Fetal Erythropoiesis Following Bone Marrow Transplantation

By Blanche P. Alter, Joel M. Rappeport, Titus H. J. Huisman, Walter A. Schroeder, and David G. Nathan

"Fetal" erythrocytes are present in older children and certain adults with hematologic disorders. To determine if regenerating bone marrow produces such cells, we examined the blood of seven allogeneic bone marrow transplant recipients. Six patients were engrafted with donor cells, while one patient recovered autologous bone marrow after rejection of a marrow transplant. All seven patients had fetal hemoglobin levels of up to 10% by 100 days after transplant. In three patients, the $\gamma$ to $\delta$ ratio in the fetal hemoglobin was "newborn," while in one it was "adult." Gamma chain synthesis in blood and bone marrow never exceeded 20% of total non-alpha globin synthesis. The fetal hemoglobin was heterogeneously distributed in the cells. High titer i antigen also appeared. All fetal characteristics declined by 200 days. Erythropoiesis during bone marrow recovery appears to be associated with an accelerated, albeit partial, recapitulation of ontogeny.

ERYTHROCYTES WITH FETAL CHARACTERISTICS are present in older children and adults with a variety of hematologic disorders, such as leukemia, bone marrow failure, and refractory anemias. To determine if regenerating marrow produces such erythrocytes, we studied seven consecutive patients with aplastic anemia or acute leukemia who were immunosuppressed and had been engrafted with allogeneic bone marrow. An increase in fetal hemoglobin had been previously noted in some of these patients after transplantation.

The characteristics of fetal erythrocytes are summarized in Table 1. Fetal hemoglobin, $\alpha_2\gamma_2$, predominates in intrauterine life, but is replaced in infancy by the major adult hemoglobin $\alpha_2\beta_2$, and the minor adult hemoglobin $\alpha_2\delta_2$. The gamma chain of fetal hemoglobin is heterogeneous at the 136

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position because of production by nonallelic genes. Approximately 70% of the gamma chains in each individual newborn have glycine residues at the 136 position, and 30% have alanine. This $G\gamma$ to $A\gamma$ ratio of approximately 3:1 characterizes the “newborn” type of fetal hemoglobin. In adults, the small amount of gamma chains have 0.4 glycine residue at 136, and a consequent $G\gamma$ to $A\gamma$ ratio of 2 to 3. The membranes of fetal erythrocytes are characterized by their content of $i$ antigen. Adult erythrocytes have $I$ antigen, which is thought to be a modification of the $i$ matter. The red cells of fetuses and newborns are larger than those of adults, and have a shorter life span. They have less carbonic anhydrase and have distinctive levels of other enzymes.

Fetal hemoglobin levels of over 50% and as much as 85% have been reported in juvenile chronic myelogenous leukemia, and in some cases of erythroleukemia. Less extreme increases in hemoglobin F have been reported in other types of leukemia as well, both in children and in adults. Patients with congenital aplastic anemias, such as Fanconi’s pancytopenia or pure red cell aplasia, have had fetal hemoglobin levels of up to 30%. Increased fetal hemoglobins have been seen in people with untreated acquired aplastic anemia and in those with refractory anemia or “preleukemia.”

The glycine value at the 136 position of the gamma chain in juvenile chronic myelogenous leukemia indicates that in this disorder expression of the gamma genes is of the “newborn” type. In other types of leukemia, some patients have had “newborn,” while others have had “adult” gamma chains. Similarly, the gamma chain has been either newborn or adult in acquired aplastic anemia or in Fanconi’s anemia, although it has been only “newborn” in Diamond-Blackfan anemia. All of these patients have been older children or adults.

The fetal red cell membrane $i$ antigen has been observed in leukemia and aplastic and refractory anemias. Fetal hemoglobin and $i$ antigen are usually seen in the same disorders. However, $i$ antigen can appear as a manifestation of bone marrow stress in the absence of fetal hemoglobin. This observation has been demonstrated by Hillman and Giblett in normal adults who had been phlebotomized extensively and had developed $i$ antigen but no fetal hemoglobin.

Characteristic fetal patterns of red cell carbonic anhydrase have been observed in juvenile chronic myelogenous leukemia. Other glycolytic enzyme activities characteristic of the fetal nature of the erythrocytes have been observed in erythroleukemia and in refractory anemia.

