ABSTRACT. Two patients with severe rheumatoid arthritis (RA) were treated with high dose chemotherapy and autologous blood stem cell transplantation. Hematopoietic stem cells mobilized readily with cyclophosphamide and granulocyte-colony stimulating factor. Both patients achieved an American College of Rheumatology (ACR) 50% response before starting high dose therapy. The transplantation regimen included 200 mg/kg cyclophosphamide and 6 doses of equine antithymocyte globulin. Transplantation was well tolerated and both patients recovered neutrophils on day 7 post-transplant. At one month post-transplant both patients had an ACR response of 80%. Both individuals relapsed at 6 months and responded well to a combination of disease modifying antirheumatic drugs that was previously ineffective. At 12 months ACR responses were 80% and 60%, respectively. The first patient developed a flare at 18 months when she was found to be hypothyroid; she regained an 80% ACR response at 24 months with therapy of thyroproiodism. The second patient progressed relentlessly 15 months post-transplant. Immunological reconstitution showed a continuous inversion of the ratio of CD4 and CD8 lymphocytes with a predominant expansion of memory T cells. (J Rheumatol 2001;28 Suppl 64:13-20)
Daily 12 liter leukaphereses were started when white blood cell count reached > 1000/µl, and the minimum goal was to collect 4.0 × 10^6 CD34 cells/kg recipient weight. The unmanipulated collected product was cryopreserved in 5% dimethylsulfoxide plus 6% hydroxyethylstarch.

**Preparative regimen.** The goals of the preparative regimen were to maximally suppress the immune system with minimal toxicity. Three weeks after stem cell collection, patients received cyclophosphamide 50 mg/kg/day on days –5 to –2, and equine antithymocyte globulin (ATG) 20 mg/kg IV (Pharmacia-Upjohn, Peapack, NJ, USA) on days –3, –2, –1, and on posttransplant on days 1, 2, and 3. Prednisone was given at a dose of 0.5 mg/kg/day in a single dose until day 14, with taper to 10 mg PO QD on day 28, subsequent taper was performed slowly and per the directions of the treating rheumatologist.

**Supportive care.** Prior to each dose of ATG patients were premedicated with diphenhydramine 25 mg IV, ranitidine 50 mg IV, acetaminophen 650 mg PO, and methylprednisolone 100 mg IV; G-CSF 5 µg/kg SC was given daily from day 1 until absolute neutrophil count (ANC) reached > 500/µl, cefepime was given IV during the period of posttransplant neutropenia. Oral sulfamethoxazole-trimethoprim and acyclovir were given until 6 months posttransplant, and oral fluconazole until the steroid dose was lowered to 10 mg/day. Patients negative for cytomegalovirus (CMV) serology received irradiated CMV negative blood products, and peripheral blood buffy coat and urine were tested weekly for the CMV early antigen reactivation until 100 days posttransplant.

**Study design.** This was a pilot study designed to eventually enroll a maximum of 10 patients. The major endpoints were extent of grade III transplantatation related toxicities and ACR 50% improvement at 3 months posttransplant. Secondary endpoints were to determine efficacy and toxicity of the stem cell mobilization procedure, the rate and nature of hematological and immunological reconstitution, and the impact of the transplantation on RA. The predefined goal was to have 50% patients maintain a 50% ACR response at 2 years after transplant.

**RESULTS**

**Patient enrollment.** Since the opening of the protocol in October 1997, 5 patients with severe RA were screened for the study. Three were not transplanted for the following reasons: one female patient refused because of concerns of infertility posttransplant, one patient was not eligible for psychosocial reasons, and one patient died of sepsis while waiting to be evaluated for the study. Two patients were transplanted. The referrals for this study declined at the end of 1998, coinciding with the US Food and Drug Administration (FDA) approval of anti-tumor necrosis factor (TNF) drugs. The treatment protocol has been subsequently modified and now the eligibility criteria require a previous failure of anti-TNF therapy.

