Isolation and In Vitro Differentiation of Human Erythroid Precursor Cells

By H. C. Kim, P. A. Marks, R. A. Rifkind, G. M. Maniatis, and A. Bank

There is decreased β-globin production in β-thalassemic reticulocytes and nucleated erythroid cells. In this study, we have examined whether unbalanced globin synthesis is expressed at all stages of human erythroid cell maturation. In order to determine the pattern of globin synthesis in early erythroid cells during erythroid cell maturation, an in vitro culture system using human bone marrow erythroid precursor cells has been developed. Early erythroid precursor cells (proerythroblasts and basophilic erythroblasts) have been isolated from nonthalassemic and thalassemic human bone marrows by lysing more mature erythroid cells, using complement and a rabbit antiserum prepared against normal human red cells. In the presence of erythropoietin, differentiation and proliferation of erythroid cells is demonstrable in liquid suspension culture for 24–48 hr, as determined by morphological criteria and by an increase in globin synthesis. The ratio of α- to β-globin chain synthesis in nonthalassemic cells is approximately 1 at all stages of erythroid cell differentiation during culture. In cells from four patients with homozygous β-thalassemia there is decreased β-globin synthesis compared to α-globin synthesis, both in early erythroid precursor cells and during their maturation in culture. These findings indicate that unbalanced globin chain synthesis is expressed at all stages of red cell maturation in homozygous β-thalassemia.

IN HOMOZYGOUS β-THALASSEMIA, decreased β-globin synthesis has been found in association with decreased amounts of β-globin mRNA in peripheral blood and bone marrow cells. In order to determine whether β-globin mRNA activity is decreased from the start or only during later stages of erythroid cell development in β-thalassemia, the isolation of early erythroid precursor cells from bone marrow was undertaken. In previous studies of human bone marrow nucleated erythroid precursor cells, the total marrow cell population has been used. Human bone marrow contains a mixed population of erythroid cells at all stages of maturation, including proerythroblasts and basophilic erythroblasts (precursor cells), as well as more differentiated hemoglobinized cells, the polychromatophilic and orthochromatic erythroblasts, reticulocytes, and mature red cells.

This paper describes the purification of a relatively homogeneous population of early erythroid precursor cells and maturation and proliferation of these cells in vitro in a liquid suspension culture. To do this, we have modified a method originally described for mouse fetal liver erythroid cells in which antisera against mature red cells is used to lyse erythroid cells differentiated be-
Beyond the basophilic erythroblast stage. Isolated erythroid precursor cells from human bone marrow proliferate and differentiate in culture over 24-48 hr. During this time there is a 5- to 20-fold increase in globin synthesis, and the majority of erythroid cells become hemoglobinized. The results indicate that the synthesis of α- and β-globin chains is relatively equal at all stages of erythroid differentiation in nonthalassemic nucleated erythroid cells. In homozygous β-thalassemia, on the other hand, decreased β-globin synthesis is present in erythroid precursor cells and persists throughout maturation of erythroid cells in culture.

**Materials and Methods**

**Preparation of Rabbit Antihuman RBC Antibody**

Rabbit antihuman red cell antibodies were prepared according to a modification of the procedure of Cantor et al. Group A, B, and O blood samples were obtained from the blood bank, washed three times with saline, the packed cells were diluted 1:1 with saline, and equal volumes of group A, B, and O cells were mixed before use. One to 1.5 ml of this erythrocyte suspension was injected intravenously into the ear vein of several rabbits, three times a week for 4 wk. One wk after the last injection, the rabbits were bled by cardiac puncture, the serum sterilized by Millipore filtration (0.45 μm pore size), and stored in small aliquots at −20°C. Rabbit antihuman erythrocyte antiserum (called antiserum) was tested for its complement-dependent hemolysin titer, using each of the A, B, and O human red cell suspensions at 4 x 10⁶ cells/ml; antisera with titers of 1:2000 or greater were used to isolate precursor cells. Antisera made against mixed group A, B, and O red cells had similar titers when tested against different A, B, and O red cells.