The enzymatic characteristics of fetal red cells described above are difficult to
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detect in the blood of patients who have received recent transfusions. Since all of our bone marrow transplant recipients had been transfused, we confined our studies to those items that could be evaluated reliably in this setting: the amount and distribution of fetal hemoglobin, the $\Delta\gamma$ to $\beta\gamma$ ratio of the fetal hemoglobin, globin chain biosynthesis, and i antigen titer. These fetal features appeared and then declined in our patients. In six of the transplant recipients, the blood was shown to be of donor origin, and thus the erythrocytes with fetal characteristics were derived from normal donor stem cells. One patient rejected the graft and recovered his own bone marrow. He also showed the same pattern of transient fetal erythropoiesis.

MATERIALS AND METHODS

Seven patients received bone marrow transplants, three for acute myelogenous leukemia and four for aplastic anemia. Diagnostic criteria and transplant preparations and subsequent management have been described previously. All donors were HLA-matched MLC nonreactive siblings. Identifying characteristics of the patients and donors are outlined in Table 2.

Following transplantation, several parameters were assessed at frequent intervals. Blood counts, indices, and reticulocytes were measured with standard methods. Fetal hemoglobin was quantitated by alkali denaturation and its distribution assessed by the acid elution technique, using a prepared reagent kit from Boehringer-Mannheim. Analysis of the $\Delta\gamma$ to $\beta\gamma$ ratio was done according to published procedures.

Globin chain synthesis was evaluated by incubation of peripheral blood or bone marrow with $^3$H-leucine (New England Nuclear Corporation, 80 Ci/m mole) or $^{35}$S-methionine (New England Nuclear Corp., 400 Ci/m mole). Chain separation was done by chromatography on carboxymethylcellulose in 8 M urea. The globin peaks from the columns were recovered and fingerprinted in some experiments.

Anti-i serum (Den) was kindly provided by Marie Crookston. It was diluted 1/50 in normal saline, then in fivefold serial dilutions. The red cells were washed three times with saline, and resuspended at a concentration of 2% in saline. One drop of cell suspension was mixed with one drop of diluted anti-i in a 10 x 75-mm test tube. The suspensions were left stationary for 4 hr. and were gently resuspended by tapping. They were spun at 1000 rpm (300 g) for 1 min, resuspended again, and then read on a microscope slide. The agglutinates were

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Table 2. Patient Identification Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>Age</th>
<th>Phenotypes</th>
</tr>
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<tbody>
<tr>
<td>J.F. AML</td>
<td>9</td>
<td>13</td>
<td>Fy$^b$, C$^+$</td>
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<tr>
<td>M.L. AML</td>
<td>19</td>
<td>26</td>
<td>XY</td>
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<tr>
<td>T.V. AML</td>
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<td>24</td>
<td>YY</td>
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<td>XY</td>
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<tr>
<td>R.S. Aplastic</td>
<td>15</td>
<td>17</td>
<td>XY</td>
</tr>
<tr>
<td>P.G. Aplastic</td>
<td>17</td>
<td>17</td>
<td>XX</td>
</tr>
</tbody>
</table>

AML, acute myelogenous leukemia; TBI, total body irradiation; ATS, antithymocyte serum.
graded from ± to 4+.\textsuperscript{45} Red cell antigen "titer" was based on the last positive antibody dilution. All procedures were performed at 20°C.

RESULTS

Six patients had successful bone marrow engraftment. The data are shown in Fig. 1. Reticulocytes appeared between 21 and 31 days, and reached their peaks at 45-82 days. The highest values were 6%-19%. The fetal hemoglobin rose with the reticulocyte count and attained a maximum at 31-100 days following transplantation. The peak fetal hemoglobin ranged from 4% to 10%, which corresponded to 400-1200 mg/100 ml. The mean corpuscular volume reached the maximum of 105-118 cu μ at between 48 and 159 days.

Four patients had several measurements of the $\delta^\gamma$ to $\gamma^\gamma$ ratio. Three had
glycine values above 0.65 in the "newborn" range. One patient's glycine ratio was 0.5 or below (i.e., "adult") on three occasions over a 6-mo interval.

The three patients in whom the i antigen was measured (the same three who had "newborn" \( \gamma \) to \( \gamma \) ratios) had titers of 1/1250 on at least one occasion. Normal adult erythrocytes always had a titer of below 1/50, while cord cells were 1/6250. Thus the patients had significant amounts of i antigen.

One patient had a late rise in reticulocytes, fetal hemoglobin, and i antigen; these occurred during an episode of Coombs'-positive hemolytic anemia.

