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Carbonic Anhydrase Isozymes in Cultured Friend Leukemic Cells[1] (39873)

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Introduction. Friend virus-induced mouse erythroleukemia cells grown in the presence of dimethyl sulfoxide (Me_2SO) produce globin, heme, and erythrocyte-specific surface antigens (1). We have now quantitated levels of carbonic anhydrase I (CA I) and carbonic anhydrase II (CA II), the second-most-abundant proteins in the erythrocyte, in these cells. Each isozyme is the product of a separate gene (2) and together they contribute two additional biochemical markers for characterizing different clones and stages of differentiation of Friend cells.

Materials and methods. Four different experiments were performed. Cells for experiments 1–3 were grown in New Haven. They were all from clone 745 and were grown by the procedures of Friend *et al.* (3) except for the use of Dulbecco's modified Eagle medium (GIBCO) supplemented with glutamine. For experiments 1–3, exponentially growing inocula were seeded at 10^5 cells/ml on Day 0 in the presence or absence of 2% redistilled Me_2SO. Clone 745A cells used in experiment 4 were grown, with and without 2% Me_2SO (not redistilled), in New York. Cells were seeded at 10^5 cells/ml and Me_2SO was added at the same time the cells were seeded. In all cases, pellets containing 1 to 2 × 10^8 cells were shipped on dry ice to Baltimore. Cells from experiment 3 thawed in transit. Since enzyme levels in these samples were comparable to those in the other experiments, they are included here.

Prior to assay, cells were thawed and an approximately equal volume (0.1 ml) of 1 mM potassium phosphate buffer, pH 7.4, was added. Thereafter, cell disruption was ensured by three freeze–thaw cycles. Cellular debris was removed by 5-min centrifugation in a Beckman microfuge and supernatant fluids were stored at 4°.

Procedures for quantitative radial immunodiffusion analysis of carbonic anhydrase, as well as purification of mouse carbonic anhydrase and preparation of rabbit anti-mouse carbonic anhydrase sera for experiments 1–3, are described elsewhere (4). For experiment 4, mouse carbonic anhydrases were purified by affinity chromatography (5) and goat anti-mouse carbonic anhydrase sera were prepared. Friend cell lysates, unlike mouse erythrocyte hemolysates, consistently showed two precipitin rings with rabbit anti-mouse CA I; only the larger ring was used for calculation of CA I levels. Correlation coefficients for standard curves were greater than 0.99. The average coefficient of variation in quadruplicate assays of each of 12 samples was 8.6% for CA I and 10% for CA II.

Cell lysate protein concentrations were estimated by the procedure of Lowry *et al.* (6) using known concentrations of purified bovine serum albumin as standards.

For experiments 1–3, hemoglobin concentrations were determined by spectrophotometry following addition of Drabkins so-

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lution (1 g of $NaHCO_3$, 50 mg of KCN, and 200 mg of $K_3Fe(CN)_6$/liter of water). Because such mixtures were unusually turbid, the area of the absorbance curve above a line connecting optical density at 510 and 600 nm was used to estimate hemoglobin levels. Hemoglobin concentrations for experiment 4 were estimated by radial immunodiffusion analysis (7) using goat anti-human hemoglobin A crossreactive with mouse hemoglobin. Correlation coefficients for mouse hemoglobin standard curves were greater than 0.99.

Results. Friend cell carbonic anhydrase levels are shown for both Me_2SO-treated and untreated cultures in Fig. 1, where they are expressed as the percentage of soluble protein. Corresponding changes in percentage hemoglobin are illustrated for Me_2SO-treated cultures in Fig. 2. The numbering of the experiments corresponds to the hemoglobin concentration observed in Me_2SO-treated cultures of the final day, a measure of the degree of erythroid differentiation attained. If the final CA I levels for the experiments are expressed in terms of micrograms of CA I per milligram of hemoglobin (93, 19, 24, and 3.3), the cultures with the higher hemoglobin levels have CA I levels closer to those of a DBA/2J mouse erythrocyte (6.8) (8). Similarly, for CA II levels (89, 40, 21, and 9.6), cultures with the higher hemoglobin levels are also more like DBA/2J erythrocytes (7.6) (8).

Despite variability between experiments, two general patterns emerge: First, CA I levels remain constant or decrease somewhat during treatment with Me_2SO, whereas they increase in most untreated control cultures; second, CA II levels increase in Me_2SO-treated cultures and control cultures.

Discussion. Our studies are somewhat different from those of Kabat *et al.* (9) who

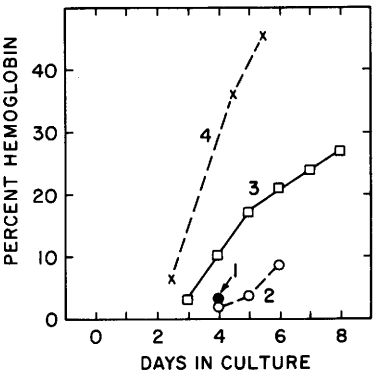

FIG. 2. Percentage of soluble protein represented by hemoglobin in four different cultures of Me_2SO-treated Friend cell cultures. Numbers denote individual experiments.