**Priming.** The lowest ANC during the priming regimen was zero, lasting only one day in both patients (Figure 1). On day 10, both patients had rapid reappearance of myeloid precursors in the peripheral blood smear, including 2–4% blasts. The lowest platelet counts during the priming process in patients 1 and 2 were 92,000 and 175,000/mm³, respectively. There were no flares of RA during the G-CSF administration and the only side effect in both patients was a short lived, grade III low back pain on day 9. Each patient required only one leukapheresis procedure (Table 1).

**Transplantation.** The neutrophil recoveries to ANC > 500/µl were rapid and occurred in both patients on day 7 posttransplant (Figure 2). Platelet recovery to > 20,000/mm³ was rapid and occurred in both patients on day 8. Each patient received 4 single donor irradiated platelet transfusions for platelet count below 20,000/mm³. Patient 1 received one transfusion of packed red blood cells (RBC) for a hemoglobin below 8 g/dl; patient 2 did not require RBC transfusions. The entire procedure was well tolerated and conducted in the outpatient setting. The only toxicities were that both patients had grade III low back pain the night before onset of the ANC recovery and patient 2 developed deep venous thrombosis of the left calf on day 14 that required 6 month anticoagulation with coumadin.

**Clinical response. Patient 1.** A 25-year-old white woman with a 7 year history of RA had failed prior therapies with intramuscular gold, methotrexate, hydroxychloroquine, sulfasalazine, minocycline, and the triple drug combination. She worked part time at an administrative job and was taking NSAID continuously, oral and intraarticular steroids, and narcotic analgesics. At the time of transplant she had 15 tender and 10 swollen joints, morning stiffness of 90 minutes, and her Clinical Health Assessment Questionnaire (CLINHAQ) disability score was 1.5 (on the 0–3.0 scale). Her erythrocyte sedimentation rate (ESR) was 33 mm/h (normal < 20), C-reactive protein (CRP) was 2.7 mg/dl (normal < 0.5), and rheumatoid factor (RF) 46 IU/ml (normal < 40). After completing the priming regimen prior to starting high dose therapy she already had a 50% ACR response. At one month posttransplant her ACR response was 80%, with no morning stiffness and RF within normal range (Figure 3). An 80% ACR response was maintained at 3 months posttransplant. At 4 months she started showing signs of disease recurrence, oral prednisone was increased from 6 to 10 mg/day, and she started oral doxycycline 100 mg PO QD. At 6 months posttransplant her ACR response lowered to 20% and RF became positive, 89 IU/ml. She then started a 3 drug combination including sulfasalazine 3000 mg/day, hydroxychloroquine 400 mg/day, and oral methotrexate 20 mg/wk². Her symptoms improved gradually and at one year followup she was working full time, had an ACR response of 80%, with CLINHAQ disability score of zero. At 17 months posttransplant, 11 months after starting the triple drug regimen, she developed a severe flare of RA and a complete loss of the clinical response. This flare was associated with the de novo onset of hypothyroidism and markedly elevated thyroid stimulating hormone. At the time of flare her ESR was 22 mm/h, CRP 2.4 mg/dl, and RF 45 IU/ml. Thyroid hormone replacement was started and the triple DMARD reg-

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**Table 1. Stem cell mobilization variables in 2 patients with RA.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukapheresis days</td>
<td>Day 10</td>
<td>Day 11</td>
</tr>
<tr>
<td>Liters of blood processed</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>CD34 cell dose, 10^6/kg</td>
<td>8.5</td>
<td>17.5</td>
</tr>
<tr>
<td>CPU-GM dose, 10^6/kg</td>
<td>23.3</td>
<td>114.8</td>
</tr>
<tr>
<td>CD3 cell dose, 10^6/kg</td>
<td>0.90</td>
<td>1.72</td>
</tr>
<tr>
<td>MNC dose, 10^6/kg</td>
<td>1.04</td>
<td>2.46</td>
</tr>
</tbody>
</table>
imen was continued. She improved rapidly and on the second anniversary of transplant, her ACR response was again 80% with the CLINHAQ disability score of 0.12, ESR 35 mm/h, CRP 2.0 mg/dl, and RF 41 IU/ml.

