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Changes in Isoenzyme Patterns Expressed by the Erythroleukemia Cell Lines K-562 and HEL After Induction of Differentiation

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A. Introduction

Stable human leukemia–lymphoma cell lines provide model systems to study the processes involved in leukemic and normal cell differentiation [10]. Leukemic cells, arrested at a certain stage of differentiation, can be triggered to differentiate to functionally and morphologically more mature cells [7]. Both "fresh" leukemic cells and cells maintained in long-term culture are sensitive to in vitro induction of differentiation. Furthermore, cell differentiation is a novel concept in the treatment of acute leukemias and several substances such as low dose Ara-C and the physiologic compound retinoic acid have been shown to act as differentiating agents in vivo [7].

Two human erythroleukemia cell lines, K-562 [8] and HEL [9], were used to study the effects of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and of a differentiation inducing factor-(DIF)-containing medium for induction of differentiation.

B. Materials and Methods

Conditioned medium was prepared by stimulating cultured lymphocytes of the T cell line HUT-102 with TPA for 5 h. The TPA-free supernatant from the stimulated cells (harvested after 48 h) had multiple biologic activities including the DIF. A concentration of 5×10^5 cells/ml was incubated in the presence of 10^{-8}–10^{-11} *M* TPA or of 5%–10% DIF-containing medium in RPMI 1640 medium supplemented with 5% Fetal Calf Serum at 37 °C in 5% CO_2 humidified atmosphere.

Cells were harvested after 0, 24, 48, 72, and 96 h and examined for the following parameters: cell growth and viability by trypan blue dye exclusion test, morphology (cytospin preparation stained with Wright–Giemsa), nitro blue tetrazolium (NBT) reduction test, and main emphasis on isoenzyme patterns of the enzymes carboxylic esterase (Est, EC 3.1.1.1), acid phosphatase (acP, EC 3.1.3.2), hexosaminidase (Hex, β-*N*-acetylglucosaminidase, EC 3.2.1.30) and lactate dehydrogenase (LDH, EC 1.1.1.27). Enzymes were separated into isoenzymes by isoelectric focusing (IEF) on horizontal thin layer gels containing 4.8% polyacrylamide [1]. Isoenzymes were visualized by histocytochemical staining techniques as described in detail earlier [1–3].

C. Results

I. Morphology

The most striking alterations in the morphology of the HEL cells during treatment with TPA was the prominent size change. HEL cells became larger with more cyto-

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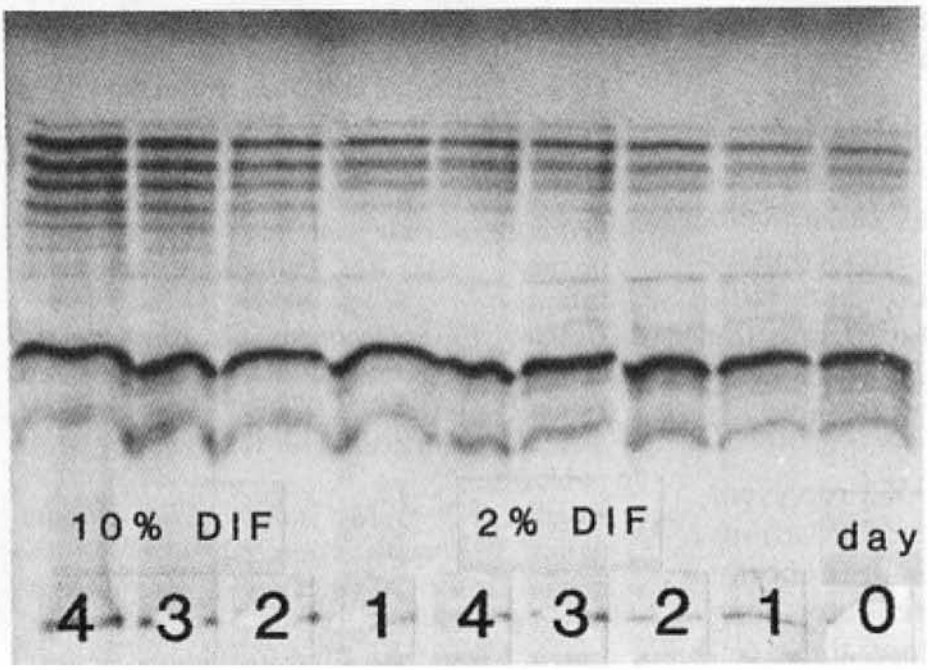

Fig. 1. Induction of differentiation of K-562 with a differentiation inducing factor (DIF). Esterase isoenzyme profiles of K-562 at day 0, 1, 2, 3, and 4 during exposure to 2% and 10% DIF in the medium. *Left side* 10% DIF; *right side* 2% DIF (*top* cathode, *bottom* anode)

plasm. Some cells were extremely large with a tendency to spread out accompanied by adherence to plastic surfaces and development of pseudopodia. In most cells the cytoplasm was vacuolated; many multinucleated cells were seen. DIF did not induce such strong cytoplasmic changes, but enlargement and vacuolization of the cytoplasm were also detected. TPA led to vacuolization and increase of cytoplasm in K-562. No change of size was found after exposure to DIF, only larger and more vacuoles were seen in K-562. K-562 cells did not show surface adherence or pseudopodia.

II. NBT Reduction

A maximum of 40% of the HEL cells became NBT positive with either 10^{-9} or 10^{-10} *M* TPA or 10% DIF. Maximally, 12% of the K-562 cells showed positivity with 10^{-9} *M* TPA.

III. Cell Growth

TPA was very cytotoxic (for HEL more than for K-562). DIF was less cytotoxic (for K-562, no effect on HEL).

IV. Isoenzymes

TPA and DIF induced the new expression and a stronger staining intensity of several Est isoenzymes in K-562 (Fig. 1). A stronger staining intensity and one new Est isoenzyme were seen in HEL with TPA. An Est isoenzyme which is specific for monocytes [2] could not be detected in K-562 or HEL after TPA or DIF treatment. TPA and DIF increased the intensity of all AcP isoenzymes in K-562, including a tartrate-resistant AcP band [1], whereas no changes in the AcP profile occurred in HEL. TPA and DIF (TPA > DIF) led to the new expression of the Hex A isoenzyme, an increase in the intensity of Hex B, and the loss of Hex I in K-562. An increase of Hex A was seen in HEL. One new isoenzyme was induced in the LDH pattern (LDH 1) in both K-562 and HEL by TPA and by DIF.

D. Discussion

Phenotypic changes could be induced by use of the chemical agent TPA and the physiologic inducer DIF in K-562 and HEL. Although not identical, the changes seen after exposure to TPA were similar to those induced by DIF. Recently, a DIF obtained from the same cell line which we used (HUT-102) has been extracted, purified, and characterized [11]. The changes seen in K-562 were quantitatively and qualitatively stronger than in HEL when compared with their original features. HEL cells differentiated mainly along the myeloid–macrophage cell lineage (mor-

phological changes, NBT positivity, adherence to plastic surfaces, development of pseudopodia, and typical isoenzymatic alterations), but also along the erythroid cell axis as we detected the expression of hemoglobin (data not shown). K-562 cells which appear to be originally arrested at an earlier stage of differentiation than HEL cells [4–6] probably differentiated into the myeloid and erythroid series. A monocyte-specific isoenzyme [2] was not found in K-562 or HEL.

In conclusion, HEL and K-562 represent cell lines which can be induced by various agents such as TPA, DIF, hemin (data not shown), and others to differentiate along different cell lineages. Therefore, these cell lines have been termed multipotential stem cells.

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