**Isolation of Bone Marrow Precursor Cells**

Human bone marrow samples (usually 3–5 ml) from patients with sickle cell disease and thalassemia were aspirated and mixed with heparin (1000 U/ml). Three patients with β-thalassemia major and one with thalassemia intermedia were studied. All were of Italian ancestry. P.M. was a 12-yr-old male, M.O. a 10-yr-old female, and R.N. a 14-yr-old male with typical thalassemia major requiring frequent transfusions. These three patients currently participate in hypertransfusion programs to maintain pretransfusion hemoglobin levels above 9–10 g/100 ml. One patient, M.D., a 7-yr-old boy diagnosed as having thalassemia intermedia on the basis of a relatively stable hemoglobin of 9–10.5 g/100 ml did not require blood transfusions until age 6. He developed hepatosplenomegaly, and at age 7 underwent splenectomy because of an increasing transfusion requirement. Both of his parents had elevated HbaA₂ levels, and he was thought to represent a "mild" expression of homozygous β-thalassemia. Marrow samples on all four patients were obtained at the time of splenectomy. Marrow cells were disaggregated by passage through progressively smaller bore needles from 20 to 25 gauge. The cells were washed twice with Hank’s balanced salt solution (HBSS) (Gibco, Grand Island, N.Y.), then resuspended at a concentration of 12 x 10⁶ cells/ml in NCTC-135 culture medium (Gibco), supplemented with 10% fetal calf serum (Microbiological Associates, Bethesda, Md.), 1% penicillin-streptomycin (5000 units of penicillin and 5000 μg of streptomycin per ml), and made 15 mM in Hepes buffer (N-2-hydroxyethyl-piperazine-N-2-ethane sulfonic acid, Microbiological Associates). Guinea pig complement (Hyland Lab, Los Angeles, Calif.), 0.05 ml/ml of total reaction volume, was added dropwise to a suspension of the bone marrow cells at 4°C, with continuous stirring. The marrow cell suspension was brought to room temperature, antiserum was added to about 1:500 dilution and the suspension was passed through a nylon filter of pore size 10 μm (Nitex HC-10) in order to disrupt large cell clumps. The suspension was then stirred for 30 min at room temperature, centrifuged at 150 g for 5 min, and the supernatant, containing bare nuclei, was discarded. The pellet of precursor cells was washed three times in culture medium. The entire procedure was performed under sterile conditions.
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Culture of Human Erythroid Precursor Cells

The concentration of erythroid precursor cells was adjusted to 1-2 x 10^6 cells/ml with NCTC-135 medium containing 20% human AB serum, 20% fetal calf serum, 1% penicillin-streptomycin, and 20 mM Hapes. The cells were cultured with human urinary erythropoietin* at a final concentration of 0.34 units/ml of cell suspension. Cells without erythropoietin were cultured under identical conditions. Ten milliliters of cell suspension were cultured in T25 tissue culture flasks (Falcon, Oxnard, Calif.) at 37°C in 5% CO2. Cells were harvested after 10, 24, and 48 hr of culture. In each culture 0.6 ml of the suspension was used to determine the viable cell count by trypan blue dye exclusion in a hemocytometer, and to make slides (Cytocentrifuge, Shandon Instruments, London) for staining with benzidine-Wright-Giemsa stain. At least 300 stained cells were counted on each of two slides. The remainder of the cultured cells were used to determine globin synthesis.

Measurement of Globin Synthesis

The cultured cells were washed twice with HBSS and incubated with one volume of Krebs-Ringer-bicarbonate buffer (KRB) without leucine14 and 100 µCi/ml of [4,5-3H] leucine (30 Ci/m mole, New England Nuclear, Boston, Mass.), adjusted to pH 7.4. Incubation was for 60 min at 37°C with shaking. At the end of incubation, ten volumes of ice-cold HBSS were added, the cells were washed three times, and lysed with ten volumes of distilled water, and three cycles of freezing and thawing. Cell membranes and nuclei were removed by centrifugation at 20,000 g for 20 min. Unlabeled carrier human hemoglobin was added to lysates prior to preparation of globin. Globin was prepared with acid acetone at -20°C, and chromatographed on carboxymethylcellulose in 8 M urea-phosphate buffer at pH 6.7, as described previously.