Table 2 lists the information which indicates that the six patients presented above were reconstituted with homologous donor bone marrow stem cells. One patient (P.G.) recovered his own hematopoietic cells. The white cell chromo-

![Graph](image-url)  

**Fig. 2.** Data in one patient whose own bone marrow recovered. The data are superimposed on the data from the six patients shown in Fig. 1; ——, patients who recovered with transplanted cells; ——, patient (P.G.) who recovered with autologous cells.
somes were male, whereas his donor was female, and his red cells failed to express Fy⁺, an antigen present on the donor’s cells.

The course of the patient (P.G.) who rejected a bone marrow graft and recovered with his own marrow cells is compared with the courses of the six engrafted patients in Fig. 2. Bone marrow examination at 2 wk following transplant indicated engraftment, as did leukocyte cytogenetics, but the graft was lost by 3 wk. At 4 wk, antithymocyte serum was administered in preparation for a second transplant. This transplant was not done because granulocytes and reticulocytes appeared by day 55 (9 days after the start of antithymocyte serum), and the patient began to recover. Reticulocytes reached the maximum of 17% on day 104 (dated from the original transplant). Fetal hemoglobin was 10% at that time, and the i antigen titer was 1/6250. The increased cell size, fetal hemoglobin, and i antigen have persisted in this patient despite achieving a normal hemoglobin level.

Incubation of this patient’s blood with ³H-leucine was performed on days 64 and 91. Globin chain analysis from the day 64 specimen revealed the production of 12% gamma chain (Fig. 3). At this time, his blood contained only 0.7% fetal hemoglobin (Fig. 2), a value that rose to 10% 1 mo later. At this later time, a globin synthesis study indicated the production of 18% gamma chain. Incubations of both blood and bone marrow with ³⁵S-methionine were also performed on day 64, and the globin chains were isolated on carboxymethylcellulose columns and fingerprinted. The putative gamma chain contained only gamma- and no beta-methionine peptides. Similar studies were performed in two of the patients who had been reconstituted with donor cells with identical results.

A representative acid elution pattern is shown in Fig. 4, taken 75 days after a successful transplant. At this time, 27% of the cells had some fetal hemoglobin, while the fetal hemoglobin measured by alkali denaturation was only 9%. From the earliest time at which fetal cells could be seen, the heterogeneous distribution demonstrated in the figure was apparent. Some cells were definitely fetal and some were ghosts (probably transfused cells), while some cells were intermediate in staining intensity and presumably contained both fetal and adult hemoglobin, indicating that some of the erythrocytes produced following bone marrow transplant were not entirely fetal in their characteristics.

Fig. 3. Radiochromatogram of globin synthesis in the patient (P.G.) who recovered with his own bone marrow. Blood was incubated with ³H-leucine on day 64.
Fig. 4. Acid elution preparation of blood on day 75, from a patient (T.F.) with a successful bone marrow transplant. The dark cells contain predominantly fetal hemoglobin, the gray have both fetal and adult, and the ghosts have only adult hemoglobin.

DISCUSSION

Total bone marrow obliteration and regeneration with transplanted marrow is the most extreme clinical example of an acquired hematopoietic “stress.” In this circumstance, previously normal stem cells replicate rapidly in a new environment. We have demonstrated that this situation leads to the production of red blood cells which reflect this transient stress by the presence of certain fetal characteristics. These are exemplified by the presence of fetal hemoglobin, as measured by resistance to acid elution or alkali denaturation. Gamma-chain synthesis can be detected by column chromatography and confirmed by fingerprinting. In three of the four patients from whom data are available, the $G_\gamma$ to $A_\gamma$ ratio of the newly produced gamma chains identified them as being “newborn” in type.

In addition to fetal hemoglobin, the red blood cells following transplantation had almost as much i antigen as do the cells of newborn infants. The slightly lower titer in the patients in comparison to newborns was probably due to dilution of their i-positive cells by i-negative cells from previous transfusions. Macrocytosis, another characteristic of fetal red cells, was also a consistent finding in the patients, with mean cell volumes above 100 cu $\mu$. This macrocytosis persisted even after reticulocytosis had subsided.

