Brief Communication: Successful Treatment of Pure Red-Cell Aplasia with an Anti-Interleukin-2 Receptor Antibody (Daclizumab)

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Background: Pure red-cell aplasia (PRCA) is a rare hematologic disease characterized by anemia, reticulocytopenia, and absence of bone marrow erythroid precursors. Most patients respond to some form of immunosuppressive treatment, but few prospective clinical trials have been performed.

Objective: To examine the efficacy of a humanized monoclonal antibody to the interleukin-2 receptor (daclizumab) for treating PRCA.

Design: Pilot study.

Setting: Federal government research hospital.

Patients: 15 patients with idiopathic PRCA.

Intervention: Daclizumab, 1 mg/kg of body weight, every 2 weeks for a total of 5 infusions. Pure red-cell aplasia was defined as transfusion-dependent anemia with a reticulocyte count of 60 × 10^9 cells/L or less and bone marrow showing absent or diminished erythroid precursors.

A
cquired pure red-cell aplasia (PRCA) is characterized by isolated severe anemia and reticulocytopenia (1) and by absent or markedly diminished erythroid precursors in the bone marrow, which is otherwise normal. It is associated with many disorders, including parvovirus B19 infection (2), lymphoproliferative disorders (3, 4) rheumatologic diseases (5), thymoma (6), and (rarely) erythropoietin administration due to antitherpoietin antibody formation (7). Most cases of PRCA are idiopathic. With the exception of parvovirus infection (8), which results to intravenous immunoglobulin, PRCA seems to have an autoimmune pathophysiology. Antibodies directed against early erythroid precursor cells (9, 10) and cytotoxic T cells with specificity for erythroid progenitors (11) have been described. In early studies in a patient with T-cell large granular lymphocytic leukemia, malignant T lymphocytes inhibited erythroid colony formation (12), which was reversed in vitro by antithymocyte globulin and complement. Immune-mediated PRCA shares many clinical associations and pathophysiological mechanisms with aplastic anemia. In PRCA associated with thymoma, a thymectomy may produce responses in as many as 40% of patients (13). Plasmapheresis has successfully treated PRCA associated with systemic lupus erythematosus and after major ABO-mismatched bone marrow transplantation (14). Various immunosuppressive treatments have been reported as effective, such as corticosteroids, splenectomy (15), thymectomy (16), cyclophosphamide (17, 18), and azathioprine (19). Corticosteroids produce responses in about 50% of patients (20), but long-term use is associated with clinically significant morbidity. Patients have responded to treatment for large granular lymphocytic leukemia, as well as cyclosporine alone. In PRCA associated with large granular lymphocytic leukemia, 28 of 47 patients treated with cyclosporine (21) had a hematologic response that may reflect the responsiveness of the underlying disorder to cyclosporine. Of 9 steroid-refractory patients, 6 patients (66%) responded to antithymocyte globulin (22). Less toxic but equally effective therapeutic strategies could avoid the need for hospitalization (required for antithymocyte globulin) and monitoring of drug levels (required for cyclosporine) and avoid potentially serious collateral organ damage (seen with long-term use of corticosteroids). Recently, responses to the anti-CD20 monoclonal antibody (rituximab) (23, 24) have been reported in patients with chronic lymphocytic leukemia and in PRCA associated with erythropoietin use. In both cases, PRCA has been attributed to antibodies against erythroid precursors.

We have reported that daclizumab is effective for moderate aplastic anemia, producing durable responses in more than 50% of patients (25). Unlike antithymocyte globulin, which requires hospitalization and has several side effects, daclizumab can be administered in an outpa-

Measurements: Response to therapy was assessed by transfusion independence, increments in reticulocyte count, and nontransfused hemoglobin.

Results: Daclizumab had little toxicity. Of the 15 patients, 6 patients (40%) responded to treatment. All responders became transfusion-independent and achieved normal or near-normal hemoglobin values and normal reticulocyte counts.

Limitations: The study was unblinded, and the number of patients was too small to assess drug safety reliably.

Conclusions: Daclizumab seems safe. Its efficacy in this pilot protocol suggests that expanded study in PRCA and other bone marrow failure syndromes is warranted.
Patient eligibility was assessed after obtaining informed consent, according to a protocol approved by the institutional review board of the National Heart, Lung, and Blood Institute, Bethesda, Maryland. We treated 15 patients (9 men and 6 women) with PRCA, as defined by anemia requiring transfusion, reticulocyte count of $60 \times 10^9$ cells/L or less, absent or diminished erythroid precursors in the bone marrow, and an untransfused hemoglobin level less than 100 g/L. We used the average of 3 blood count measurements that we obtained within a 2-week period before enrollment to assess eligibility for entry into the study. We defined transfusion dependence as a requirement of at least 2 units of red cells per month for at least 2 consecutive months before enrollment. We excluded patients with a current diagnosis or history of the myelodysplastic syndrome, abnormal cytogenetics, Fanconi anemia, lymphoma, or other lymphoproliferative disease. Patients received daclizumab, 1 mg/kg of body weight, infused intravenously every other week for a total of 5 doses. We defined response at 3 months by transfusion independence or a stable increase in hemoglobin level by 15 g/L, absolute reticulocyte count by $50 \times 10^9$ cells/L or more, or both. Patients had a bone marrow evaluation with cytogenetics performed before and at 1 month after treatment. We obtained weekly blood counts during the course of the study. All participants visited the National Institutes of Health at 1 month, 3 months, and 6 months after the last dose of daclizumab and annually thereafter. We administered red-cell transfusions to maintain the hemoglobin level greater than 70 g/L or at a higher level if necessary to control symptoms of anemia.

