In Vitro Differentiation of a Human Erythroid Cell Line (KMOE) Induced by Some Metabolic Inhibitors

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Summary. KMOE-2/05, a continuous human erythroid cell line derived from a patient with acute erythremia was capable of differentiating into benzidine positive cells following exposure to cytosine arabinoside (CA), mitomycin C, or daunorubicin. Among the three substances, CA was the most effective inducer. Other compounds, reported as effective inducers on human and murine erythroid cells, were also tested but they were ineffective.

Benzidine positive cells were counted to be approximately 500/1 x 10^5 cells after 10 days incubation with CA at its optimal concentration of 1 x 10^-5 M. Under the same conditions, the hemoglobin (Hb) concentration quantitated by radioimmunoassay (RIA) was more than 500 ng/1 x 10^6 cells. Quantitative kinetics of synthesized Hb and of benzidine-positive cell counts, after exposure to CA were closely correlated.

Key words: Hemoglobin A synthesis – KMOE cells – Human erythroid cells – Differentiation induction

Introduction

Continuous erythroid cell lines of human origin provide a good model system for elucidating specific events occurring during differentiation. The K562 cell line, derived from a patient with chronic myelogenous leukemia in blast crisis (Lozzio and Lozzio 1975) is capable of erythroid differentiation (Andersson et al. 1979). Recently, another human erythroleukemic cell line, HEL, derived from a patient with Hodgkin’s disease who later developed erythroleukemia, has been reported as capable of erythroid differentiation (Martin and Papayannopoulou 1982). K562 cells produce fetal and embryonic hemoglobin (Hb) induced by hemin or butyric acid (Rutherford et al. 1979; Cioe et al. 1981) and HEL cells synthesize Hb Bart’s (Hb γ4) induced by hemin (Martin and Papayannopoulou 1982).

The KMOE cell line, established from a patient with acute erythremia also has an erythroid nature since benzidine positive cells have been found within colonies formed on a soft agar plate (Okano et al. 1981).

The present report describes the evidence of Hb synthesis of KMOE cells in suspension culture, detected by benzidine staining or quantitated by radioimmunoassay (RIA), after the addition of various compounds known as inducers. Synthesized Hb was preliminarily characterized by isoelectric focusing analysis (Kaku et al. 1984).

Material and Methods

Cells

Original KMOE cells in suspension culture were negative for benzidine staining. To try to obtain clones with a greater differentiation potential cell cloning was attempted by the separation of clones from benzidine positive colonies formed within semi-solid culture plates. Repeated cell clonings resulted in clone KMOE-2/05, which showed the highest benzidine positivity among the clones obtained even without the addition of the inducer, and was used throughout the experiments.

Culture Conditions and Study of Hb Synthesis

Cells were maintained in alpha-MEM (GIBCO, USA) supplemented with 15% fetal bovine serum (Flow Labs., USA) at 37 °C in a humidified atmosphere with 5% CO2. The cells were seeded on 60-mm glass Petri dishes containing 8 ml of culture medium, at a density of 5 x 10^4 cells/ml. All chemicals tested as inducers were added to the plates 2 days after the seeding. At appropriate intervals, benzidine positive cells were assessed, and the amount of Hb was quantitated by RIA. The viability of the cells was tested by trypan blue dye exclusion.
Benzidine Staining

Hb synthesis was scored by benzidine staining according to the method described by Orkin et al. (1975).

Quantitation of Synthesized Hb by RIA

Cells were pelleted by low speed centrifugation and disrupted by ultrasonication. The cell lysates were then centrifuged again at 2,000 rpm for 10 min. The supernatant thus prepared was used as the sample for RIA. Human Hb (Sigma Chem. USA) was radioiodinated by the chloramine T method. Radioiodinated Hb solution (100 μl) was mixed with 100 μl of the sample in a plastic tube (Falcon, 2038, USA) and coated with guinea pig antibody against human Hb. The tubes containing the mixture were rotated for 5 h at room temperature, then rinsed three times with phosphate buffered saline containing 0.5% bovine serum albumin. The radioactivity of each tube was counted and the synthesized Hb was estimated.

Results

Response of KMOE-2/05 Cells to Various Compounds

a) Detection of Hb Synthesis by Benzidine staining. A total of 14 compounds known as effective inducers of K562 and/or Friends Cells were tested for their ability to induce Hb synthesis of the KMOE-2/05 cells. As an indication of Hb synthesis, benzidine positive cells/1 x 10^5 cells were counted. The results are summarized in Table 1.

Benzidine positive cells within a population of KMOE-2/05 cells in suspension culture without the presence of any inducers were counted as 1-2 cells/1 x 10^5 cells. A significant increase in the number of benzidine positive cells was noticed after the addition of cytosine arabinoside (CA), mitomycin C or daunorubicin, CA was the most effective inducer of the three. Mitomycin C and daunorubicin showed a positive but weak effect. Other metabolic inhibitors, 5-fluorouracil, 6-mercaptopurine, bleomycin, methotrexate and actinomycin D were not effective. The polar compounds such as dimethyl sulfoxide (DMSO), and hexamethylene-bis-acetamide (HMBA) were also ineffective.

The effect of CA, the most effective chemical, was tested at 5 different concentrations (Table 1). The number of benzidine positive cells at any concentration was higher than that of the control culture. The maximum effect of CA was observed at a concentration of 1 x 10^-5 M. At concentrations of CA greater than 1 x 10^-5 M, a severe cytotoxic effect was noted resulting in lower inductive effects.

Figure 1 shows the proportion of benzidine positive cells after various exposure periods to CA at a concentration of 1 x 10^-3 M. Both the numbers of total cells and benzidine positive cells were counted every other day. A significant increase in number of benzidine positive cells was first noted on day 5 and reached a maximum on day 10 after the addition of CA. At that time, benzidine positive cells were estimated to be 500/10^5 cells. The viability of cells and the count of positive cells declined at starting day 10 of treatment.

b) Quantitation of Synthesized Hb by RIA in the Presence of Some Inducers. The Hb synthesized after the addition of CA was quantitated by RIA. CA was added to the culture on day 2, at various concentrations, and the synthesized Hb was quantitated on day 12. As shown in Fig. 2, the maximum effect of CA on the synthesis of Hb was observed at a concentration of 1 x 10^-5 M. Figure 3 also gives the quanti-