Heterogeneous Ontogeny of Erythropoiesis After Bone Marrow Ablation and Allogeneic Bone Marrow Grafting

By Karl J. Winkler, Christopher D. Rea, Samuel Rahbar, L. Robert Hill, and Karl G. Blume

Thirty-two patients who underwent bone marrow transplantation for hematologic malignancies were studied to evaluate the use of fetal hemoglobin synthesis as an indicator of fetal erythropoiesis. Two different patterns of response were noted. One group of patients who had an elevated HbF level prior to marrow grafting later showed a marked reactivation of HbF synthesis, whereas the other group of patients who had normal HbF levels prior to transplantation failed to exhibit such an increase. This phenomenon occurred independently of age of marrow graft donors or recipients, the type of underlying hematologic malignancy, or remission induction therapy prior to preparation for transplantation, pretransplant hemoglobin levels, transfusion with red blood cells, red cell volume, and production of reticulocytes.

HUMAN and several other mammalian species sequentially express different globin genes during ontogeny, or during periods of “stress,” such as hypoxemia or anemia. Hemoglobin ontogeny in humans includes three distinct phases. The embryonic phase predominates from the 5th to the 16th week of gestation. In this period, embryonic hemoglobin chains are ε and δ. Throughout the remainder of gestation (“fetal” phase), the major hemoglobin is hemoglobin-F (αγ2), but gradually increasing amounts of HbA (α2β2) can be detected during this phase. After birth, fetal hemoglobin is gradually replaced by adult hemoglobin (HbA), and HbF represents approximately 1% of the total hemoglobin (“adult” phase). Residual hemoglobin-F contains two different γ-chains designated Gγ and Aγ, which contain either glycine (Gγ) or alanine (Aγ) at position 136. The synthesis of the two types of chains is coded by two nonallelic structural gene complexes with the linkage arrangement ψβ2, ε, Gγ, Aγ, ψβ1, δ, β. The ratio of Gγ:Aγ synthesized at birth is approximately 7:3 and declines during the first 6 mo of life.

In certain hematologic disorders such as leukemia, aplastic anemia, childhood erythroblastopenia, and juvenile chronic granulocytic leukemia, as well as in in vitro culture systems, a reactivation of fetal hemoglobin synthesis occurs. A transient manifestation of fetal erythropoiesis also has been documented in a small number of patients recovering from bone marrow transplantation (BMT) that had been performed as a treatment for aplastic anemia and leukemia.

We have undertaken a study to analyze the following aspects: (1) Is a repeated ontogeny a constant factor of erythropoiesis after BMT? (2) Do factors of donor or host influence the pattern of recovery?

MATERIALS AND METHODS

EDTA blood samples were obtained at weekly intervals from 32 patients undergoing allogeneic BMT for hematologic malignancies (20 patients with acute myelogenous leukemia, 10 patients with acute lymphoblastic leukemia, and 2 patients with chronic myelogenous leukemia in accelerated phase). Preparation for BMT and posttransplant care was performed as described previously. All bone marrow donors were matched for the major histocompatibility complex with their respective recipients. Blood samples were also studied in 19 of the bone marrow donors. Electrophoretic screening for abnormal hemoglobins was negative in all donors and recipients. Fetal hemoglobin was determined by alkali denaturation. For quantitation of Gγ and Aγ, fetal hemoglobin was prepared by alkali denaturation and precipitation of HbA followed by filtration. The filtrate that contained HbF was extensively dialyzed against distilled water to remove ammonium sulfate and alkali, concentrated by ultrafiltration and lyophilization.

Fetal hemoglobin prepared in this way was comparable to HbF prepared by DEAE cellulose chromatography. Alkali denaturation did not interfere with electrophoretic separation of Gγ and Aγ chains. Carbonic anhydrase was present in all preparations. Polyacrylamide gel electrophoresis was carried out at 125 V for 20 hr in the cold. All gels were stained with Coomassie blue for 24 hr. After destaining, the gels were scanned at 550 nm. Aγ and Gγ were determined by planimetry.

RESULTS

Hemoglobin-F levels were followed in the 32 marrow transplant recipients for 2 wk before and for 13–76 wk after successful bone marrow engraftment and in 19 of the bone marrow donors. The other 13 marrow donors were not examined.

Two phenotypic groups of transplant recipients were detected, based on high or low HbF production after BMT. Those patients (n = 13) whose mean HbF rose to 1.9% or higher after erythroid production started (third week after BMT) were considered high responders (HR), whereas those patients (n = 19) who remained below that limit were regarded as low responders (LR). The cut-off point of 1.9% was chosen because it represents the upper limit of normal (n = 20;
mean ± 2 SD = 0.9% ± 1.0%). Figure 1 illustrates HbF levels separately for high and low responders.

