In contrast to severe aplastic anemia (sAA), the appropriate management of patients with moderate pancytopenia is unclear. In this study, we examined the efficacy of a humanized monoclonal antibody recognizing interleukin-2 receptor (daclizumab), which has proven to be a successful immunosuppressive agent in solid organ and bone marrow transplantation. We treated 17 patients with moderate aplastic anemia (mAA) with 1 mg/kg every 2 weeks for 3 months. mAA was defined as depression of 2 of the 3 blood counts: absolute neutrophil count 1200/mm³ or less, platelet count 70 000/mm³ or less, hemoglobin level 8.5 g/dL or lower, and absolute reticulocyte count 60 000/mm³ or less. The primary end point of our protocol was a hematologic response in at least one affected peripheral blood value. Daclizumab had little toxicity. Six of the 16 (38%) evaluable patients responded to treatment. Two patients with previous chronic disease showed complete return of normal counts, which were sustained for more than 2 years following treatment. Four patients had single-lineage responses. Two previously transfusion-dependent patients became transfusion independent; one patient with many neutropenia-related infections had a normal neutrophil count following treatment. Daclizumab appears safe; its efficacy in this pilot protocol suggests that expanded study of this monoclonal antibody in immune-mediated bone marrow failure syndrome is warranted. (Blood. 2003; 102:3584-3586)

© 2003 by The American Society of Hematology

Study design

Patients

Eligible patients with mAA were entered into the study after obtaining informed consent according to protocol approved by the Institutional Review Board of the National, Heart, Lung and Blood Institute (Bethesda, MD). We treated all consecutive patients over the age of 7 years with mAA, defined according to the Consensus Conference on Treatment of Aplastic Anemia,² by a hypocellular bone marrow (cellularity < 30%) and depression of at least 2 of 3 blood counts below the normal values: ANC 1200/mm³ or less, platelet count ≤ 70 000/mm³ or less, anemia with hemoglobin level 8.5 g/dL or less, and absolute reticulocyte count 60 000/mm³ or less. Patients with severe pancytopenia were excluded from the protocol. The average of 3 measurements of blood counts obtained within a 2-week period prior to enrollment into the study was used to assess study eligibility. Transfusion independence was not an exclusion criterion.
Intracellular staining for interferon γ (IFN-γ) expression was performed using the PharMingen intracellular staining kit (San Diego, CA). Double-color surface staining was first performed with phycoerythrin (PE)–conjugated anti-CD4 and anti-CD8 mAbs and then cells were permeabilized using a saponin-based method (PharMingen) and stained with fluorescein isothiocyanate (FITC)–anti–IFN-γ or IL-4 mAbs (PharMingen; BioSource, Camarillo, CA). Specificity of the antibody was confirmed by showing elimination of staining with blocking antibody. To perform this test 10 μg of a purified unconjugated antibody was added to the fixed/permeabilized cells, which was then incubated for 20 minutes prior to addition of the conjugated antibody. Samples were analyzed using the Coulter EPICS V flow cytometer (Hialeah, FL). Lymphocytes were gated initially by forward scatter/side scatter; secondary gates were set based on staining with isotypic control mAbs such that less than 1% of cells stained positive. Staining was compared to that for 30 normal controls. Results of 30 normal controls as well as the reproducibility of the technique were previously published by our laboratory.17

Results and discussion

Six of 16 evaluable patients (38%) with mAA responded to treatment within 90 days of receiving the last dose of daclizumab (Table 2; Figure 1). One patient, lost to follow-up, was excluded from analysis and was not evaluated at the 3-month evaluation period. Of the 16 evaluable patients with mAA (one patient was lost to follow-up), 3 were newly diagnosed and 13 had chronic mAA. Patients no. 1 and 2 with previously chronic disease showed complete return of normal blood counts within the first 4 months of receiving daclizumab. Single-lineage responses were seen in 3 patients with chronic disease (patients no. 3, 9, and 16). Two patients (nos. 1 and 9) previously dependent on RBC transfusions no longer require transfusions after more than 2 years of follow-up. Another patient (no.3), who had experienced many neutropenia-related infections, now has a normal white blood cell count. Only one patient progressed despite treatment and was later treated with ATG/CsA and responded. Although follow-up time was limited, only one patient had a relapse, but she responded promptly to further daclizumab treatments. Daclizumab was associated with no toxicity except for a mild first infusion reaction in one patient (itching), which did not require treatment and did not recur. As in a previous study of sAA17 CD8 cells from 11 patients were tested for the presence of IFN-γ; 5 of the 6 testing positive by the assay

Table 1. Criteria for response

<table>
<thead>
<tr>
<th>Neutrophils</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>If baseline ANC below 500/mm³</td>
<td>Increase in ANC ≥ 300/mm³</td>
</tr>
<tr>
<td>If baseline ANC above 500/mm³</td>
<td>Increase in ANC ≥ 500/mm³</td>
</tr>
<tr>
<td>If baseline platelet count 70 000/mm³</td>
<td>Increase in platelet count ≥ 30 000/mm³</td>
</tr>
<tr>
<td>or below but above 50 000/mm³</td>
<td>Increase in platelet count ≥ 20 000/mm³</td>
</tr>
<tr>
<td>If baseline platelet count 50 000/mm³</td>
<td></td>
</tr>
<tr>
<td>or below</td>
<td></td>
</tr>
</tbody>
</table>

Transfusion dependence

<table>
<thead>
<tr>
<th>Transfusion independence</th>
<th>Decreased requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>No transfusions for &gt; 8 wk</td>
<td>Decrease in transfusion number over time</td>
</tr>
</tbody>
</table>
responded to daclizumab, whereas all 5 patients testing negative failed to clinically respond to daclizumab (Figure 2).

The goal of immunosuppressive regimens in AA is elimination of T cells effecting the immune-mediated destruction of hematopoietic progenitor and stem cells. Although this study included only patients who were symptomatic from their bone marrow failure, immunosuppressive therapy, if sufficiently innocuous and also convenient, could potentially be used to prevent stem cell depletion in patients with evidence of immune-mediated bone marrow destruction in an early phase of hematologic disease. The relapse rate after treatment with daclizumab is not known at this point, but the one relapse reported here might be indicative of the need for further immunosuppression in some patients. Further long-term studies are needed both to determine systematically the effect of early treatment on the course of mAA, as well as to establish the optimal dosing regimen for daclizumab and the need for further immunosuppression. Other roles for daclizumab in AA can now be envisioned including treatment of relapsed sAA, in myelodysplastic syndrome, for pure RBC aplasia and other single-lineage failure diseases, and as a replacement for CsA in treatment-dependent patients with sAA where CsA is contraindicated or has shown toxicity.

References

Recombinant humanized anti-IL-2 receptor antibody (daclizumab) produces responses in patients with moderate aplastic anemia

Jaroslaw P. Maciejewski, Elaine M. Sloand, Olga Nunez, Carol Boss and Neal S. Young