. The table (fig. 3) shows the distribution of agar colonies of 7 cases related to the different regions of EPM. Definitely higher colony counts were found with cells of high EPM. In the cases of normal and chronic myelocytic leukemia bone marrow ten to twenty times as much colonies were counted in comparison to the slow EPM region. The other cases showed the same tendency but in a lesser degree.

In parallel the nucleated cells of the two separated fractions were implanted into millipore chambers which were repeatedly grafted into sublethally irradiated mice. After two weeks all the chambers were colonized. No differences were detected in cell composition and number of cells in the chambers regardless wether the chamber content was derived from bone marrow cells with high or low EPM.

Our results may allow the following tentative conclusions:

There are existing two murine and probably two human bone marrow cells with stem cell properties. One is characterized by a higher EPM, a sensitivity against antimitotic drugs like vinblastine and a limited capacity of differentiation. The other one shows a lower EPM, vinblastine resistence and a possible higher capacity of differentiation. It seems probably that under certain conditions this slow migrating cell transforms into a faster migrating one.

We feel that the assumption of an electrophoretically slow migrating omnipotent hemic stem cell is allowed. This fact might be the key for isolating these cells from the faster migrating, graft versus host reactivity mediating T-cells. Thus the different electrophoretic behavior of bone marrow cells might gain importance in bone marrow transplantation.

Literature:

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