Differing Responses of Globin and Glycophorin Gene Expression to Hemin in the Human Leukemia Cell Line K562

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The human leukemia cell line, K562, produces embryonic and fetal hemoglobins and glycophorin A, proteins normally associated only with erythroid cells. Hemoglobin accumulation is enhanced by exposure of the cells to 0.05 mM hemin. We have examined K562 cells before and after exposure to hemin to determine whether expression of these erythroid proteins was shared by all cells or confined to specific subpopulations. Globin gene expression was examined by quantitation of globin mRNA sequences, using a 3H-globin cDNA molecular hybridization probe. Constitutive cells produced globin mRNA, the content of which was increased 3–4-fold by hemin. Cell-to-cell distribution of globin mRNA was determined by in situ hybridization of 3H-globin cDNA to constitutive and hemin-treated K562 cells. Virtually all cells in the culture exhibited grain counts above background, indicating globin gene expression by all cells, rather than a confined subpopulation. Virtually all hemin-treated cells had 3–5-fold higher grain counts, indicating uniformly increased globin gene expression. The glycophorin content of K562 cells was estimated by fluorescence-activated cell sorting (FACS) of cells labeled with fluorescein-labeled anticycloglycophorin antiserum. The vast majority of constitutive cells contained glycophorin, but exhibited no apparent increase in glycophorin accumulation after hemin exposure. Thus, glycophorin and globin genes exhibited differential responses to hemin. These differences could reflect normal differences in the patterns of specialized gene expression in stem cells. Alternatively, different aberrations of gene expression could be occurring in response to the determinants of the neoplastic properties of K562.

The K562 cell line, originally isolated by Lozzio and Lozzio from the pleural effusion of an adult in terminal blast crisis of chronic myelogenous leukemia, exhibits some phenotypic features typical only of the erythroid series among normal human hematopoietic cells. These include production of embryonic and fetal hemoglobins and globin messenger RNAs; accumulation of glycophorin A; lactate dehydrogenase (LDH) isoenzyme patterns characteristic of fetal red cells; and alterations of heme metabolism in response to exogenous heme that resemble those of erythroid precursors. Some of these erythroid phenotypic features are selectively enhanced when the cells are grown in the presence of heme or butyric acid. However, K562 cells differ in several respects from normal adult erythroid stem cells, since expression of other erythroid features is either absent or aberrant: adult hemoglobins are not produced, intrinsic polypeptides of the cell membrane are aberrantly glycosylated, erythroid blood group antigens and HLA antigens are not expressed, carbonic anhydrase is not produced in quantities encountered in erythroid cells, and the cells do not undergo terminal differentiation or acquire morphological features of recognizable normal erythroblasts. Moreover, in the case of globin gene expression, levels of globin mRNA and hemoglobin produced represent only about 10%–20% of the level achieved in marrow erythroblasts. The K562 cell line does not appear to be a human cell line analogous to the Friend mouse erythroleukemia cell line, which exhibits erythroid commitment and maturation more closely resembling the normal pattern.

Several fundamental questions concerning K562 cells remain unresolved. First, the low level of erythroid phenotypic expression, as well as the variability reported from different laboratories using different strains of K562 cells, could indicate either restricted expression of these genes in all K562 cells or expression by only a limited "erythroid" subpopulation. This subset might include cells whose erythroid properties are more typical of normal erythroid progenitors than the majority of the cells. Second, the erythroid features of these cells could reflect an incomplete expression of the normal genetic program for erythroid differentiation, or could reflect the response of specific genes to the determinants that derange gene expression in neoplastic cells. Third, the enhancement of erythroid features seen in response to agents such as hemin and butyric acid could result from a uniform increase in expression of specific genes in all cells exposed, or only in a responsive subpopulation. Finally, the coexpression of erythroid phenotypes in cultures of K562 could represent coexpression within the same cells or separate...
rate expression of some phenotypes in some populations and of others in other subpopulations.

