Fetal Hemoglobin in Mixed Hemopoietic Colonies (CFU-GEMM), Erythroid Bursts (BFU-E) and Erythroid Colonies (CFU-E): Assessment by Radioimmune Assay and Immunofluorescence

By A. A. Fauser and H. A. Messner

Colony assays are now available to study erythroid differentiation at three different levels. Mixed hemopoietic colonies represent progeny of pluripotent progenitors (CFU-GEMM). Erythroid bursts and colonies are derived from early (BFU-E) and late precursors (CFU-E) that are committed towards erythropoiesis. The three different types of colonies were examined for their content of fetal hemoglobin (HbF) by radioimmune assay and/or immunofluorescence. A comparison of both methods showed the radioimmune assay to be more sensitive. Frequency analysis revealed more than 90% of all mixed hemopoietic colonies positive for HbF. The proportion was significantly lower for erythroid bursts and even further reduced for erythroid colonies. Quantitative assessment established a range of HbF concentrations for erythroid colonies between 0 and 500 pg. For erythroid bursts, concentrations between 0 and 5000 pg were measured. The amount of HbF did not correlate with the size of the examined bursts. The addition of stimulators other than erythropoietin, provided in our culture system by media conditioned with leukocytes in the presence of phytohemagglutinin (PHA-LCM), increased the frequency of HbF-containing bursts. However, the quantitative distribution of HbF was not affected. These results are consistent with the view that more primitive erythroid progenitors are more likely to give rise to HbF-containing progeny. The similarity in distributions of HbF concentrations suggests a regulatory mechanism responsible for HbF synthesis that is intrinsic to the progenitor cells and appears to be independent of cell division.

ERYTHROID PRECURSORS of varying states of differentiation give rise in culture to different types of colonies. In addition to the well established assays for CFU-E and BFU-E, a culture method is now available for human pluripotent progenitors (CFU-GEMM). In all three types of colonies, erythropoietin is required for the final development of mature hemoglobinized erythroblasts. However, stimulators other than erythropoietin influence significantly the growth of these colonies. Mixed colonies that contain granulocytes, erythroblasts, megakaryocytes, and macrophages require media conditioned by leukocytes in the presence of phytohemagglutinin (PHA-LCM) for their development. The same material, and to a lesser degree leukocyte-conditioned medium (LCM) prepared in the absence of PHA, enhance erythroid burst information. Evidence is available that suggests T cells as a possible cellular source of these stimulators. It is not known whether these stimulators improve culture conditions for human cells nonspecifically or whether they interact with heterogeneous precursor subpopula-

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Measurements of fetal (HbF) and adult (HbA) hemoglobin in individual erythroid colonies and bursts led to further appreciation of heterogeneity among CFU-E and BFU-E. Usually, a higher proportion of erythroid bursts contained HbF than erythroid colonies. This observation might suggest that immature erythroid progenitors have a greater potential to produce progeny capable of HbF production. If such a link could be verified, the presence of HbF in adult red blood cells might serve as a marker for regulatory events occurring at the level of primitive hemopoietic progenitors.

This problem was approached by investigating the influence of different culture conditions on the distribution of HbF in colonies derived from progenitors believed to be at different differentiation levels; that is, erythroid colonies, bursts, and mixed hemopoietic colonies. A radioimmune assay is described, which is suitable for quantitation of HbF in individual colonies.

Materials and Methods

Patient Material

Bone marrow specimens were obtained from six normal bone marrow transplant donors, two transplant recipients after engraftment, and five individuals during investigation for a variety of hematologic disorders. The samples were aspirated into heparinized syringes. Cord blood was collected into heparinized test tubes immediately following the transection of the umbilical cord. Peripheral blood samples from normal adult volunteers were obtained by venipuncture into heparinized syringes.

Preparation of Leukocyte-Conditioned Medium

Conditioned media were prepared under two conditions from peripheral leukocytes of normal individuals or patients with hemochromatosis. First, conventional leukocyte-conditioned medium (LCM) was produced as described by Iscove et al. Briefly, 10⁶ leukocytes/ml were immobilized in 0.5%(w/v) agar with 10% fetal calf serum (FCS) (v/v) in alpha medium (Flow Laboratories, Rockville, Md.). This semisolid agar base was overlaid with alpha medium containing 10% (v/v) FCS. The supernatant was harvested after 7 days of incubation at 37°C in humidified atmosphere with 5% CO₂.

