Erythropoiesis in Patients With Refractory Diamond-Blackfan Anemia


In two previous studies, we observed that recombinant human interleukin-3 (rIL-3) induced an increase in marrow burst-forming unit-erythroid–derived colonies in vitro in some patients with Diamond-Blackfan anemia (DBA). To determine whether a similar erythropoietic response could be induced in vivo, we treated 13 patients with DBA (aged 4 to 19 years) with two preparations of IL-3. All patients had absent absolute reticulocyte counts and markedly reduced to absent recognizable bone marrow erythroid elements; patients with circulating reticulocytes in the previous 12 months were excluded from study. All patients except 1 had failed steroid therapy and had been transfusion-dependent since infancy; 1 patient was maintained on high-dose prednisone at the time of enrollment. On the first arm of the study, IL-3 (Immunex Corp, Seattle, WA) was administered subcutaneously using a dose escalation regimen of 125 to 500 μg/m²/day in divided dosage at 12-hour intervals, coadministered with 1.5 mg/kg/d of oral ferrous sulphate. Of the 13 patients that entered the trial, 4 stopped prematurely because of adverse side effects. In the other 9 evaluable cases, reticulocytes increased transiently in 1 patient from 0 to 65 × 10⁹/L after 35 days of IL-3 therapy at 250 μg/m², but transfusion dependency persisted. One transient peak in absolute reticulocyte count was noted in 6 other patients, but no erythroid response was observed after completion of a full course of IL-3. Oral prednisone at 0.5 mg/kg/d was then coadministered with IL-3 at 500 μg/m² to 5 of the patients without effect, and treatment was stopped. In 2 patients, a second preparation of IL-3 (Sandoz Canada Inc, Dorval, Quebec, Canada) was initiated in a dose escalation regimen of 2.5 to 10 μg/kg and was coadministered with ferrous sulphate. No erythroid response was observed in either patient, and in one of the two, alternate-day subcutaneous recombinant erythropoietin at 300 U/kg was administered for 3 weeks in combination with daily IL-3 at 10 μg/kg, but no increased erythropoiesis was seen. Significant increases in white blood cell and eosinophil counts during administration of both preparations of IL-3 were observed in all patients. These data show that the response of DBA patients to IL-3 in vivo is heterogeneous and cannot be predicted from in vitro studies. The absence of a corrective effect of IL-3 in these patients with DBA indicates that a deficiency of the cytokine is not central in the pathogenesis of the disorder.

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From The Hospital for Sick Children, Toronto, Ontario, Canada; Gwynne Hazen Cherry Memorial Laboratory, UCDA-Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, CA; Immunex Corp, Seattle, WA; and Sandoz Canada Inc, Dorval, Quebec, Canada.

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Address reprint requests to Nancy F. Olivieri, MD, FRCPC(C), Director, Haemoglobinopathy Program, Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada MSG IXS.

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CONSTITUTIONAL pure red blood cell (RBC) aplasia, or Diamond-Blackfan anemia (DBA), is a severe congenital hypoproliferative anemia characterized by macrocytosis and reticulocytopenia, and is associated with a wide variety of clinical and in vitro abnormalities. Markedly reduced or absent RBC precursors are observed in the bone marrow of these patients, in the presence of normal marrow cellularity, and in the preservation of other hematopoietic cell lineages. Several therapeutic approaches have been used in DBA, but, in standard practice, treatment options are limited to two: steroid therapy or RBC transfusions. Although more than half of patients respond to steroid therapy with an increase in hemoglobin concentration and a decrease in transfusion requirement, only approximately half of these can be maintained on low-dose steroid therapy or enter a steroid-free remission. A small number of patients may respond to treatment with androgens, cyclosporine A, or chemotherapeutic agents. The remainder, approximately one-quarter of all patients, must be maintained on a program of RBC transfusions with its associated complications and therefore on iron-chelation therapy. Bone marrow transplantation has offered a curative option in a few patients worldwide to date, but remains limited to the small proportion of patients with HLA-matched marrow donors.