In this article, we describe experiments designed to address some of these questions by analysis of globin messenger RNA and glycophorin accumulation, using methods that permit comparison of the relative amounts of each substance accumulating in individual cells in constitutive and hemin-treated cultures of K562 cells. Our data suggest that the vast majority of K562 cells express both genes in the constitutive state. There is a uniform low level of expression, rather than highly efficient expression by a limited subpopulation. However, globin and glycophorin genes respond to hemin differently, since expression of the former is clearly enhanced in most cells by hemin, whereas expression of the latter is virtually unchanged.

MATERIALS AND METHODS

Cell Lines and Culture Conditions

K562 cells were the kind gift of Drs. T. R. Rutherford and J. B. Clegg. This subculture of K562, designated "clone R" in a previous report from our laboratory,5 produces embryonic and fetal hemoglobin, but does not produce the adult hemoglobins, HbA or HbA2. Moreover, the pattern of hemoglobin production exhibits an imbalance of the "α-thalassemic type" with predominance of the "non-α-chains" (γ and ε) and a relative paucity of α chains and the embryonic α-like chain (ζ chain).4,6,7 HL-60 cells, a promyelocytic leukemia line with capacity for granulocytic maturation,17# were the kind gift of Dr. Joseph R. Bertino.

Cells were maintained at 37°C in a humidified atmosphere in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells maintained in the above state are designated "constitutive K562 cells" in this article.

"Hemin-treated K562 cells" were obtained as follows: cells grown to a density of approximately 10^6 cells/ml were exposed to hemin by addition of hemin to a final concentration of 0.05 mM. Hemin, prepared as described previously,21 was sterilized by filtration prior to use. Hemin exposure was maintained for 5 days. Cells were then harvested by centrifugation, washed with 0.9% saline, and resuspended in the appropriate buffers for analysis. Constitutive K562 cells exhibited essentially 100% viability as assayed by trypan blue exclusion; hemin treatment reduced viability by about 10%.

Preparation of mRNAs and cDNAs

Extraction of RNA from K562 cells and reticulocytes, synthesis of radioactive complementary DNA copies (cDNAs) from mRNA preparations, purification of α and β globin cDNAs, and molecular hybridization analysis of RNA preparations in aqueous solutions were performed using methods we have described in detail elsewhere.15,24

Analysis of globin mRNA content in individual K562 and HL-60 cells was accomplished by the in situ hybridization technique developed by Brachic and Haase.25 K562 cells were collected by cytocentrifugation onto microscope slides pretreated with dilute hydrochloric acid to reduce nonspecific hybridization background. The preparations were pretreated with proteinase-K as described,25 and incubated with approximately 10^6 cpm of HcDNA prepared from cord blood reticulocytes (specific activity 50.4 Ci dCTP/ mmole) for 48 hr at 37°C. The cDNA preparations used consist of over 90% α, β, and γ globin mRNA sequences which, under the conditions employed, hybridize efficiently with globin mRNAs, but not with other mRNAs (unpublished results). After hybridization, the slides were washed as described by Brachic and Haase,25 covered with photographic emulsion (Kodak NT-3 Nuclear Track Emulsion), incubated in light-tight containers for 52 hr, developed in Kodak D-19 developer and Kodak fixative, and stained with Wright-Giemsa stain. To generate the quantitative data discussed in the Results section, the number of grains present over each cell were individually counted. A minimum of 1200 grains were counted on each slide. Grains adhering in regions where no cells were present were negligible. When the method was applied to cytocentrifuge preparations of reticulocyte or bone marrow cells, only reticulocytes and erythroblasts accumulated grains (unpublished results).