Second, conditioned medium was prepared by incubating peripheral leukocytes for 7 days in 20% FCS with α-medium and 1% PHA (Borroughs Wellcome, Research Triangle Park, N. C.). This material, PHA-LCM, has been used extensively in this laboratory in studies of leukemic blood cells in culture. Phytohemagglutinin by itself will also increase burst information in culture, but at a higher concentration than that present in PHA-LCM.

Culture Assays for Erythropoietic Progenitors (BFU-E, CFU-E)

Erythropoietic progenitors formed erythroid bursts and colonies when immobilized in culture in the presence of appropriate stimulators, as previously described. Nucleated bone marrow cells were obtained by buffy coat preparation and were counted with a hemocytometer. Appropriate cell numbers, usually 2–3 × 10⁵ nucleated cells, were mixed with 30% FCS, alpha medium, and 2.5 U of erythropoietin (Step III, Connaught Laboratories, Toronto, Canada). Dose-response curves of suitable stimulators, such as LCM or PHA-LCM, were obtained for concentrations up to 20%. If not specifically indicated, LCM was used at 10% and PHA-LCM at a concentration of 5%. The suspensions were mixed to yield a final methylcellulose concentration of 0.8%. Quadruplicate cultures containing a volume of 1 ml/35-mm plastic Petri dish were incubated at 37°C in a moist atmosphere with 5% CO₂. The CO₂ concentrations were routinely monitored with a Bacharach Fyrite test kit. Colony formation was assessed using an inverted microscope. Erythroid colonies (CFU-E) were enumerated after 7 days in culture. They consist of 8–64 hemoglobinized cells in a compact cluster. On days 12–14, erythroid bursts
Colony appeared predominantly red, while translucent cells in other areas of the same colony lacked this feature typical for hemoglobin-containing cells. In addition, within some colonies cells of much larger size could be observed. Colonies of mixed appearance were picked by micropipette and examined for HbF by immunofluorescence, as outlined below. Subsequently, the presence of multiple hemopoietic lineages in individual colonies was confirmed by Wright stain. This assessment precluded the use of the radioimmunassay for HbF analysis of mixed colonies.

Preparation of Hemoglobin

Hemoglobin preparations were obtained from hemolysates of cord blood or peripheral blood samples of normal adult individuals by passage through DEAE Sephadex-A-50 (K9/20; Pharmacia Fine Chemicals, Piscataway, N.J.) and rechromatography of appropriate fractions on CM-Sephadex-C-50 (K9/20; Pharmacia Fine Chemicals). The resulting hemoglobin preparations were assessed for purity by SDS polyacrylamide gel electrophoresis and isoelectric focusing using LKB-Ampholine.

Preparation of Antibodies

Antibodies directed against HbF were prepared as described by Wood et al. Two milligrams of purified HbF were injected subcutaneously into each rabbit with complete Freund’s adjuvant. Injections of 2 mg HbF on day 14 and 10 mg on day 28 were repeated with incomplete Freund’s adjuvant. Titers were assessed by double diffusion in agar, and sera were usually harvested after 3–5 wk following the final injection. The crude antisera were cross-reactive with HbA and required purification of the activity directed against HbF.

Crude antisera were dialyzed for 24 hr against a 0.2-M buffer at pH 8 with 3 exchanges and passed through a Sepharose 4B immunoabsorption column (K16/20) coupled with purified HbA. Antibodies directed against HbA were removed by this procedure, while the anti-HbF activity remained in the unbound portion of the serum, as assessed by double diffusion in agar. This unbound material was concentrated to the original volume using a UM 30 Amicon filter and then passed through a second Sepharose 4B immunoabsorption column (K16/20) containing purified HbF. The procedure absorbed the anti-HbF activity. Both columns were extensively washed with a 0.2-M borate buffer before eluting the bound antibodies with CO2 water at a pH of 4.0. Of the protein recovered from the Sepharose 4B columns, 40% was recovered after adherence to an HbA column and 60% after adherence to an HbF column. Small amounts of hemoglobin present in the latter preparation were removed by a CM 52 cellulose (Whatman) column (K26/12) chromatography. During this procedure, 6%–10% of the protein was lost. The remaining antibodies were dialyzed against tris-HCl buffer (0.01 M, 0.2-M NaCl) at pH 7.4, concentrated using a UM 30 Amicon filter, and stored at −70°C for further use in the radioimmunonassay. The preparations were reassessed for specificity in double diffusion agar plates with serial dilutions (1:1–1:32) of antigen and antibody. No cross-reactivity with HbA was observed in any dilution.