The observed wide heterogeneity in associated clinical abnormalities and the variable response of the erythroid progenitors in vitro to the addition of recombinant human interleukin-3 (rHuIL-3) and to stem cell factor in patients with DBA suggest that DBA may represent a disorder resulting from different mutations in a single gene or several distinct diseases resulting from mutations in different genes. The observation that the hematologic abnormalities of DBA patients resemble those seen in Steel and W mice stimulated interest in the characterization of these genes in patients with DBA, but neither Southern blotting of c-kit and Steel genes nor nucleotide sequencing of c-kit and Steel mRNAs have shown abnormalities in patients with DBA.

Therapy with various cytokines has been attempted in patients with DBA both because of the difficulties associated with standard therapy in this disease and because of data suggesting that pharmacologic doses of certain cytokines might stimulate in vitro growth of normal and DBA bone marrow. The effects of granulocyte-macrophage colony-stimulating factor on proliferation, maturation, and differentiation of normal and DBA bone marrow-derived progenitors have been reported. The role of IL-3 in the pathogenesis of DBA remains to be defined.
stimulating factor (GM-CSF) and rHuIL-3 have been studied singly and in combination on the in vitro proliferation of bone marrow erythroid progenitors from patients with DBA. Data from these studies demonstrate that rHuIL-3, but not GM-CSF, markedly increased the number and size of the burst-forming unit erythroid (BFU-E)-derived colonies in bone marrow erythroid progenitors from patients with DBA? Patients with several treatment combinations of rHuIL-3.* Patients with DBA were treated with daily GM-CSF followed by rHuIL-3 effect in vitro, a small cohort of patients with refractory DBA and reported the achievement of transfusion independence with prednisone, and rHuIL-3 (Sandoz) in combination with corticosteroids or erythropoietin. Patients were entered in cohorts of 3 or 4 at escalating doses of rHuIL-3 (125 µg/m²/day to a maximum dose of 500 µg/m²/day) administered in divided dosage every 12 hours until a packed RBC transfusion was required or to a maximum of 42 days at each dose, after which the dose was escalated. Packed cell transfusions were administered if the peripheral hemoglobin level decreased to less than 7 g/dL or if clinical intolerance of anemia was noted by the principal investigator.

A total of 11 patients received more than one dose of rHuIL-3 (provided by Hoechst-Roussel Pharmaceuticals [Somerville, NJ) in collaboration with Immunex). Before initiation of rHuIL-3 therapy, each patient received a packed RBC transfusion to a hemoglobin of 13.0 g/dL. The day after transfusion, the patient began treatment with rHuIL-3. Of the 9 patients that completed the trial, 4 began rHuIL-3 at a divided dose of 125 µg/m²/day, 3 at 250 µg/m²/day, and 2 at 500 µg/m²/day. rHuIL-3 was coadministered with daily oral ferrous sulphate at 1.5 mg/kg, despite increased serum ferritin concentrations in most patients on study. Iron supplementation was prescribed during IL-3 therapy because of the theoretical concern that tissue-bound iron stores as insoluble hemosiderin, even in heavily iron-loaded patients, may be unavailable to an expanding erythron. Administration of nightly subcutaneous deferoxamine was stopped during the period of administration of rHuIL-3. After a full course of 500 µg/m² was completed, oral prednisone (0.5 mg/kg/d) was coadministered with this dose of rHuIL-3 in 5 patients. An example of the

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**MATERIALS AND METHODS**

**Patient Characteristics**

Thirteen patients were enrolled; 9 completed the therapeutic trial and 4 were eliminated because of adverse reactions (see Results). All patients had well-documented DBA, all but 1 had failed steroid therapy, and the rest had been transfusion-dependent since infancy with a transfusion requirement of (mean ± SD, range) 177 ± 27 (134 to 214) mL/kg body weight to maintain a pretransfusion hemoglobin concentration of 8.7 ± 1.4 (6.8 to 10.2) g/dL over the 1 year before enrollment on study. The mean age of the patients was 11.9 ± 4.5 (4 to 19) years. Two patients were full siblings (patients no. 2 and 3, Table 1) whose older sister had died of corticosteroid-related complications during treatment for DBA 10 years previously; no other patient was known to have a relative with DBA. All patients had Karnofsky scores of greater than 80%, normal renal function, liver enzymes less than twice normal, and no history of severe allergic disorders, asthma, cardiac disease, or hypertension. Excluded from the study were patients with atypical DBA associated with pancytopenia, and patients with transient periods of erythropoiesis, ie, all patients in whom circulating reticulocytes were observed in the 12 months preceding the study. Patient characteristics for the 9 cases who completed the trial are shown in Table 1.