Fetal hemoglobin production, as determined by the relative amount of gamma chain synthesis, did not exceed 20% in any of these patients, and hence
fetal erythropoiesis was incomplete. The differential stain for fetal hemoglobin revealed that some cells had intermediate stain intensity and contained both fetal and adult hemoglobin, another indication that the stem cells of the normal donor produced erythroid cells that partially expressed fetal characteristics. This heterogeneous pattern of fetal hemoglobin accumulation was also observed in aplastic anemia managed without transplantation.\textsuperscript{19,22,26} In contrast, in juvenile chronic myelogenous leukemia, fetal hemoglobin has been heterogeneously distributed only early in the disease. In its later stages, almost all red cells become filled with fetal hemoglobin. This finding suggests that the fetal cells in this disorder may be produced by a malignant clone of erythroid stem cells.\textsuperscript{11}

A recent report by Papayannopoulou et al.\textsuperscript{48} showed induction of fetal hemoglobin synthesis in vitro. Bone marrow from normal adults was cultured, and gamma-chain synthesis increased from about 1\% to as much as 20\% of non-alpha production. These in vitro experiments resembled our in vivo findings. It is possible that the erythroid stem cells, which can rapidly repopulate initially in an environment with a high amount of erythropoietin, are the ones that are programmed for more gamma-chain production than the average bone marrow cell. That is, these cells may have a selective advantage in the in vitro culture system and in the empty bone marrow of the transplant recipient. All cells that replicate in vitro or in vivo may produce both gamma and beta chains; in vivo these cells may be overgrown in time by cells that produce mostly beta and little gamma chain.

The amount of fetal hemoglobin which accumulated in the transplanted patients never exceeded 10\%, and began to decline after 50-100 days, as did the i antigen titer. These observations indicated that the fetal erythropoiesis was transient, as well as incomplete. It subsided as hematopoiesis recovered more completely. In one patient in whom hematopoietic stress recurred in the form of an immune hemolytic anemia, the fetal erythropoietic characteristics also reappeared (Fig. 1).

Hillman and Giblett\textsuperscript{33} have suggested that the appearance of the i antigen is related to shortened bone marrow transit time. Premature release of red cells from the marrow might prevent complete conversion of i to I, or interfere with the development of the i to I switching mechanism. A decrease in the i titer later in the patients’ courses would be explained by a dilution of the i cells by normal, i-negative cells subsequently released from the marrow, rather than by loss of i from the original cells as they matured in the circulation.

A similar theory concerning erythroid maturation and the switch from fetal to adult hemoglobin has been discussed by Baglioni.\textsuperscript{46} He has elaborated on the suggestion of Marks and Burka\textsuperscript{47} that all erythroid cells synthesize gamma chains when hemoglobin synthesis begins. Normal cells lose this capacity early in the development of the cell within the bone marrow. In conditions in which maturation is accelerated and the cells emerge from the marrow prematurely, the ability to synthesize gamma chains is retained. When the erythropoietic stress decreases, cells remain within the bone marrow longer and complete more of the switch from fetal to adult status.

In this paper we report data which indicate that transient and incomplete fetal erythropoiesis follows bone marrow transplantation. A similar pattern
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has been observed in patients with leukemia as they recover from chemotherapeutically induced aplasia.49 The transience is demonstrated by the gradual acquisition and subsequent decline of fetal hemoglobin and i antigen. The incompleteness is apparent from the heterogeneous distribution of fetal hemoglobin and from the observation that fetal hemoglobin production is never near the level of fetal synthesis observed in the blood of newborn infants. In addition, while three aplastic patients did have “newborn” fetal hemoglobin, one (AML) had “adult” fetal hemoglobin, as determined by the $^6\gamma$ to $^A\gamma$ ratios. These ratios have been consistent on more than one occasion. Presumably, the “newborn” $^6\gamma$ to $^A\gamma$ ratios revert to “adult” as the otherwise fetal erythrocytes disappear. This parameter has not been measured when the hemoglobin F level has decreased to normal because of the large quantity of blood that would have been required.

The pattern of transient fetal-like erythropoiesis was observed in homologous red blood cells that proliferated after bone marrow engraftment, as well as in the autologous cells of the one patient who rejected his graft. This partial and accelerated recapitulation of ontogeny presumably reflected the stressful erythroid development in a completely repopulating marrow and resembled the findings in other dyserythropoietic states. Whether these fetal characteristics are found in the same or in different cells remains to be fully investigated.

After completion of this work, it came to our attention that Thomas et al., and Speck et al. have recently reported one patient each who recovered with autologous bone marrow following immunosuppression and rejection of donor marrow.

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