Preparation of Anti-glycophorin A Antiserum

Asialoglycophorin-A, prepared from human red cell ghosts according to the procedure of Schulte and Marchesi,26 was mixed with complete Freund's adjuvant prior to intradermal injection into each thigh of New Zealand white rabbits. Five injection treatments, administered at 3-4-day intervals, were performed. The rabbits were bled by cardiac puncture; the serum obtained served as anti-glycophorin-A antiserum. The reactivity of this antiserum to formed elements of the blood was determined by standard indirect immunofluorescence, after labeling of the antiserum with fluorescein, using a Zeiss standard microscope 19 IV FL vertical fluorescent illuminator. The anti-glycophorin-A antiserum reacted with red cells and nucleated marrow elements but not with any other cellular elements present in suspensions of normal human peripheral blood or bone marrow cells. Barely discernable reactivity to nonerythroid cells by the crude antiserum was removed for the experiments reported herein by preabsorption with HL-60 cells. The specificity of the antiserum to glycophorin-A was shown by immunoprecipitation with...
purified preparations of glycophorin kindly provided by Dr. Vincent Marchesi, Yale University School of Medicine. For these experiments, anti-glycophorin antiserum was reacted with authentic glycophorin or erythrocyte membrane protein suspensions and the antigen–antibody complexes precipitated by binding to staphylococcal protein A immobilized on Sepharose CL-4B (Pharmacia, Uppsala, Sweden). The proteins bound to Sepharose were then analyzed by SDS-polyacrylamide gel electrophoresis. The antisera specifically bound glycophorin in this assay.

Analysis of Cell Surface Glycoprotein

Cell surface characterization of K562 cells and HL-60 cells was performed by standard indirect immunofluorescence assay with subsequent analysis of each population by a fluorescence-activated cell sorter (FACS IV, Beckman Dickinson, Mountain View, Calif.) according to the protocols described by the manufacturer. The forward angle light scatter (a measure of cell volume) and the intensity of fluorescence of each cell (a measure of cell surface glycophorin density) were simultaneously measured. FACS data accumulation of profile histograms was presented in a dual mode whereby light scatter and fluorescence intensity parameters were simultaneously collected and displayed as frequency distributions, as shown in the figures. For quantitation of fluorescence intensity data, the intensity signals of the individual cells were grouped into 256 channels, representing linear increases in fluorescence intensity, and the number of cells exhibiting intensity within each channel enumerated.21,22

RESULTS

Analysis of Globin mRNA Accumulation in K562 Cells

Figure 1 compares the ability of cytoplasmic mRNA from K562 cells and cord blood reticulocytes, prepared by oligo-d(T) cellulose chromatography,21,22 to hybridize to 3H-globin cDNA. Hybridizations were conducted at 65°C in 0.2 M sodium phosphate, 0.5% SDS—conditions shown by us previously to permit extensive crosshybridization among various embryonic, fetal, and adult mRNA sequences.6 Saturating hybridization analysis was employed so that the slopes of the hybridization curves shown in Fig. 1 reflect the relative content of globin mRNA sequences in each preparation. Hemin-treated K562 cell mRNA efficiently saturated the cord blood cDNA probe, indicating presence of globin mRNA. The slope of hybridization was only about one-eighth of that of the slope obtained from the cord blood reticulocyte RNA template used to prepare the 3H-cDNA. These results indicate that K562 cells stimulated with hemin contain only about 10% of the content of globin mRNA present in cord blood reticulocytes. HL-60 cell mRNA did not saturate the globin cDNA probe, demonstrating the specificity of the annealing of K562 cell mRNA with the cDNA probe and indicating virtual absence of globin mRNA sequences from HL-60 cells.