**Patient 2.** A 45-year-old white man with a 17 year history of RA had previously failed gold, intramuscular methotrexate, hydroxychloroquine, sulfasalazine, minocycline, and the triple regimen. He was a wheelchair bound construction inspector receiving full disability benefits. He was able to achieve short periods of meaningful ambulation only by using pulses of 30–60 mg oral prednisone and intraarticular steroids in large joints. At the time of transplant he had 25 tender and 15 swollen joints, morning stiffness of 180 minutes, and CLINHAQ disability score of 2.1 (0–3 scale). His ESR was 53 mm/h, CRP 3.5 mg/dl, and RF was positive. After completing the priming regimen he had already achieved 50% ACR response. At one month posttransplant, his ACR response was 80%, with no morning stiffness and CLINHAQ score of 0.37 (Figure 4). His laboratory variables remained abnormal, with ESR 30 mm/h, CRP 3.0 mg/dl, RF 2535 IU/ml. At the 3 month evaluation the ACR response dropped to 40% and he was started on oral doxycycline. The overall functional status continued to be perceived by the patient as excellent and he returned to his full time non-sedentary work as a construction inspector. At 6 months his ACR response remained 40% but CLINHAQ increased to 1.37, and he started the 3 drug regimen. At one year followup he was working full time, having 60% ACR response and CLINHAQ disability score zero. His RF posttransplant was never lower than 180 IU/ml. At 14 months posttransplant, he progressed, and at 15 months his ACR response was zero. He failed to respond to addition of leflunomide and etanercept and at 20 months posttransplant he returned to full disability, with ESR 39 mm/h, CRP 4.4 mg/dl, and RF 1710 IU/ml.

**Immunological recovery.** Both patients had low absolute lymphocyte counts and reversed CD4/CD8 ratios at one year posttransplant (Figure 5). CD8 cells were elevated and CD4 cells decreased for 12 months posttransplant in both patients. The number of the CD4/CD45RA naive T cells was severely suppressed compared to CD4/CD45RO memory T cells, and the count seemed to be much lower in patient 2 (age 45 yrs) than in patient 1 (age 25 yrs). Patient 1 developed positive CMV

![Figure 1. Absolute white blood cell count and lymphocyte count in 2 patients with RA after priming with 2 g/m² cyclophosphamide and granulocyte colony-stimulating factor. WBC1/ALC1 and WBC2/ALC2: absolute white blood count/absolute lymphocyte count in patients 1 and 2.](https://www.jrheum.org)
early antigen in urine at one month posttransplant, but urine cultures converted to negative at 5 months posttransplant. Her blood buffy coat remained constantly negative for the early antigen, although the longterm culture of the one month blood specimen revealed a cytopathic effect. She never developed CMV disease and never received CMV-specific antiviral therapy. Patient 2 had negative CMV serology pretransplant. Immunoglobulins IgA, IgM, and IgG were never profoundly suppressed in either patient and were within normal range at 12 months posttransplant (data not shown).

DISCUSSION
The rationale for performing hematopoietic stem cell transplantation in RA is based on increasing evidence of a poor longterm outcome in some patients with RA and improved safety of transplants\(^3,4\). Hope for the success of transplantation in severe autoimmune diseases has been inspired by the success of autologous stem cell transplantation in treating lymphoid malignancies, where malignant lymphocytes can be eliminated by using high dose chemotherapy, resulting in prolonged disease-free survival or cure\(^5\). In autologous transplantation the graft has been considered a vehicle for rescue, preventing complications of prolonged neutropenia. Such high dose procedures eliminate the patient’s immune system and promote de novo replenishment of the immune system with cells derived from transplanted hematopoietic stem cells\(^6\). However, mature lymphoid cells may survive and expand following autologous stem cell transplantation. Consequently, there are questions as to whether this strategy will benefit patients with autoimmune disease. The current report confirms the safety and feasibility of autologous stem cell transplantation for patients with severe RA. The priming regimen with cyclophosphamide and G-CSF was very efficient, both in providing large numbers of hematopoietic progenitors and achieving a rapid control of RA. The only significant posttransplant toxicity was the low back pain attributed to the rapid growth factor promoted hematopoietic expansion. Growth factor administration posttransplant may in fact be unnecessary when high doses of CD34 cells are infused\(^7\).