Antibody preparations to be used for HbF immunofluorescence analysis were processed to obtain the Fab’2 fragment. After chromatography through a CM 52 cellulose column, the monospecific antibody preparations were dialyzed against 0.1 M acetate buffer at a pH of 4.5, cleaved with pepsin (1:60,000, Sigma, St. Louis, Mo.) for 20 hr at 37°C, neutralized with solid tris to a pH of 8, and then chromatographed in tris-HCl buffer through a Sephadex G-200 column (K26/70; Pharmacia Fine Chemicals). Material containing the Fab’2 fraction was concentrated as described above and stored frozen at −70°C. The purification procedures were monitored by SDS polyacrylamide gel electrophoresis, and specificity was tested by double diffusion in agar using serial dilutions of antigens and antibodies up to titers of 1:32. Again, no cross-reactivity of the Fab’2 fragment with HbA was observed under these conditions.
Indirect Immunofluorescence of HbF-Containing Erythroid Progenitors in Human Erythroid Burst and Colonies

CFU-E colonies were picked by micropipette on day 7; bursts were harvested on day 14. Some 20–50 colonies of either type were taken at random in each experimental group and placed directly on glass slides. In some experiments, colonies were added to a 1% bovine serum albumin (BSA) solution in phosphate-buffered saline (PBS), and slides were prepared by cytocentrifugation using a Shandon Cytocentrifuge. The air-dried specimens were fixed by adding cold ethanol twice for 15 min, and the fixed slides were washed for 2 × 10 min in tris-HCl buffer (0.01 M tris, 0.5 M NaCl, 0.02% Na-azide) at pH 7.4. At this stage, the slides may be immediately processed for immunofluorescence or stored at 4°C for future examination.

For immunofluorescence, slides were wetted with tris-HCl buffer. The specimens were incubated with 5μl of rabbit anti-human HbF Fab'2 fragment at concentrations of 200 μg/ml for 1 hr at 37°C and washed twice thoroughly in the same buffer. These slides were then exposed for 1 hr at 37°C to 10 μl of fluoroisothiocyanide (FITC) conjugated Fab'2 fragment goat anti-rabbit IgG Fab'2 fragment (Cappel, Cochranville, Pa.). After being washed twice in buffer, the wet slides were mounted with a coverslip to avoid rapid drying. Colonies were evaluated with a fluorescence microscope that is equipped with a specific filter combination for FITC-labeled specimens.

Radioimmune Assay for HbF Hemolysates of Erythroid Bursts and Colonies

The assessment of HbF levels by radioimmune assay required the production of specific anti-HbF antiserum, iodination of purified HbF molecules, and the preparation of hemolysates of individual colonies.

Antiserum against HbF were raised in rabbits as described above. Two micrograms of purified HbF were iodinated as outlined by Sonoda and Schlamowitz. Briefly, the reaction mixture contained 0.1 mCi of Na-125I, 2 μg Chloramine-T in a total volume of 45 μl of 0.3 M phosphate buffer at a pH of 7.4. The reaction was carried out at 0–2°C and terminated after 12 min by adding 75 μg of DL-tyrosine. Free 125I was removed by passage through a Sephadex G-50 column. Rechromatography of the iodinated protein on a Sephadex G-100 column yielded a single peak, demonstrating that no significant aggregation of proteins had occurred during the iodination procedure.

Twenty to fifty erythroid bursts and colonies were harvested from the culture plates by micropipette and hemolyzed in 10 μl of distilled water. After overnight storage at 4°C, the following mixture was added to each hemolysate: 32 μl of a solution that contained 0.3 M phosphate buffer, 0.1% Triton-X 100, 0.02% Na-azide, 10 μl/ml BSA, and 3 μl of rabbit serum. This mixture remains stable for several weeks when stored at 4°C.