**Table 1. Clinical Data on Patients No. 1 Through 9 With DBA**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Mean Hb 6 mo Before Tx</th>
<th>ARC</th>
<th>Transfusion Requirement (mL/kg/yr)</th>
<th>Other Rx</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>F</td>
<td>8.5</td>
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<td>162</td>
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<td>3</td>
<td>17</td>
<td>F</td>
<td>9.1</td>
<td>0.0</td>
<td>0</td>
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<td>M</td>
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<td>0.0</td>
<td>0</td>
<td>157</td>
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<td>5</td>
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<td>F</td>
<td>9.5</td>
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<td>0</td>
<td>134</td>
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<tr>
<td>6</td>
<td>12</td>
<td>F</td>
<td>10.2</td>
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<td>M</td>
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<td>—</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>M</td>
<td>7.0</td>
<td>0.0</td>
<td>214</td>
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<tr>
<td>9</td>
<td>10</td>
<td>M</td>
<td>6.8</td>
<td>0.0</td>
<td>208</td>
<td>DFO</td>
</tr>
</tbody>
</table>

Mean ± SD 11.9 ± 4.5 8.7 ± 1.4 177 ± 28

Abbreviation: DFO, deferoxamine.

**Study Protocol**

The study was approved by the Human Subjects' Review Committee of the Hospital for Sick Children and of The UCLA School of Medicine. Written informed consent was obtained from each patient or a parent. This was a two-center, open-label, nonrandomized, dose escalation phase I-II study using rHuIL-3 by subcutaneous administration twice daily in patients with DBA. rHuIL-3 was supplied as a lyophilized powder in vials of 250 or 500 µg rHuIL-3, 40 mg mannitol USP, 10 mg sucrose NF, and 1.2 mg tromethamine USP. During maintenance therapy, patients or their parents were taught to administer subcutaneous rHuIL-3 and were kept in regular contact with the research nurse between clinic visits.

rHuIL-3 (Immunex). Patients were entered in cohorts of 3 or 4 at escalating doses of rHuIL-3 (125 µg/m²/day to a maximum dose of 500 µg/m²/day) administered in divided dosage every 12 hours until a packed RBC transfusion was required or to a maximum of 42 days at each dose, after which the dose was escalated. Packed cell transfusions were administered if the peripheral hemoglobin level decreased to less than 7 g/dL or if clinical intolerance of anemia was noted by the principal investigator.

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The Immunex rHuIL-3 treatment protocol for DBA is shown, as administered to patient no. 1, in Fig 1.

rHuIL-3 (Sandoz). When no erythroid response was observed with the Immunex preparation of rHuIL-3 alone and with prednisone, therapy was stopped. Two patients (nos. 2 and 3) subsequently entered a trial of rHuIL-3 (Sandoz Canada Inc) using a dose escalation regimen of 2.5, 5, and 10 μg/kg coadministered with ferrous sulphate at 1.5 mg/kg body weight. The dose was increased, as in the previous protocol, when an RBC transfusion was required during administration of the lower dose.

rHuIL-3 (Sandoz) and rHuEPO. When no erythroid response to this preparation in either patient treated with rHuIL-3 (Sandoz) was observed, administration of rHuEPO at 300 U/kg was performed to 1 of the patients (no. 2) on alternate days by a separate subcutaneous injection coadministered with daily 10 μg/kg rHuIL-3 (Sandoz).