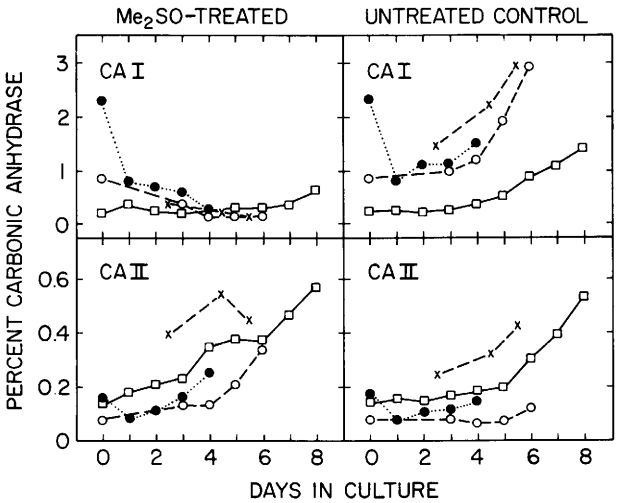

FIG. 1. Percentage of soluble protein represented by immunologically measured CA I and CA II in cultures of Friend virus-infected mouse erythroleukemic cells. Symbols for the four different sets of experiments are the same as those used in Fig. 2. Data for experiment 4 are offset to the left by a half-day for graphic presentation both here and in Fig. 2.

recently examined total carbonic anhydrase enzymatic activity in FSD-1 mouse erythroleukemia cells. After 4 days in culture, Me_2SO-treated cultures had approximately three times the activity of control cultures. Comparison with our findings is difficult as a different cell line and culture procedure were used, the carbonic anhydrase levels attained in Me_2SO-treated cultures (expressed as percentage of mouse erythrocyte levels) were higher in our cultures, and prediction of combined enzyme activity in unfractionated extracts from protein levels of individual carbonic anhydrase isozymes is unreliable. Despite such difficulties, it is possible, in view of the considerably greater specific activity of CA II (2), that the approximately twofold-greater level of CA II in Me_2SO-treated versus untreated cultures at 4 days led to an equivalent difference in enzymatic activity.

Two other studies on carbonic anhydrase activity indicate that the presence of activity and its increase in Me_2SO-treated cultures are related to the erythroid nature of these cell lines. Conscience, Miller, Henry, and Ruddle (submitted for publication) have shown that a number of nonerythroid cultured mouse cells show no carbonic anhydrase activity, while a noninducible Friend cell line fails to show an increase in carbonic anhydrase activity on Me_2SO treatment. Hybridization with nonerythroid cells, which results in a loss of hemoglobin and globin mRNA, produces a considerable decrease or disappearance of carbonic anhydrase activity. Scher, Parkes, and Friend (manuscript in preparation) have shown that butyric acid, another inducer of erythroid differentiation, also produces an increase in carbonic anhydrase activity. Bromodeoxyuridine, which inhibits erythroid differentiation, also inhibits the rise in CA activity produced by Me_2SO or butyric acid.

Our results may also be compared with those of the two available studies of carbonic anhydrase biosynthesis by authentic erythroid tissue. One of these studies (10) deals with biosynthesis of radiolabeled CA I and CA II in different density fractions of marrow cells from *Macaca nemestrina* (pigtailed macaque). The other study (11), whose analysis was limited to assay of total enzymatic activity, was based on rabbit bone marrow cell fractions with different sedimentation properties. In the study of macaque marrow, the biosynthetic ratios between CA I and CA II and hemoglobin were approximately the same as the concentration ratios for these proteins in the mature erythrocyte, suggesting coincident increases in concentrations of the three proteins. The study in the rabbit showed coincident increases in CA II and hemoglobin concentrations provided it is assumed, as is true in the mature erythrocyte (2), that a high proportion of total carbonic anhydrase enzymatic activity is due to CA II. Thus, the coincident increases in concentrations of hemoglobin (Fig. 2) and CA II (Fig. 1) seen in Me_2SO-treated cell cultures agree with observations in macaque and rabbit bone marrow cells. The results for CA I and Me_2SO-treated cells do not, however, correspond directly to the data from macaque bone marrow cells.

Although these comparisons suggest that the developmental changes observed in Me_2SO-treated cells *in vitro* do not duplicate those observed *in vivo,* the CA I decreases and CA II increases do result in enzymatic patterns like those of mouse erythrocytes. Cultures with higher final hemoglobin levels had resemblance to erythrocytes in isozyme patterns. The attainment of erythroid isozyme patterns, despite the differences between marrow and Friend cell baseline values, suggests that the mechanisms governing these in cultured cells may be the same as those operating in marrow cells.

The reasons for the changes in CA I and CA II seen in control cultures are not known, but are likely due to a depletion or change in pH in the media. In an experiment in which cultures were diluted with fresh media on Day 4 (data not shown), the increase in CA I and CA II levels in the control culture was not observed. Dilution of a Me_2SO-treated culture had no effect.

Summary. Carbonic anhydrase isozymes I and II (CA I and CA II) were assayed in cultured Friend leukemic cells by radial immunodiffusion. In dimethyl sulfoxide-treated cultures, CA I levels remain constant or decrease somewhat, while CA II

levels increase. However, in untreated control cultures both CA I and CA II levels increase.

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