The radioimmune assay was performed as outlined by Kung et al. Briefly, the above mixture was incubated for 1 hr at 37°C with 5 μl of a 1:400 dilution of the anti-HbF antiserum. This concentration was sufficient to precipitate 50% of the input 125I-labeled HbF. Subsequently, a known amount of 125I-labeled HbF was added for 1 hr to compete for the remaining specific anti-HbF antiserum. At that time, 25 μl of goat anti-rabbit antiserum was added and the mixture transferred to 4°C. The precipitates formed after 8 hr were washed twice with buffer and counted in a gamma counter (LKB 80,000).

Standard curves were obtained for purified HbF (Fig. 1). As can be seen, very low concentrations of HbF could be measured, making the radioimmune assay suitable for HbF analysis in single colonies. As demonstrated in Fig. 1, no competition with HbA was observed up to a concentration of 10 ng. Granulocytic colonies derived from CFU-C and samples of cell-free tissue culture medium from the same culture plates were negative for HbF.

A comparison of both assays for HbF on erythroid colonies and bursts grown from specimens of 6 individuals (Table 1) yielded the radioimmune assay more sensitive.

RESULTS

Influence of Varying Concentrations of LCM and PHA-LCM on Erythroid Burst Formation

The plating efficiencies of CFU-E and BFU-E can be altered by varying the culture conditions. Bone marrow cells were grown in culture with optimal erythropoietin levels of 2.5 U and increasing concentrations of LCM or PHA-LCM. The
addition of both types of stimulator molecules enhanced the number of erythroid bursts beyond the values obtained in cultures that contained only erythropoietin. Typical dose–response curves are displayed in Fig. 2. Plateau levels of bursts grown in the presence of PHA-LCM appeared higher than those observed with optimal concentrations of conventional LCM. Neither LCM nor PHA-LCM promoted growth of hemoglobin-containing erythroid bursts or colonies in the absence of erythropoietin.

Influence of Differing Culture Conditions on Hemoglobin F Distribution in Erythroid Bursts and Colonies

The influence of these three different culture conditions on the amount of HbF in individual bursts and colonies was examined. Bone marrow specimens of 4 patients

![Graph](image)

**Fig. 1.** Precipitation of $^{125}$I-labeled HbF with increasing concentrations of HbF and HbA added in a volume of 10 μl to the reaction mixture. No competition with purified HbA was observed.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Hb</th>
<th>Retics</th>
<th>F Cells*</th>
<th>CFU-E IF</th>
<th>RIA IF</th>
<th>BFU-E IF</th>
<th>RIA IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR†</td>
<td>Normal erythropoiesis</td>
<td>13.6</td>
<td>65,000</td>
<td>&lt;1%</td>
<td>0%</td>
<td>6%</td>
<td>28%</td>
<td>37%</td>
</tr>
<tr>
<td>MO</td>
<td>IASA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc(1)‡</td>
<td>Bone marrow transplant</td>
<td>12.2</td>
<td>100,000</td>
<td>12%</td>
<td>12%</td>
<td>34%</td>
<td>34%</td>
<td>63%</td>
</tr>
<tr>
<td>recipient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mc(2)‡</td>
<td>Bone marrow transplant</td>
<td>13.2</td>
<td>300,000</td>
<td>3%</td>
<td>14%</td>
<td>30%</td>
<td>38%</td>
<td>65%</td>
</tr>
<tr>
<td>recipient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BE</td>
<td>ALL (remission)</td>
<td>14.5</td>
<td>55,000</td>
<td>3%</td>
<td>3%</td>
<td>13%</td>
<td>31%</td>
<td>53%</td>
</tr>
<tr>
<td>IA</td>
<td>Lymphoma</td>
<td>15.4</td>
<td>47,000</td>
<td>7%</td>
<td>8%</td>
<td>34%</td>
<td>16%</td>
<td>49%</td>
</tr>
<tr>
<td>LA</td>
<td>Idiopathic neutropenia</td>
<td>15.1</td>
<td>67,000</td>
<td>4%</td>
<td>15%</td>
<td>43%</td>
<td>30%</td>
<td>81%</td>
</tr>
</tbody>
</table>

All colonies were grown in the presence of 2.5 U of erythropoietin and 10% conventional LCM. Colonies that contained more than 200 pg of HbF by RIA were considered positive.