Table 1. Hemin Effect on Globin mRNA Content of K562 Cell Cytoplasm

<table>
<thead>
<tr>
<th>RNA Content/Cell (pg)</th>
<th>Percent Poly-(A) + mRNA</th>
<th>Poly-(A)' mRNA (%)</th>
<th>pg Globin mRNA/Cell</th>
<th>Molecules/Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutive K562 cells</td>
<td>13.1</td>
<td>0.87</td>
<td>0.29</td>
<td>3.7 x 10^4</td>
</tr>
<tr>
<td>Hemin-treated K562 cells</td>
<td>15.9</td>
<td>1.0</td>
<td>0.80</td>
<td>1.3 x 10^4</td>
</tr>
<tr>
<td>Hemin-treated/constitutive</td>
<td>1.21</td>
<td>1.15</td>
<td>2.76</td>
<td>3.80</td>
</tr>
</tbody>
</table>

Over 90% of the total cellular globin mRNA was present as cytoplasmic poly-(A)' mRNA (data not shown). Thus, data from Figs. 1 and 2 were used to derive the amounts of globin mRNA shown in the table. RNA content/cell was estimated by measuring the total RNA recovered/3 x 10^6 cells from constitutive (39.3 mg) and hemin-treated (47.6 mg) cells. Percent poly-(A)' mRNA was taken to be the percent of this RNA binding to oligo-d(T) cellulose in 0.5 M KCl but recovered after elution by H2O. The percent of poly-(A)' mRNA which is globin mRNA, was calculated from Fig. 2, noting the input in ng of poly-(A)' mRNA required to bind into mRNA-cDNA hybrids of 1 ng globin cDNA (1 ng cDNA = 2 x 10^6 cpm, based on specific activity [50.4 Ci/mmol], counting efficiency (16%), and length of hybridizing sequence [500 bases]). The number of pg globin mRNA/cell was calculated by multiplying the pg total RNA/cell by the percent globin mRNA. Molecules globin mRNA/cell were calculated by dividing the pg globin mRNA/cell by 200,000 (molecular weight of globin mRNA in pg/mole) and multiplying by 10^-11 x Avogadro’s number (6.02 x 10^23 molecules/mole). Application of these calculations to the cord blood reticulocyte RNA data shown in Fig. 1 yielded an estimate of 31,000 molecules of globin mRNA/reticulocyte, a finding in agreement with others.17,23
plasmic poly-(A)^+ mRNA show a somewhat higher degree of globin mRNA increase when expressed as molecules of cytoplasmic globin mRNA/cell, because of a higher total RNA content per cell, as well as a higher percentage of total RNA sequences represented as polyadenylated mRNAs in the hemin-treated cells. Poly-(A) RNA contained less than 5% of the globin mRNA present in the cells (data not shown). There is thus actually about a fourfold increase in the average number of globin mRNA molecules present in hemin-treated K562 cell cytoplasm (Table 1). These results are similar to those reported by ourselves and others and correlate well with levels of hemoglobin and globin production.

As shown in Table 1, the approximate average number of globin mRNA molecules per cell can be derived from these results by using the globin mRNA content of cord blood reticulocyte mRNA as a standard of reference. We and others have estimated that there are approximately 30,000 molecules of globin mRNA present in each reticulocyte. Using this figure, comparison of the slopes of hybridization obtained with constitutive K562 cells, hemin-treated K562 cells, and cord blood reticulocytes suggests that there are about 4000 molecules of globin mRNA present after hemin treatment, as compared to approximately 1000 present in the constitutive state. The increased cytoplasmic globin mRNA represents a true increase in content (molecules/cell), in relative percentage of total RNA and poly-(A) RNA, and in globin mRNA concentration (molecules/cell volume), since the increase is more than can be accounted for by changes in cytoplasmic RNA content of poly-(A) RNA content (Table 1) or the small changes in cell volume due to hemin (cf. Fig. 5, lefthand panel).