Figure 2 demonstrates the mean HbF levels and 95% confidence limits for low and high responders before and after BMT. In high responders, a mean HbF level of 3.5% ± 2.0% was present when the marrow ablative therapy was started (week -2), compared to 1.2% ± 0.6% HbF in low responders. This difference is significant at the level of \( p < 0.001 \) (two-tailed t test). After transplantation, the two groups of responders again behaved differently, with the initially low group showing no or only little elevation of HbF (LR) and the other group showing a marked rise of HbF (HR) after a nadir had been
reached during the third posttransplant week. During the second 6-mo period of the first year after BMT, HbF levels of HR patients returned to normal in 2 patients, whereas it remained high (3.5%-5.8%) in 3 patients even after 1 yr. In the other 8 patients, HbF production either decreased or the follow-up period was too short for meaningful evaluation. The two patients with the highest HbF levels (7.5%) later suffered leukemic relapse with persisting increased HbF synthesis. Four patients of the LR group also suffered a leukemic recurrence after BMT.

Figures 1 and 2 also show HbF levels of the donors. The donors for high responder recipients had a HbF level of 1.5% ± 0.8%, whereas HbF was 0.8% ± 0.3% in the donors for low responder recipients (p < 0.02).

No statistical difference was found in respect to age of the recipients or donors in both groups. Also, the type of leukemia or form of pretransplant chemotherapy had no influence on HbF synthesis before and after transplant. There was no difference between the two groups in respect to clinical or hematologic condition before BMT, nor was there any difference in respect to the outcome of transplantation. Transfusion requirement (see top panel of Fig. 2), reticulocyte counts, and mean corpuscular red cell volume was comparable in both groups. Pertinent clinical data on high responders and low responders and/or their respective donors are presented in Table 1.

Sequential measurements of Gγ:Aγ ratios did not reveal a clear-cut pattern. Analysis of individual courses show that some patients had a fetal type Gγ:Aγ ratio with elevated Gγ, whereas other patients did not respond with a relative increment of Gγ. High or low HbF responders did not exhibit a clear association to fetal or adult pattern of the γ-chains, except that high responders 10 wk after BMT had a significant increase in %Gγ compared to their pretransplant values (p < 0.002). Low responders lacked such increment.

DISCUSSION

The result of our study differs from an earlier report. Whereas a uniform reactivation of fetal erythropoiesis was observed in a group of seven children and adults after allogeneic bone marrow transplantation, our patients show a heterogeneous pattern unrelated to the ages of donors and recipients. Two different phenotypic groups of responders were detected, one with and one without a phase of HbF elevation after marrow grafting. This phenomenon did not depend on the age of donors or recipients, type of leukemia, or other hematologic parameters.

The findings of an elevated hemoglobin-F level before BMT in some of our patients is consistent with the observation that some patients have an elevation of HbF after combination chemotherapy. Enhanced synthesis of HbF after induction chemotherapy has been found to be associated with a better chance of achieving a remission in patients with acute myelogenous leukemia. No difference in HbF at the time preparation for BMT was started nor after BMT was noticed between the types of leukemia. A comparison of HbF levels of low and high responders in respect to pretransplant HbF levels shows that 9 of the 13 patients of the high responding group had a clearly increased pretransplant HbF level (>1.9%), in contrast to only 2 of the 19 in the low responder group. This result might indicate persisting, yet undefined, host factors in the bone marrow microenvironment.

The observation of an influence of donor HbF levels on HbF synthesis in the recipient is in agreement with earlier clinical and experimental observations that indicate that elevated HbF levels may have a genetic basis. Further detailed family studies, including determination of chain synthesis rates and Gγ:Aγ ratios of parents and siblings in addition to the donor–recipient pairs, are needed to establish a clear-cut relationship between donor HbF and HbF synthesis in the recipient after marrow grafting.

Table 1. Clinical Data on High Responders and Low Responders and on Their Respective Donors

<table>
<thead>
<tr>
<th></th>
<th>High Responders (n = 13)</th>
<th>Low Responders (n = 19)</th>
</tr>
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<tbody>
<tr>
<td><strong>Transplant recipients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>2</td>
<td>0</td>
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<tr>
<td>Age (yr: median; range)</td>
<td>20; 2-41</td>
<td>21; 2-39</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>8:5</td>
<td>13:6</td>
</tr>
<tr>
<td>Pre-BMT Hb (g/dl: median; range)</td>
<td>11.0 (10.8-17.6)</td>
<td>12.3 (8.9-16.7)</td>
</tr>
<tr>
<td>Red cell transfusions after BMT (median; range)</td>
<td>7; 3-12</td>
<td>7; 5-18</td>
</tr>
<tr>
<td>Survival in remission (&gt;1 yr post-BMT)</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><strong>Transplant donors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr: median; range)</td>
<td>24; 2-48</td>
<td>26; 3-37</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>7:6</td>
<td>9:10</td>
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REFERENCES


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