Clinical Monitoring

Before each dose cycle of rHuIL-3 in both trials, each patient was evaluated by complete history and physical examination; complete blood count (CBC); differential and reticulocyte count; serum chemistry profile including electrolytes, blood urea nitrogen (BUN), creatinine, serum aspartate transaminase (AST), serum alanine transaminase (ALT), total protein, and albumin; prothrombin time (PT); partial thromboplastin time (PTT); Hb f quantitation; RBC i antigen; serum EPO level; serum ferritin, iron, and iron binding capacity; serum sample for determination of rHuIL-3 antibody; urinalysis; electrocardiogram; and chest x-ray. Bone marrow aspiration was obtained for cellularity, myeloid/erythroid ratio, and morphology before the first cycle of rHuIL-3. Before an increase in rHuIL-3 dose, and within 72 hours of the last dose of rHuIL-3, physical examination and all the above, including bone marrow aspirates, were repeated.

Statistical Analysis

Initial values were compared with those after IL-3 therapy, using the Student's t-test for paired data. Data are presented as mean ± standard deviation.

RESULTS

Response to Administration of rHuIL-3 (Immunex/Hoechst-Roussel)

The mean duration of rHuIL-3 administration for the 9 patients was 136 ± 61 days. The mean duration of therapy at daily doses of 125 to 500 μg/m², coadministered with oral prednisone, and of total days on rHuIL-3 is shown in Table 2.

Biologic Response to rHuIL-3 (Immunex)

Table 3 shows the change in absolute reticulocyte count, total white blood cell (WBC) count, neutrophil count, and eosinophil count in the 9 patients who completed the course of rHuIL-3 (Immunex). A significant increase in the mean peripheral blood total WBC count (P < .025) and in the eosinophil count (P < .005) during rHuIL-3 administration was observed in all patients (Table 4). No significant change in the mean neutrophil or platelet count was observed in any patient. Patients no. 1 through 7 demonstrated variable, transient increases in absolute reticulocyte count (ARC; from 3 to 79 X 10⁹/L), but in no patient was this response adequate to increase peripheral hemoglobin concentration, and no patient showed an increase in marrow erythroid elements after rHuIL-3 (Immunex) when compared with pretreatment specimens.

Erythroid Response to rHuIL-3 (Immunex)

In patients no. 2 and 5 (Figs 2 and 3), a solitary measurement of the ARC was recorded to be increased significantly over baseline during administration of daily doses of rHuIL-3 (Immunex) of 500 and 250 μg/m², respectively. In neither patient was this increase sustained for more than 1 week. In patient no. 6, the ARC increased from 0 to 65 X 10⁹/L after 35 days of administration of rHuIL-3 at 250 μg/m². This patient maintained a mean hemoglobin concentration of 11.0 g/dL over 41 days without transfusion; at day 50, the patient's ARC was 0, and thereafter she again required regular transfusions. In patients no. 7, 8, and 9, no increase in ARC has been noted using rHuIL-3 up to 500 μg/m²/d. The addition of prednisone at 0.5 mg/kg/d orally to rHuIL-3 at 500 μg/m²/d did not induce a significant erythroid response in any patient.
FAILURE OF IL-3 IN DIAMOND-BLACKFAN ANEMIA

Tabla 2. Dosing Schedules of Immunex and Sandoz Preparations of rHuIL-3 for Nine Patients With DBA

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Days at 125 mg/ml²</th>
<th>Days at 250 mg/ml²</th>
<th>Days at 500 mg/ml²</th>
<th>Days at 250 mg/ml² Prednisone</th>
<th>Days at Sandoz IL-3 2.5 µg/kg</th>
<th>Days at Sandoz IL-3 5.0 µg/kg</th>
<th>Days at Sandoz IL-3 10.0 µg/kg</th>
<th>Total Days on Study</th>
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<td>—</td>
<td>19</td>
<td>—</td>
<td>—</td>
<td>19</td>
<td>62</td>
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</tbody>
</table>

Mean ± SD: 35 ± 4.8, 41 ± 7.3, 44 ± 22, 30 ± 12.8, 136 ± 61

Adverse Reactions to rHuIL-3

Two patients developed urticaria and generalized wheezing in response to the test dose of rHuIL-3 and were not entered on study. Mild chills, headaches, flushing, and redness at the injection site not requiring withdrawal from the study were observed in 5 patients in response to administration of rHuIL-3 (Immunex). In 2 other patients, serious side effects permitted administration of less than 4 weeks of treatment. In the first patient in whom administration of rHuIL-3 began at a dose of 250 µg/ml², treatment was discontinued after 5 days of treatment because of a severe serum sickness reaction. In the second patient who began rHuIL-3 at an initial dose of 500 µg/ml², rHuIL-3 was discontinued after 27 days of treatment because of severe local reactions to subcutaneous administration.