*Measured by immunofluorescence.
†A 3-yr posttransient bone marrow aplasia.
‡Bone marrow transplant for aplastic anemia (1) 40 days and (2) 92 days postengraftment.
with a variety of clinical diagnoses were exposed to: (1) 2.5 U of erythropoietin, (2) 2.5 U of erythropoietin and 10% LCM, and (3) 2.5 U of erythropoietin and 5% PHA-LCM. Erythroid colonies and bursts were enumerated, and a random sample of individual colonies was assessed by radioimmune assay for their HbF content.

The HbF concentrations measured for individual bursts grown under different culture conditions are plotted in Fig. 3. Each experimental group yielded a heterogeneous distribution of values with a spread between 0 and 5000 pg of HbF. In addition, patient-to-patient variation was observed. In 3 of the 4 individuals, only occasional bursts of HbF levels of more than 1500 pg were encountered. In one patient, more than half of all bursts contained an excess of 1500 pg, with maximal values of 5000 pg. The addition of LCM or PHA-LCM did not significantly
influence the distribution of HbF among bursts. Representative data from one of the patients [Mc(1)] are shown as a cumulative distribution (log probit plot) for values obtained for bursts grown under these three different culture conditions (Fig. 4). Very similar slopes were observed for each of the experimental conditions, indicating that the distribution of HbF among bursts was not significantly altered by the culture conditions.

Although the distribution of HbF among bursts was not altered by culture conditions, total plating efficiencies for three of the four patients were increased in cultures with PHA-LCM (Fig. 5). From Fig. 5, it is evident that the added stimulator permitted the detection of more bursts that contained HbF; thus, the distribution was determined more completely, since a higher number of HbF-containing bursts was available for its determination.

**Distribution of HbF in Colonies of Different Sizes**

The increased number of HbF-positive bursts observed in cultures with PHA-LCM might reflect increased proliferation. To test the relationship between the...
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Fig. 6. Concentration of HbF in individual bursts as function of size. Bursts grown from 2 individuals (GM, open symbols; Mc(1) closed symbols) with 2.5 U of erythropoietin and 5% PHA-LCM were assessed.

number of divisions and the presence of HbF, burst size and HbF concentration were compared for individual bursts from cultures of marrow from two patients. One patient (GM) was analyzed 2 mo after serving as a bone marrow transplant donor; the second [Mc(2)] was studied 92 days after successful engraftment. Individual erythroid colonies were assessed for HbF by radioimmune assay after 7 days of culture; bursts were analyzed after 14 days. Erythroid bursts were judged by eye to be small (<250 cells), intermediate (250–500 cells), and large (>500 cells). Erythroid colonies regularly contained 20–80 cells. Similar results were obtained for both patients (Fig. 6). Of all bursts, 68% and 76% were found positive for HbF. The HbF content in positive bursts varied from 500 to 3000 pg. The distribution was independent of the size of the bursts. The amount of HbF present in positive erythroid colonies was significantly lower and varied between 200 and 600 pg.

HbF Analysis in Mixed Hemopoietic Colonies

The question of whether or not more primitive progenitors have a greater probability to produce progeny capable of HbF synthesis was directly approached by analyzing mixed hemopoietic colonies for HbF. Six bone marrow specimens and one peripheral blood sample obtained from four normal bone marrow transplant donors and one recipient after engraftment were examined for their ability to promote growth of mixed hemopoietic colonies that contain granulocytes, erythroblasts, and, in lower frequency, also megakaryocytes and macrophages. These colonies were identified by their morphological appearance and examined for HbF by immunofluorescence. Subsequently, their composition of various hemopoietic lineages was determined by cytologic analysis using a Wright stain. In three of the samples, the frequency of HbF was compared in mixed hemopoietic colonies and erythroid bursts grown in the presence of PHA-LCM and erythropoietin (Table 2).