Cell-to-Cell Distribution of Globin mRNA Sequences in K562 Cells

As shown in Fig. 3, in situ hybridization analyses with ^3H-cord-blood cDNA revealed that globin mRNA sequences were essentially absent from HL-60 cells (Fig. 3A). As shown in Fig. 3B, a substantial increase above background in grain counts over individual cells was observed for the vast majority (>90%) of constitutive K562 cells. Moreover, as shown in Fig. 3C, a readily detectable increase in the grain counts over the majority of hemin-treated K562 cells was apparent. These data demonstrate that the low level of globin mRNA production detected in liquid hybridization experiments (Figs. 1 and 2) was due to relatively uniform expression of globin genes in all K562 cells examined. The results were not compatible with the expression, at higher levels, of the globin genes in only a limited subpopulation of cells. Moreover, the
increase in globin mRNA content detected in liquid hybridization experiments (Fig. 2) after hemin treatment appeared to be due to a relatively uniform increase in globin mRNA content of every cell, rather than more extensive "induction" of only a small subpopulation. Thus, globin gene expression and a hemin-mediated increase in the efficiency of globin gene expression appear to be properties of the majority of K562 cells. These phenomena were not confined to a subpopulation.

Quantitative estimates of the cell-to-cell distribution of globin mRNA sequences in individual cells were made by plotting the frequency of cells with a given number of grains present over nucleus, cytoplasm, and total cells. The results are displayed in Fig. 4. There was almost no overlap between results observed for constitutive and hemin-treated cells. As indicated in Table 2, the data obtained from the in situ hybridization experiments suggest an approximately fourfold induction of globin mRNA sequences, regardless of whether one utilized median or mean values for the distribution of grain counts in each state. The differences in means were statistically significant by the Student's t test at a 1% level of confidence (cf. Table 2), and correlate well with the liquid hybridization measurements (Table 1). No evidence for selective increases in nuclear or cytoplasmic globin mRNA was observed (Fig. 4); however, cytoplasm spread over the nuclei of these cells could easily obscure such compartmental change.

**Analysis of Glycophorin Accumulation in K562 Cells**

Several control experiments were performed to demonstrate the specificity of reaction between K562 cells and antiglycophorin-A antiserum (data not shown). K562 cells demonstrated no fluorescence intensity above background when incubated with a 1:10 dilution of normal rabbit serum (preimmune serum). HL-60 cells and other nonerythroid cells did not react with the antiserum, whereas erythrocytes, erythroblasts, and reticulocytes did. Each of these control experiments was performed using both fluorescence microscopy and fluorescence-activated cell sorting.

When constitutive K562 cells were incubated with fluorescein-labeled glycophorin A antiserum, the vast majority of cells exhibited substantial fluorescence, appreciable by fluorescence microscopy—a result in agreement with others.23 No evidence for major subpopulations of nonreacting cells could be appreciated. However, no qualitatively apparent increase in fluorescence was evident in hemin-treated cells. As shown by Fig. 5, cell sorter analysis confirmed these preliminary impressions. The histogram labeled A shows uninduced cells, and the histogram labeled B shows induced cells. For these experiments, we used antiglycophorin-A antiserum that had been repeatedly absorbed with HL-60 cells to reduce background fluorescence. Although a subpopulation of cells in the constitutive K562 cell population could be detected as
Table 2. Average Grain Counts per Cell or Cellular Compartment in K562 Cells Analyzed by In Situ cDNA-mRNA Hybridization

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
<th>Grain Counts/Cell or Cell Compartment</th>
<th>Ratio (Induced/Uninduced)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median</td>
<td>SD</td>
</tr>
<tr>
<td>HL-60 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>3–16</td>
<td>8.8</td>
<td>8</td>
<td>3.7</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>3–19</td>
<td>12.1</td>
<td>12</td>
<td>4.8</td>
</tr>
<tr>
<td>Total cells</td>
<td>11–32</td>
<td>20.9</td>
<td>21</td>
<td>6.9</td>
</tr>
<tr>
<td>Constitutive K562</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>3–26</td>
<td>12.5</td>
<td>12</td>
<td>4.4</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>8–57</td>
<td>32.4</td>
<td>30</td>
<td>12.9</td>
</tr>
<tr>
<td>Total cells</td>
<td>14–82</td>
<td>44.9</td>
<td>42</td>
<td>16.1</td>
</tr>
<tr>
<td>Hemin-treated K562</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>28–40</td>
<td>22.5</td>
<td>22</td>
<td>3.9</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>55–97</td>
<td>75.9</td>
<td>76</td>
<td>11.6</td>
</tr>
<tr>
<td>Total cells</td>
<td>76–122</td>
<td>96.9</td>
<td>97</td>
<td>12.8</td>
</tr>
</tbody>
</table>