RESULTS

Isolation of Erythroid Precursor Cells by Antibody Lysis

The initial bone marrow cell suspensions consisted predominantly of mature red cells and variable numbers of nucleated precursors. Bone marrows from patients with hemolytic anemias including sickle cell disease and thalassemia contained a higher proportion of nucleated erythroid precursors than normal marrow (Fig. 1A). Hemoglobinized erythroblasts, reticulocytes, and mature erythrocytes were selectively lysed by the reaction with anterythrocyte antiserum and complement, while early erythroid precursor cells remained unlysed (Fig. 1B). The level of contamination of early erythroid precursor cells by residual nucleated hemoglobinized erythroid cells varied from culture to culture (Tables 1, 2). Nonerythroid nucleated cells were not lysed by antiserum. Nucleated erythroid cells in unfractionated bone marrow comprised 3% of P.M., 2.5% of M.O., 1.2% of R.N., and 1.0% of M.D. samples (Table 2). The per cent of nonnucleated red cells decreased from 97% to 99% in unfractionated marrow to between 0.4% and 3.1% in 0-hr specimens after antibody lysis.

Characterization of Cultured Erythroid Precursor Cells

Precursor cells (proerythroblasts and basophilic erythroblasts) were cultured for up to 48 hr in the presence of erythropoietin. Between 0 and 24 hr of cul-

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*Provided by the Committee on Erythropoietin, NIH, procured by the Department of Physiology, University of Northwest, Corrientes, Argentina, processed by the Hematology Research Laboratory, Children's Hospital of Los Angeles, for distribution by the National Heart Institute under Research Grant HE-10880.
Fig. 1. (A) Total bone marrow cells before antibody lysis. (B) Cells after antibody lysis consisting predominantly of early erythroid precursors, proerythroblasts, and basophilic erythroblasts. (C) Cells after 24 hr of culture with erythropoietin. (D) Cells after 48 hr of culture with erythropoietin. × 600.

ture in the presence of erythropoietin, the number of erythroid cells increased 1.2- to 2-fold (Tables 1, 2). Between 24 and 48 hr in culture with erythropoietin, the total number of erythroid cells remained relatively constant or showed a modest decline. By 24 hr in culture, approximately 50\% of erythroid cells were hemoglobinized (benzidine-positive polychromat and orthochromat erythroblasts); more than 60\% were hemoglobinized by 48 hr (Fig. 1C, D, Tables 1, 2). At the same time, the number of early erythroid precursors was fairly well maintained in cultures with erythropoietin (Tables 1, 2). The kinetics of proliferation and differentiation of nonthalassemic (sickle cell cultures) and β-thalassemia erythroid precursor cells in culture were similar.

**Globin Synthesis During Differentiation of Erythroid Precursor Cells**

Globin synthesis in the erythroid precursors from the bone marrow of two patients with sickle cell disease (cells from patient D.T. were cultured twice) at 0 hr showed small amounts of β5- and α-globin synthesis (Table 1, Fig. 2). After 10 hr in culture, there was a 4- to 5-fold increase in globin synthesis, with the increase in β5- and α-globin synthesis continuing for at least 24 hr. At 24 hr of culture, the increase in globin synthesis was 5- to 20-fold over the 0-hr cells. The relative rates of α- and β-globin synthesis (α/β ratio) remained approximately 1 during 48 hr of culture (Table 1).
Table 1. Erythroid Cell Counts and Globin Synthesis in Bone Marrow of Patients With Sickle Cell Disease

<table>
<thead>
<tr>
<th>Patients</th>
<th>Duration of Culture (hr)*</th>
<th>Total Erythroid Cell Counts (x 10^3/ml)</th>
<th>Early Erythroid Precursors (B-) (x 10^3/ml)†</th>
<th>Late Erythroid Precursors (B+) (x 10^3/ml)†</th>
<th>Per Cent of B+ Cells</th>
<th>α</th>
<th>β</th>
<th>α/β Ratio</th>
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<td>D.T.</td>
<td>0</td>
<td>50.0</td>
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<td>292,367</td>
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<td>270,811</td>
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<td>D.G.</td>
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<td>146.8</td>
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<td>98,444</td>
<td>1.07</td>
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</table>