A total of 68 mixed colonies was obtained. Almost all (93%) were positive for HbF. This degree of positivity was significantly higher than that for erythroid bursts or colonies obtained from the same specimens or the frequency of HbF-positive bursts observed in previous experiments (Table 1). Only 25% of bursts were HbF-positive in cultures of marrow from 3 normal subjects.
Table 2. Identification of HbF in Mixed Granuloerythrocytic Colonies and Erythroid Bursts by Immunofluorescence

<table>
<thead>
<tr>
<th>Name</th>
<th>Diagnosis</th>
<th>Specimen</th>
<th>No. HbF-Positive Colonies</th>
<th>Total No. Colonies Examined</th>
<th>No. HbF-Positive Colonies</th>
<th>Total No. Colonies Examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.P.</td>
<td>Bone marrow donor</td>
<td>BM</td>
<td>9</td>
<td>10</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>G.H. (1)†</td>
<td>Bone marrow donor</td>
<td>BM</td>
<td>10</td>
<td>10</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>D.J. (1)*</td>
<td>Bone marrow transplant recipient</td>
<td>BM</td>
<td>3</td>
<td>3</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>D.J. (2)*</td>
<td>Bone marrow transplant recipient</td>
<td>BM</td>
<td>8</td>
<td>10</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>G.H. (2)†</td>
<td>Bone marrow donor</td>
<td>BM</td>
<td>15</td>
<td>16</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>J.M.</td>
<td>Bone marrow donor</td>
<td>BM</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>R.Mc.</td>
<td>Bone marrow donor</td>
<td>BM</td>
<td>9</td>
<td>10</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Total no. of colonies</td>
<td></td>
<td></td>
<td>63</td>
<td>68</td>
<td>9</td>
<td>36</td>
</tr>
<tr>
<td>Proportion HbF-positive</td>
<td></td>
<td></td>
<td>93%</td>
<td>25%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND, not done.
*Transplant for aplastic anemia (1) 8 mo and (2) 11 mo following engraftment.
†Two samples of the same transplant donor were studied.

DISCUSSION

A number of investigators have reported the presence of HbF in erythroid colonies and bursts derived from human bone marrow and peripheral blood. We were able to confirm these observations using a radioimmune assay. This method also permitted quantitation of HbF in individual bursts. Regrettably, a comparable measurement of HbA per burst was not feasible. However, it is unlikely that the presence of HbA would greatly alter the quantitation, since HbA did not compete in the assay with HbF up to concentrations of 10 μg (Fig. 1). If higher relative concentrations were competitive in individual bursts, this might have occurred more frequently in larger bursts; yet no relationship was seen between burst size and HbF content.

Papayannopoulou et al. have suggested that a link may exist between the level of differentiation of erythropoietic progenitors and their ability to give rise to bursts containing HbF. Our studies employed two methods to examine this hypothesis. First, by using a quantitative radioimmune assay, it was possible to determine the distribution of HbF among individual bursts. Second, culture conditions were varied; particularly, PHA-LCM was used. This material increased the plating efficiency of erythroid bursts, and since it is essential for the production of mixed colonies, it may be active on very early progenitors.

We found that, although cultures stimulated by PHA-LCM contained an increased number of HbF-positive bursts, the distribution of HbF among bursts was not affected (Fig. 4). This distribution is markedly asymmetrical, with many bursts containing little HbF and few containing large quantities. Skewed distributions of this form may be generated by random events occurring during colonial growth. Thus, in the present context, molecular events leading to HbF synthesis...
occurring early might lead to bursts containing large quantities of that hemoglobin, while late events would yield bursts with little or no detectable HbF. Regardless of the mechanism, the similarity in distributions observed in cultures with varying concentrations of erythropoietin and PHA-LCM is consistent with a mechanism of regulation of HbF synthesis that is intrinsic to progenitor cells and not greatly affected by culture conditions. As might be expected for a molecular mechanism, the event leading to HbF synthesis appeared to be independent of the number of cell divisions occurring during burst formation, since no correlation was observed between HbF content and burst size.

Mixed hemopoietic colonies were examined by HbF only by immunofluorescence; thus, quantitative data are not available to determine whether the distribution of HbF among such colonies resembles that for bursts. However, it was apparent that even using this less sensitive assay, almost all mixed colonies contained HbF. The initiation of HbF synthesis may be considered to be a component in an erythropoietic differentiation program and more probable for primitive cells than for more differentiated cells.

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Fetal hemoglobin in mixed hemopoietic colonies (CFU-GEMM), erythroid bursts (BFU-E) and erythroid colonies (CFU-E): assessment by radioimmune assay and immunofluorescence

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