Data were obtained as in Figs. 3 and 4, by counting 35 cells of each category. Mean = total grains counted/number cells counted. Medians were derived from Fig. 4. Standard deviation and standard error of the mean (SEM) were calculated assuming the data shown in Fig. 4 represent samples of normal distribution. Student’s t testing yielded p values <0.01 for the hypotheses that the means of HL60 cells = mean constitutive K562, and mean of constitutive K562 cells = mean of hemin-treated cells for nuclei, cytoplasm, and total cells. Note that comparison of nuclear to cytoplasmic grain counts is compromised by the predominance of cytoplasmic mRNA and the spreading of cytoplasm over nuclei during preparation of the slides.

Table 3. Effect of Hemin on Glycophorin-A Content of K562, Estimated by Fluorescent Antiglycophorin Antibody Binding and Fluorescence-activated Cell Sorter Analysis

<table>
<thead>
<tr>
<th></th>
<th>Fluorescence Intensity (Intensity Channel)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Constitutive K562 cells</td>
<td>112.8</td>
</tr>
<tr>
<td>Hemin-treated K562 cells</td>
<td>127.7</td>
</tr>
<tr>
<td>Ratio hemin-treated/constitutive</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Data were obtained from computer printouts of cell distributions shown in Fig. 5 and calculations performed as discussed in Table 2.

Having only modest fluorescence (histogram A, Fig. 5), the majority of the cells exhibited fluorescence similar to hemin-treated K562 cells (histogram B, Fig. 5). No major shift in the fluorescence intensity profile exhibited by the majority of the cells was detected. The histograms for hemin-treated and constitutive cells were virtually superimposable in the higher ranges of fluorescence intensity.

Table 3 compares the mean and median value of intensity channels obtained before and after hemin treatment. These channels are linearly related to intensity and indicate a lesser quantitative increase in glycophorin accumulation after hemin treatment than was observed for globin mRNA, even though the differences in fluorescence channels was significant by Student’s t testing (p < 0.01).

The differences shown in Table 3 correspond to a ratio of glycophorin A content in hemin-treated/constitutive cells of only 1.1. As indicated by Fig. 5, the major differences between constitutive and hemin-treated cells was the presence of a small subpopulation, comprising 12% of constitutive cells, with fluorescence intensities more than 2 standard deviations lower than the mean value for the hemin-treated cells. This is evident as a low fluorescence “shoulder” in Fig. 5, histogram A. As shown in Fig. 6, simultaneous three-dimensional display of the cell volume, fluorescence, and frequency of the cells revealed that these less fluorescent cells also consisted predominantly of small...
The fluorescence intensity of erythrocytes that had been reacted with the fluorescein-labeled antiglycophorin antibody was considerably higher than that of K562 cells when assessed by microscopy. In contrast, erythrocytes exhibited mean channel intensities only about 5% as high as K562 cells in FACS experiments. The latter result reflects the much smaller volume of erythrocytes and the sensitivity of fluorescence intensity detection by the FACS system to cell volume. Therefore, although we were able to form the qualitative impression that glycophorin accumulation by K562 cells was less abundant than by erythrocytes, it has not been possible to verify this impression quantitatively. The possibility thus remains that K562 expresses the glycophorin gene as efficiently as it is ever expressed during normal erythropoiesis. Our conclusion that glycophorin and globin gene expression respond differently to hemin is not altered by this possibility.

**DISCUSSION**

Our studies suggest several conclusions about the erythroid properties of K562 cells. First, the expression of both glycophorin and globin genes appears to occur within most or all K562 cells. We observed no evidence for a subpopulation of cells not expressing these genes or expressing them at higher levels more typical of late erythroid cells. We also obtained no evidence for separate expression of one, but not the other, group of genes in distinctly separate subpopulations.