No changes in serum electrolytes, BUN, creatinine, AST, ALT, total protein and albumin, PT, PTT, Hb F quantitation, RBC i antigen, serum EPO level, serum ferritin, iron, and iron binding capacity were noted during rHuIL-3 treatment. No change in bone marrow cellularity or myeloid/erythroid ratio was noted during rHuIL-3 treatment.

Response to Administration of rHuIL-3 (Sandoz)

Two sisters (patients no. 2 and 3) began rHuIL-3 (Sandoz) treatment 8 months after a final dose of rHuIL-3 (Immunex). rHuIL-3 (Sandoz) was administered daily in escalating doses 2.5, 5.0, and 10.0 µg/kg as described in Study Protocol. During 83 days of rHuIL-3 (Sandoz), ARC remained at 0 in both patients. Total WBC count and eosinophil count, but not neutrophil or platelet count, increased to a significantly higher peak while on rHuIL-3 (Sandoz) than observed in the same patients at the highest dose of rHuIL-3 (Immunex) (data not shown). Bone marrow aspiration in these patients after the completion of a total of 83 days of rHuIL-3 (Sandoz) showed no change in the erythroid compartment.

rHuIL-3 (Sandoz) Combined With rhuEPO Therapy

Immediately after administration of 83 days of rHuIL-3 (Sandoz) alone, patient no. 2 received 3 weeks of alternate-day subcutaneous rhuEPO therapy at 300 U/kg in combination with daily rHuIL-3 at 10 µg/kg (Sandoz). No peripheral blood or bone marrow erythroid response was observed.

DISCUSSION

This report describes the lack of erythroid response in 9 patients with DBA treated with two preparations of rHuIL-3, alone and in combination with oral corticosteroid and subcutaneous rHuEPO, over a mean period of 136 days. All of the patients who entered this study had an absolute lack

Table 3. Hematologic Responses to rHuIL-3 in Patients No. 1 Through 9 With DBA

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Total WBC (x10⁹/L)</th>
<th>Eosinophil (x10⁹/L)</th>
<th>PMN (x10⁹/L)</th>
<th>Total WBC (x10⁹/L)</th>
<th>Eosinophil (x10⁹/L)</th>
<th>PMN (x10⁹/L)</th>
<th>ARC (x10⁹/L)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4.4</td>
<td>0.09</td>
<td>1.9</td>
<td>7.7 (600)</td>
<td>1.3 (500)</td>
<td>2.7 (500)</td>
<td>5 (125)</td>
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<td>2</td>
<td>4.6</td>
<td>0.09</td>
<td>2.8</td>
<td>11.9 (250)</td>
<td>6.4 (500)</td>
<td>5.1 (500)</td>
<td>79 (500)</td>
</tr>
<tr>
<td>3</td>
<td>4.2</td>
<td>0.3</td>
<td>1.9</td>
<td>12.6 (600)</td>
<td>2.4 (500)</td>
<td>4.8 (500)</td>
<td>4 (500)</td>
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<tr>
<td>4</td>
<td>4.7</td>
<td>0.05</td>
<td>2.9</td>
<td>6.2 (600)</td>
<td>2.1 (125)</td>
<td>1.6 (500)</td>
<td>8 (250)</td>
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<td>0.01</td>
<td>4.2</td>
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<td>2.8 (500)</td>
<td>20 (250)</td>
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<tr>
<td>6</td>
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<td>0.01</td>
<td>3.6</td>
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<td>4.9 (250)</td>
<td>5.6 (250)</td>
<td>65 (250)</td>
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<td>7</td>
<td>4.8</td>
<td>0.38</td>
<td>1.9</td>
<td>9.0 (600)</td>
<td>3.3 (500)</td>
<td>1.9 (500)</td>
<td>3 (500)</td>
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<tr>
<td>8</td>
<td>6.6</td>
<td>0.19</td>
<td>2.2</td>
<td>6.2 (600)</td>
<td>1.6 (500)</td>
<td>3.1 (500)</td>
<td>0 (500)</td>
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<td>9</td>
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<td>0.19</td>
<td>4.7</td>
<td>16.6 (500)</td>
<td>0.7 (500)</td>
<td>10.2 (500)</td>
<td>0 (500)</td>
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</table>