No outside funding was received for this study.

RESULTS

Patients

We treated 15 patients (9 men and 6 women) with PRCA with daclizumab (Table). Of these patients, 11 patients previously received immunosuppressive therapies but did not respond. These therapies included antithymocyte globulin, corticosteroid, and cyclosporine. The average age was 44 years, and the time from diagnosis to treatment ranged from 3 months to 10 years. All patients were transfusion-dependent before daclizumab administration. One patient previously had a short-lived hematologic improvement after removal of a thymoma. The serum specimens of all patients were negative for the presence of circulating parvovirus by DNA dot-blot hybridization.

Hematologic Response

Of the 15 patients who entered into the protocol, 6 patients (40%) responded hematologically within 90 days of receiving the last dose of daclizumab. All patients achieved a normal hemoglobin level by an average of 18 months after treatment (Table and Figure). An additional patient had a transient partial response with appearance of diminished but normal erythroid precursors in the bone marrow. This patient received an additional course of daclizumab but did not respond. Patient 1 subsequently developed myelodysplasia. One patient who did not respond to daclizumab was later successfully treated with (and responded to) horse antithymocyte globulin. Patients whose marrow showed a total absence of erythroid precursors seemed less likely to respond to daclizumab. Monoclonal antibody treatment was associated with little toxicity except for a cutaneous eruption that occurred 3 months after the last of 5 treatments in 2 patients. These patients required 3 weeks of systemic corticosteroid therapy, and their conditions cleared completely about 3 months after the last
The rash was characterized pathologically by focal spongiosis and dermal lymphocytic infiltration, and it cleared completely and did not recur after a 3-week course of oral steroid treatment. No infectious, hemolytic, or hepatic complications occurred. Bone marrow examination was normal in all responders after treatment. All nonresponders except for patients 1 and 2 (who subsequently did not respond to antithymocyte globulin) received a second course of daclizumab, and all but 1 patient did not respond.

Three patients (patients 6, 10, and 11) relapsed in the 4 months after the initial course of daclizumab, but they promptly responded when re-treated with the same regimen and have remained transfusion-independent 4 to 31 months later.

**DISCUSSION**

In our pilot trial, we demonstrate the efficacy of daclizumab in treating some patients with acquired PRCA. In 6 cases, treatment led to long-term responses, although some patients required additional courses of therapy. We considered all participants who were enrolled in the trial to have acquired PRCA because of previously normal hemoglobin values. Although we cannot determine an accurate relapse rate because of the few patients involved, those 2 recurrences that responded to re-infusion of daclizumab suggest that some cases may require periodic re-treatment. Further long-term observation of these patients will help establish the optimal dosing regimen for daclizumab and the need for further immunosuppression.

Substantial laboratory and clinical evidence supports a role for both antibody- or cellular-mediated immunity in suppressing erythropoiesis. Therapies directed against cellular (antithymocyte globulin and cyclosporine) and humoral immunity (plasmapheresis and rituximab) have been effective (27–29). Where an autoantibody has been demonstrated, the target antigen has generally not been established, except in cases related to recombinant erythropoietin administration (30, 31) and due to the production of alloantibodies after major ABO-mismatched hematopoietic stem-cell transplantation (32, 33). Suppression of erythropoiesis by T cells may be more common than humoral-mediated red-cell aplasia (5). The frequent clinical association of PRCA with lymphoproliferative disorders (especially large granular lymphocytic leukemia [34] and thymoma [6]), together with laboratory evidence that lymphocytes from these patients suppress erythropoiesis in colony culture, supports a pathophysiologic role of the T cell (35–37). Whether responsiveness to individual therapeutic regimens necessarily correlates with the pathophysiology of disease is unclear. Arguing against tailoring the therapy to the “apparent” pathophysiology of the disease are successes in treating antibody-mediated PRCA related to erythropoietin use with cyclosporine and cyclophosphamide (anti-T-cell agents), as well the responsiveness of PRCA of all etiologies to corticosteroids. Reliance on in vitro assays to designate an individual patient’s PRCA as either humoral-mediated or T-cell-mediated may not recognize the complexity of the immune system and the interplay between T cells and B cells in producing an immune response, as well as uncertainties in extrapolating in vitro experiments to clinically relevant mechanisms. Why 1 patient who had previously not responded to antithymocyte globulin improved with daclizumab is unclear. Daclizumab, an antibody with specificity to activated lymphocytes, might be more effective in eliminating the offending T-cell clone.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Age during Study, y</th>
<th>Sex</th>
<th>Race or Ethnicity</th>
<th>Duration of Disease, mo</th>
<th>Previous Treatment</th>
<th>Erythroid Marrow Precursors Present but Decreased</th>
<th>Response at 3 mo</th>
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<tr>
<td>1</td>
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<tr>
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<td>127.6</td>
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</table>

* CR = complete response; NR = no response; PR = partial response.
We have seen similar rare instances of responses to daclizumab in patients with aplastic anemia who did not respond to antithymocyte globulin.

Nonetheless, daclizumab used as an anti–T-cell agent seems to be effective in a substantial proportion of patients with PRCA in whom other forms of therapy fail, including therapies directed against both cellular and humoral immune systems. Success in treating naive patients would probably be even greater. Daclizumab may be a less toxic alternative in patients who respond to corticosteroid or...
cyclosporine therapy but relapse when these treatments are tapered or discontinued.

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