*Duration of culture with erythroid precursor cells in the presence of erythropoietin (E+).
†Early erythroid precursors are benzidine negative (B–), proerythroblasts and basophilic erythroblasts.
‡Late erythroid precursors are benzidine positive (B+), polychromatophilic and orthochromatic erythroblasts.
§Late erythroid precursors (B+) of total erythroid cell counts.
¶Total 3H incorporation into each globin chain. α/β ratios of carrier globin recovered in the above experiments were 0.99 ± 0.10.

Table 2. Erythroid Cell Counts and Globin Synthesis in Bone Marrow of Patients With Homozygous β-thalassemia

| Patients | Duration of Culture (hr)* | Total Erythroid Cell Counts (x 10^3/ml) | Early Erythroid Precursors (B-) (x 10^3/ml)† | Late Erythroid Precursors (B+) (x 10^3/ml)† | Per Cent of B+ Cells| Total cpmαβ | α | β | α/β Ratio |
|----------|--------------------------|----------------------------------------|----------------------------------------------|-------------------------------------------|------------------|---------------|----------------|-------------|
| P.M.     | 0                        | 408.0                                  | 397.2                                         | 10.8                                      | 3                | 57,802        | 8,840          | 6.46        |
|          | 10 E+                    | 476.2                                  | 471.0                                         | 5.2                                       | 1                | 104,692       | 16,430         | 6.37        |
|          | 24 E+                    | 717.5                                  | 309.2                                         | 408.3                                      | 57               | 112,548       | 16,992         | 7.55        |
|          | 48 E+                    | 664.0                                  | 237.9                                         | 426.1                                      | 64               | 130,488       | 21,108         | 6.27        |
| Total BM |                          | —                                       | —                                            | —                                         | —                | 41,120        | 2,930          | 13.90       |
| M.O.     | 0                        | 107.3                                  | 95.8                                         | 11.5                                      | 11               | —             | —             | —           |
|          | 10 E+                    | 129.0                                  | 116.1                                         | 12.9                                      | 10               | 38,707        | 10,886         | 3.56        |
|          | 24 E+                    | 179.0                                  | 130.2                                         | 48.8                                      | 27               | 47,858        | 12,180         | 3.93        |
|          | 48 E+                    | 144.8                                  | 56.2                                         | 88.3                                      | 61               | 76,288        | 18,469         | 4.13        |
| Total BM |                          | —                                       | —                                            | —                                         | —                | 21,499        | 2,839          | 7.57        |
| R.N.     | 0                        | 148.4                                  | 140.6                                         | 7.8                                       | 5                | 29,400        | 5,352          | 5.49        |
|          | 12 E+                    | 204.3                                  | 198.7                                         | 5.6                                       | 3                | 73,954        | 10,701         | 6.91        |
|          | 24 E+                    | 221.0                                  | 115.6                                         | 105.4                                     | 48               | 86,963        | 10,739         | 8.10        |
| Total BM |                          | —                                       | —                                            | —                                         | —                | 222,828       | 12,672         | 17.58       |

β- Thalassemia intermedia

| Patients | Duration of Culture (hr)* | Total Erythroid Cell Counts (x 10^3/ml) | Early Erythroid Precursors (B-) (x 10^3/ml)† | Late Erythroid Precursors (B+) (x 10^3/ml)† | Per Cent of B+ Cells| Total cpmαβ | α | β | α/β Ratio |
|----------|--------------------------|----------------------------------------|----------------------------------------------|-------------------------------------------|------------------|---------------|----------------|-------------|
| M.D.     | 0                        | 67.5                                   | 67.5                                         | 0                                         | 0                | 5,964         | 4,788          | 1.25        |
|          | 12 E+                    | 95.3                                   | 95.3                                         | 0                                         | 0                | 19,929        | 14,028         | 1.42        |
|          | 24 E+                    | 118.3                                  | 61.9                                         | 56.4                                      | 48               | 20,079        | 12,240         | 1.64        |
| Total BM |                          | —                                       | —                                            | —                                         | —                | 115,246       | 26,220         | 4.39        |