Neutrophil recovery, both during mobilization and posttransplant, was unusually rapid and steep compared to what is typically seen in patients with malignancies. The reason for the rapid expansion of neutrophils may be attributed to the lack of bone marrow exposure to prior chemotherapies and/or cytokine and cellular bone marrow milieu in patients with RA\(^8,9\). In this protocol the ex vivo T cell depletion of the graft was avoided for practical reasons. However, T cell depletion was achieved using horse ATG in vivo immediately before and after stem cell infusion. Both patients had excellent initial ACR responses after high dose immunoablation and were able

**Figure 2.** Absolute neutrophil and lymphocyte counts in 2 RA patients after autologous blood stem cell transplantation. Day 0 is the day of stem cell infusion.
Figure 3. A. Number of tender and swollen joints (maximum 28) in patient 1 after autologous blood stem cell transplant. B. ACR responses in the same patient during the posttransplant period. Base: values at baseline; Pret: values before starting high dose regimen.
Figure 4. A. Number of tender and swollen joints (maximum 28) in patient 2 after autologous blood stem cell transplant. B. ACR responses in the same patient during the posttransplant period. Base: values at baseline; Pret: values before starting high dose regimen.
to return to full time employment. However, both patients experienced recurrence at 6 months and started triple DMARD therapy. Both patients improved after reinstitution of a triple drug regimen, although this same combination was ineffective before transplant. The exact mechanism for this shift in the therapeutic sensitivity posttransplant remains unclear. The reason may be a change in the status of the cellular and functional immune system during the process of the immunological reconstitution\textsuperscript{10}. The exact mechanism of the impressive responses early posttransplant needs to be elucidated, but it appears to correlate with the period of most intensive immunosuppression. Although the transplantation procedure was safe and initially effective, the ultimate longterm benefit of this procedure remains uncertain. Hematopoietic stem cell transplantation involves heavy utilization of resources and it is likely that a more sustained benefit would be required to justify the costs and efforts. The reasons for failure of this particular regimen to control RA may include

insufficient elimination of the disease mediating cells, genetic factors, or the continuous presence of an autoantigen.

The immunological reconstitution data show that the memory cell pool is rapidly expanded after transplant in contrast to the naive T cells. The slower reconstitution of the naive CD4-CD45RA thymus dependent T cells has been described as an age dependent variable, and age may be an important factor in the selection of patients for this therapy. Intrinsic thymic dysfunction may be an additional defect specific for patients with RA. B cell secretory function seemed to be much less suppressed posttransplant as assessed by the quantitative measurements of immunoglobulins or RF. More effective strategies may be needed to eliminate memory T cells or mature B cells to improve transplant results for RA. The accrual for this protocol was interrupted by the FDA approval of anti-TNF drugs such as etanercept and infliximab. This prompted a modification of the eligibility criteria for transplant to include the failure of an anti-TNF drug prior to entering the study. Posttransplant treatment with etanercept was completely ineffective in patient 2. The long-term results of anti-TNF drugs reaffirm the need for better treatments for a fraction of patients with RA. The safety of this transplant regimen is encouraging and in reality carries a theoretical risk of early transplant mortality of only 1–2%. To capitalize on the current achievements it will be necessary to develop strategies to achieve more durable responses after transplant to justify the effort and resources invested in this procedure.

ACKNOWLEDGMENT
To colleagues and scientists at the University of Nebraska who inspired and supported the idea of stem cell transplantation for autoimmune disease.

REFERENCES