Mean ± SD: 5.9 ± 1.8, 0.15 ± 0.12, 2.9 ± 1.0, 8.8 ± 3.9, 3.3 ± 2.2, 4.2 ± 2.7

P value: ≥.25, ≤.005, ≤.1

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Table 4. Mean Hematologic Changes (± SD) for Nine Patients With DBA at the Conclusion of Immunex rHuIL-3 Therapy

<table>
<thead>
<tr>
<th>Peripheral Blood</th>
<th>Initial</th>
<th>Final*</th>
<th>Significance†</th>
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</thead>
<tbody>
<tr>
<td>Total WBC (x10^9/L)</td>
<td>5.9 ± 1.8</td>
<td>8.8 ± 3.9</td>
<td>P = .025</td>
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<tr>
<td>Neutrophils (x10^9/L)</td>
<td>2.9 ± 1.0</td>
<td>4.2 ± 2.7</td>
<td>P = NS</td>
</tr>
<tr>
<td>Eosinophils (x10^9/L)</td>
<td>0.15 ± 0.12</td>
<td>3.3 ± 2.2</td>
<td>P = .005</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
<td>179 ± 31</td>
<td>189 ± 28</td>
<td>P = NS</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation.
Abbreviation: NS, not significant.
* Initial values were compared with those after IL-3 therapy using the Student's t-test for paired data.
† Before prednisone.

activity was present did not sustain a response to rHuIL-3, consistent with the findings of our present study, in which only patients without peripheral blood and marrow erythroid activity were selected for therapy with rHuIL-3.

Gillio and Gabrilove administered a different preparation of rHuIL-3 (Sandoz) to 17 patients with DBA and reported 4 patients becoming transfusion-independent. Because a different preparation of rHuIL-3 (Sandoz) was administered in Gillio and Gabrilove's study, we hypothesized that the observed difference in response might be caused by reduced bioavailability of the glycosylated Immunex product compared with the nonglycosylated Sandoz product. We therefore administered a course of Sandoz rHuIL-3 to 2 of our patients in whom no erythroid response had been observed with administration of the Immunex preparation. Despite an increase in the total WBC count largely secondary to increases in eosinophils with both preparations (although significantly higher with the Sandoz preparation), no erythroid response to either rHuIL-3 preparation was observed in these patients.

Although urticaria and generalized wheezing in response to the test dose of rHuIL-3 prevented 2 patients from entering the study, side effects from long-term rHuIL-3 administration were minimal. Mild chills, headaches, flushing, and reddness at the injection site not requiring withdrawal from the study were observed in 5 patients in response to administration of rHuIL-3 (Immunex). In 2 other patients, more serious side effects, a severe serum sickness reaction, and continued severe local reactions prevented long-term continuation of rHuIL-3.

As previously reported, the in vivo response to rHuIL-3 could not be predicted on the basis of in vitro bone marrow BFU-E growth. Of 4 patients in the trial who demonstrated
an rHuIL-3–induced response in BFU-E-derived colonies in vitro (patients no. 1 and 4 through 6 in Halperin et al9), none demonstrated an in vivo response to either preparation of rHuIL-3. These data show that the response of DBA patients to rHuIL-3 in vivo, like that observed in vitro, is heterogeneous and cannot be predicted from in vitro studies. In the majority of DBA patients with absent ARCS and bone marrow erythroid precursors, a sustained erythroid response to treatment with rHuIL-3 is not observed. The lack of response in this small number of patients in our study who received rHuIL-3 (Sandoz) does not eliminate the possibility that different preparations of rHuIL-3 may have differing efficacy related to bioavailability altered by glycosylation. The increased eosinophilic effect of the Sandoz preparation warrants further evaluation in the treatment of this complex disorder.

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