*, †, ‡ and § as in Table 1.
¶ α/β ratios of unlabeled carrier globin recovered in the above experiments were 1.06 ± 0.06.
Globin synthesis during culture of erythroid precursor cells from three patients with homozygous β-thalassemia demonstrated unbalanced synthesis (α/β > 1) at 0 hr and at all subsequent times (Table 2, Fig. 3). At 24 and 48 hr, globin synthesis appeared to become somewhat more unbalanced (increased α/β ratio; Table 2, Fig. 3). There was no stage of erythroid cell differentiation in homozygous β-thalassemia in which α- and β-globin synthesis was balanced. In one patient with β-thalassemia intermedia, unbalanced synthesis was also observed at all stages of maturation, although the α/β ratio was closer to 1 (Table 2).

In all patients with homozygous β-thalassemia, unfractionated marrow cells displayed a more severe imbalance of α- and β-globin synthesis than the one observed in precursor cells, even after 48 hr in culture.
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A peak of radioactivity migrating prior to α globin was consistently seen in elution patterns obtained from bone marrow incubations. This “pre-α” radioactivity was less than 20% of the total α radioactivity in most cases. Calculation of α/β ratios of globin synthesis shown in Tables 1 and 2 included this “pre-α” peak radioactivity. When α/β ratios were calculated excluding the “pre-α” peak, no significant alterations in the relative α/β ratios with erythroid maturation were seen. The pre-β peak of radioactivity which cochromatographs with γ-globin represented less than 20% of total globin synthesis, and no consistent change in the amount of presumed γ-globin synthesis was noted during erythroid cell maturation (Figs. 2, 3).

DISCUSSION

Culture of human erythropoietic cells in vitro provides a useful system for the study of erythroid cell differentiation, and the molecular mechanisms controlling the synthesis of specific globin chains during normal as well as abnormal erythroid cell differentiation, including the thalassemias. Several methods have been described for culture in vitro of human erythroid cells.17-23

Previous studies have indicated that erythropoietin is required for the growth and differentiation of erythroid cells.17-23 Considerable evidence now identifies the erythropoietin-sensitive cell in fetal mouse liver erythropoiesis as an early erythroid precursor which does not synthesize hemoglobin and does not contain globin mRNA.24-27 Recognition of antigenic differences between immature and mature erythroid cells, originally suggested by the observations of Borsook et al.28-29 and Minio et al.,30 has permitted the isolation and differentiation in vitro of the erythropoietin-responsive erythroid precursor cells.12,25-27 These techniques, originally developed for the study of fetal mouse erythroid cells, have been modified for the isolation of human erythroid precursors from bone marrow.

The response to erythropoietin in culture of erythroid precursor cells from human bone marrow includes both an increase in the total number of nucleated erythroid cells as well as in the number of maturing, hemoglobin-containing cells. Globin synthesis also increases in culture under these conditions. Cultures without erythropoietin show a decline in the number of total erythroid cells, as well as the number of erythroid precursors when compared to 0-hr cells at all times after culture. Some erythroid differentiation is observed in the absence of erythropoietin, which may have been due to the presence of cells triggered by erythropoietin prior to culture.

Analysis of globin chain synthesis in cultured cells from patients with sickle cell disease demonstrates that the ratio of α/β5 chain synthesis remains close to 1 throughout the duration of culture as the proportion of maturing erythroid cells progressively increases (Fig. 2, Table 1). Thus, whatever mechanisms are responsible for the coordination of α- and β-chain synthesis appear to be active at the earliest observable stages of erythroid cell maturation and remain so throughout erythroid cell maturation.

