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K 562 Cell Line in Plasma Clot Diffusion Chambers: Changes in Cell Surface Phenotype in Relationship to Culture Conditions

B. Lau, G. Jäger, E. Korge, and P. Dörmer

A. Introduction

It is well known that proliferation and subsequent differentiation of normal hemopoietic progenitor cells require an appropriate inductive microenvironment and the addition of specific regulatory factors [7]. This is also true for differentiation of leukemic blast cells, which are considered to represent early stages of a hemopoietic cell lineage [6].

In order to examine whether the phenotypic characteristics of the K 562 cell line originally established from cells during blast crisis of a chronic myelocytic leukemia [4] can be influenced by environmental conditions of the culture, we ued the in vivo plasma clot diffusion chamber (DC) technique [8]. Normal CBA mice (group A) as well as animals rendered anemic with phenylhydrazine (group B) served as DC recipients.

B. Material and Methods

The K 562 cell line, kindly provided by Dr. L. C. Andersson, Helsinki, was maintained in RPMI 1640 medium plus 15% heat-inactivated fetal calf serum and antibiotics. Cells grew in a roller suspension and were adjusted to $1-1.5 \times 10^6$ cells/ml.

According to the method of Steinberg et al. [8] DCs (pore size 0.22 µ) were filled with 5×10^5 K 562 line cells suspended in 100 µl TC medium 199. Thereafter, 20 µl of citrated bovine plasma was added to each chamber, two of which were inserted into the peritoneal cavity of each CBA mouse (25–30 g). Animals in group A were injected intraperitoneally with 0.1 ml isotonic NaCl solution either 24 or 3 h prior to the implantation of chambers. The same time schedule was applied to CBA mice in group B, which had been pretreated with phenylhydrazine (50 mg i.p./100 g body wt.). Hematocrits and reticulocytes were determined from free-flowing tail blood of the DC recipients.

At different instances during the culture period DCs were removed, and were shaken in a 2.0% pronase solution. Besides total and differential counts one part of the chamber yield was processed for cell surface characterization with heterologous rabbit anti-glycophorin A antiserum [1], and rabbit anti-human granulocyte antiserum [3]. Evaluation was performed by applying indirect immunofluorescence. To avoid unspecific binding, all cells were preincubated with aggregated human IgG. Additionally, in situ hybridization of globin mRNA was performed using ^{3}H-cDNA derived from rat globin mRNA. Grains over individual cells were counted by incident light microphotometry [2].

C. Results and Conclusions

Whereas the hematocrits and reticulocytes of mice in group A ranged within normal levels, these parameters changed considerably in animals of group B which had been pretreated with phenylhydrazine. In this group, hematocrit values dropped from an average of 47% to a mean of 27% between day 1 and day 3 of the culture period. At the same time the number of reticulocytes increased up to 95%.

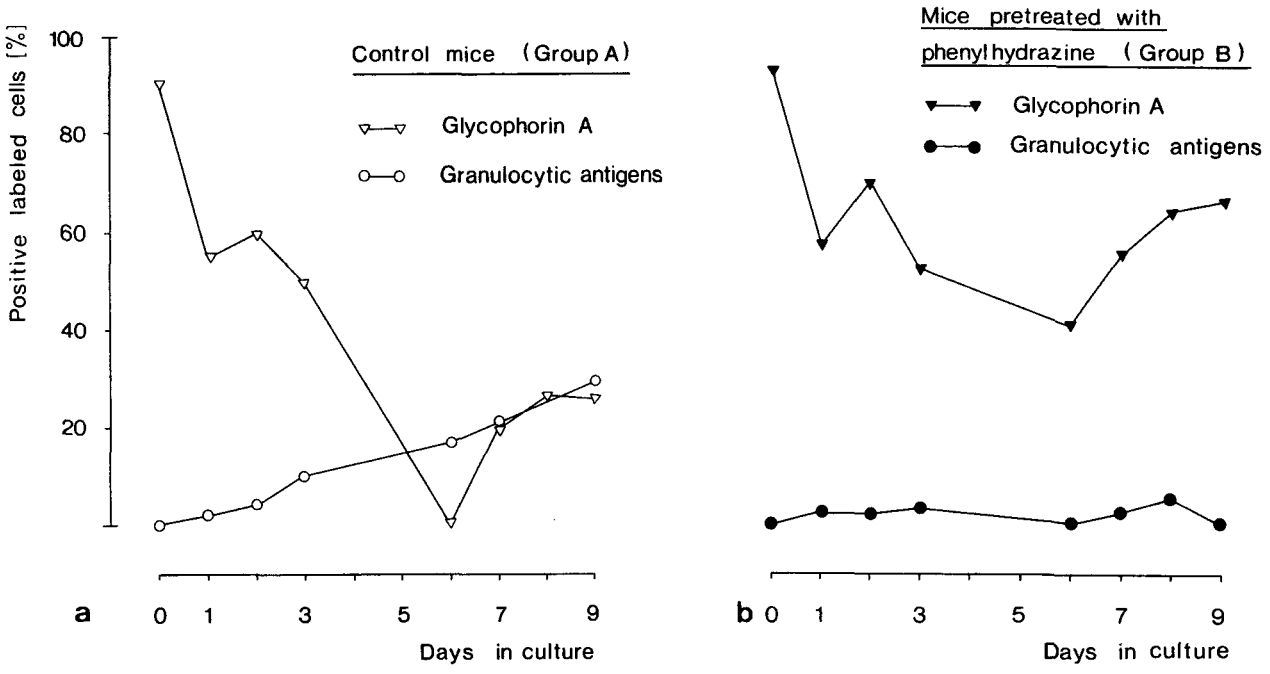

Fig. 1. Cellular phenotype characteristics of K 562 cells during a 9-day plasma clot DC culture in normal **a** and anemic **b** mice

In spite of these hematological perturbations, the growth pattern of K 562 cells was quite similar in both groups. It could be characterized by an increase in cell number until day 6, after which the chamber content obtained from anemic mice decreased slightly more than that from animals in group A. Morphological examination revealed no changes in the blast-like appearance of K 562 line cells under both culture conditions. However, differences were observed in the immunological characteristics of the chamber yield in groups A and B. As illustrated in Fig. 1, a high percentage of the chamber input positively labeled with the anti-glycophorin A antibody. After a gradual decline of glycophorin A positive cells during culture in anemic animals, there was a continuous increase from day 6 onward, reaching 70% at the end of the observation period. By using normal mice as DC hosts in group A, cells carrying glycophorin A completely disappered on day 6. Thereafter, these cells reappeared but did not exceed 30% toward the end of the culturing experiment. Concerning the reaction pattern with anti-granulocyte antiserum, a clear difference in the percentage of positive cells occurred from day 6 on, depending on whether normal or anemic mice served as DC recipients. In group A mice (Fig. 1 a) the number of cells characterized by the presence of granulocytic antigens increased up to approximately 30% on day 9, whereas few or no positive cells could be seen during culture in mice of group B (Fig. 1 b).

In accordance with the immunological findings, an increased content of globin mRNA was detected by in situ hybridization of K 562 line cells from group B. The maximum of grain counts which were equally well distributed over the nuclei and the cytoplasm was seen between day 6 and 9 of the culture.

Our observations provide evidence for a modulation in the differentiation of K 562 line cells depending on environmental conditions during plasma clot DC culture. The expression of granulocytic antigens, on one hand, and the maturation along the erythropoietic lineage, on the other hand, support the hypothesis of a bipotential differentiation capacity of K 562 line cells, a hypothesis which is in line with the findings of other groups [5].

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