Second, globin gene expression was also characterized by a relatively uniform increase in the cell-to-cell distribution of globin mRNA sequences after hemin treatment. In situ hybridization analysis yielded values for increased globin mRNA content that correlated well with data obtained by liquid hybridization.

Third, the majority of K562 cells expressed glycophorin genes in the constitutive state as efficiently as they are expressed after hemin treatment. Hemin treatment did not produce a uniform increase in glycophorin accumulation similar to that seen in the case of globin mRNA accumulation. Globin and glycophorin gene expression thus responded differently to hemin.

Erythroid cell maturation is characterized by the "coordinate" expression of many genes that determine the erythroid phenotype. However, no data exist to permit description of the actual events at the level of individual genes in early stem cells. Thus, it is not known whether expression of all the relevant genes exists at a low level in stem cells prior to maturation, whether all genes increase their expression either simultaneously or in an orderly temporal sequence, or whether some genes are already maximally expressed in the uncommitted or early committed stem cell. The latter possibility is of particular importance, since it would alter the primary conclusion drawn from Figs. 5 and 6, that little or no change in globin gene accumulation occurred as a result of hemin treatment under conditions yielding a recognizable increase in globin gene accumulation.
GLOBIN AND GLYCOPHORIN IN K562 CELLS

implies the presence of some “differentiated” markers on or within stem cells, a feature that would enhance their eventual isolation and characterization. Our data suggest that globin and glycoporin gene expression in K562 cells respond differently to treatment of these cells with hemin. To the extent (presently unknown) to which the erythroid phenotypes exhibited by K562 cells reflect normal gene regulation in erythroid progenitors, our data raise the possibility that some “highly differentiated” markers, such as glycoporin, may already be substantially expressed in primitive stem cells. Moreover, the results suggest that “coordinate” expression of erythroid-specific genes does not imply simultaneous increases in gene expression upon onset of maturation. Finally, the data imply that glycoporin-A is a potential erythroid marker that might be useful for isolation of erythroid stem cells.

Unfortunately, there is little current evidence available to verify that K562 cells exhibit a form of human erythroid maturation in the presence of hemin or butyric acid that is strictly comparable to normal erythroid maturation. K562, under culture conditions studied as of this writing, do not exhibit erythroid features nearly as striking as mouse erythroleukemia cells (MELC). MELC undergo commitment to terminal differentiation and exhibit a striking increase in globin mRNA, as well as a number of other erythroid proteins, such as heme-synthesizing enzymes, spectrin, and surface antigens, after treatment with a variety of inducing agents. It is noteworthy, however, that of the agents employed, hemin exhibits the least capacity to induce true maturation of MELC. Indeed, the major effects of hemin appear to be on globin gene expression; commitment to terminal differentiation of the cells is not induced by hemin, even though other agents have been shown to be effective in this regard. Agents other than hemin have recently been reported to increase globin gene expression in K562 cells. These include hydroxyurea and butyric acid, which are also effective for induction of erythroid maturation in MEL cells. Comparative analysis (similar to that reported in this article) of two or more genes whose expression is relevant to development of erythroid phenotype might be useful in an effort to determine whether some agent can generate maturation of K562 cells along a more normal erythroid pathway than is seen with hemin.

It is possible that the erythroid features exhibited by K562 cells do not reflect a true erythroid propensity of these primitive blasts. Rather, the expression of globin and glycoporin genes, as well as the “erythroid pattern” of heme synthetic and degradative enzymes reported by us previously, might reflect derangements of gene expression occurring as a result of the neoplastic transformation. Since the products of these genes are well characterized and quantifiable, analysis of their behavior in K562 cells should yield information as to the determinants that derange their expression. This approach could potentially lead to identification of factors whose presence or absence is relevant to the initiation and maintenance of the neoplastic state.

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