β-thalassemia is characterized by a selective deficiency of β-chain synthesis.1,2 An imbalance in globin chain synthesis can be demonstrated both in the bone marrow and in reticulocytes in the circulation.4 In the experiments reported
here, we have followed the $\alpha/\beta$ ratio of globin chains synthesized by early erythroid precursor cells, purified by the technique of immune lysis, during their differentiation in vitro in the presence of erythropoietin. In the four patients studied here, three of which were homozygous for $\beta$-thalassemia (Fig. 3, Table 2), unbalanced globin chain synthesis can be detected in the most immature cell population and persists throughout the process of maturation in vitro. This finding suggests that the defect in $\beta$-chain synthesis is present from the earliest stages of erythroid cell differentiation. There is, however, a consistent, progressive increase in the $\alpha/\beta$ ratio with differentiation in vitro.

In all four thalassemic patients examined, the $\alpha/\beta$ ratio of the cultured cells is considerably less (roughly one-half) than the $\alpha/\beta$ ratio of the whole unfractionated bone marrow. Control cultures from nonthalassemic patients exhibit a balanced $\alpha/\beta$ ratio throughout the culture period, as well as in the total bone marrow (Table 1). The more unbalanced $\alpha/\beta$ ratio of unfractionated $\beta$-thalassemic bone marrow most likely reflects contamination of these samples with peripheral blood and bone marrow reticulocytes. It previously was shown that there is a greater $\alpha/\beta$ imbalance in the reticulocytes of homozygous $\beta$-thalassemics than in erythroid precursors in their bone marrow.\(^4,8,9,31\) Patients heterozygous for $\beta$-thalassemia also show this difference in $\alpha/\beta$ ratio between bone marrow and circulating reticulocytes.\(^8,9,32,34\) In a recent study using bovine serum albumin gradients to fractionate nucleated red cells in human bone marrow,\(^33\) a progressive increase in $\alpha/\beta$ ratio from 1.0 to 2.0 has been demonstrated, with increasing erythroid maturation in heterozygous $\beta$-thalassemia cells. This study\(^33\) also documents balanced $\alpha/\beta$ synthesis at all stages of erythroid cell maturation in nonthalassemic patients.

The mechanism by which nucleated erythroid precursor cells can maintain more balanced globin chain synthesis relative to reticulocytes is not clear. However, the more balanced $\alpha/\beta$ synthesis in early erythroid precursors in $\beta$-thalassemia is not due to the presence of more $\beta$-mRNA compared to $\alpha$-mRNA in these cells, since it has been shown that $\beta$-globin mRNA content is similarly decreased in both bone marrow cells and reticulocytes.\(^8,10,35\) In heterozygous $\beta$-thalassemia, while the $\alpha/\beta$ synthesis is 1.0\(^8,9,33\) to 1.4\(^31\) in intact bone marrow and between 1.5 and 2.0 in reticulocytes\(^8,9,32,33\), the isolated globin mRNA from marrow cells and reticulocytes contains half as much $\beta$-mRNA as $\alpha$-mRNA both by assay in cell-free systems\(^8,9\) and by molecular hybridization.\(^35\) Thus, the more balanced synthesis of $\alpha$ and $\beta$ chains in bone marrow cells appears to be due to control mechanisms operative in the intact cell. Whether more balanced $\alpha/\beta$ synthesis is achieved through proteolysis of excess $\alpha$ chains in the nucleated cells,\(^32,33,36\) or by feedback regulation of the synthesis of $\alpha$ chains by interaction with free globin chains,\(^37\) or other mechanisms\(^38\) cannot be decided at the present time.

In summary, the use of an in vitro culture system has demonstrated balanced globin synthesis during erythroid cell maturation of nonthalassemic erythroid precursors in vitro and an imbalance of globin chain synthesis in early erythroid precursor cells in $\beta$-thalassemia, which persists throughout erythroid cell maturation. These data suggest that decreased $\beta$-globin mRNA content is manifest early in the development of erythroid cells in $\beta^+$-thalassemia, and it continues to be present in these cells as they mature.
ACKNOWLEDGMENT

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REFERENCES


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