AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN REFRACTORY RHEUMATOID ARTHRITIS

Sustained Response in Two of Four Patients

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Objective. To investigate the safety and efficacy of immune ablation with subsequent autologous hematopoietic stem cell transplantation (HSCT) in severe rheumatoid arthritis (RA).

Methods. Four patients with refractory RA and poor prognostic indicators were treated. Stem cells were collected and lymphocytes were depleted by 2.3–4.0 logs. The conditioning regimen included cyclophosphamide (200 mg/kg), antithymocyte globulin (90 mg/kg), and, for 1 patient, total body irradiation (TBI) with 400 cGy. Improvement was evaluated according to the American College of Rheumatology (ACR) preliminary definition of improvement in RA (ACR 20), and also according to the ACR 50 and ACR 70 criteria.

Results. HSCT was well tolerated. Three patients fulfilled the ACR 70 criteria at 1 month and 3 months post-HSCT. One patient did not fulfill the ACR 20 criteria because of persistent joint tenderness, despite improvement of the joint swelling. At 6 months post-HSCT, 1 patient fulfilled the ACR 70 criteria and 1 fulfilled the ACR 50 criteria, and these 2 patients fulfilled the ACR 70 criteria at 9 months post-HSCT. The other 2 patients (including the patient who received TBI) did not meet the ACR 20 criteria at 6 months and 9 months post-HSCT. The only patient with followup of >9 months fulfilled the ACR 70 criteria at 20 months post-HSCT.

Conclusion. In this series, autologous HSCT was safe and effective in inducing major clinical response and maintained significant benefit for 2 patients at 9 months and 20 months posttreatment, respectively. Sustained response did not occur for 2 of 4 patients. A regimen dose-response effect may exist, but the addition of TBI did not prevent disease relapse for 1 of the patients. More aggressive T cell depletion of the autograft, use of a myeloablative regimen, or use of an allograft may be necessary to decrease relapse rates.

Despite currently available therapy, some patients with rheumatoid arthritis (RA) do not respond. Although most RA patients may have a normal life expectancy, subsets of patients can be identified who have a 5-year survival rate of 40–70% (1,2). Prognostic indicators for a poor survival rate include the presence of many involved joints and the degree of functional disability as assessed by the Health Assessment Questionnaire (HAQ) (1–3). Recently, intense immunosuppression and autologous hematopoietic stem cell transplantation (HSCT) have been used to treat RA patients with poor prognosis (4–6). In a previous publication, we described 2 RA patients treated with autologous HSCT, 1 of whom had disease relapse after initial improvement (5). Here, we describe our further experience in the treatment of RA with HSCT, including the followup of the previously described patients as well as 2 additional patients and their followup.

PATIENTS AND METHODS

Patient selection. The protocol was approved by the US Food and Drug Administration under the IDE number
BB-ID 6778. The inclusion criteria were 1) age <60 years, 2) an established clinical diagnosis of RA by the 1987 criteria of the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) (7), 3) rheumatoid factor (RF) seropositivity, and 4) failure of ≥2 disease-modifying antirheumatic drugs (DMARDs). Failure of DMARD was defined as ≥6 swollen joints from active RA and either ≥30 involved joints (swelling, tenderness, deformity, pain on motion, or decreased motion) or responding to <75% of 20 HAQ questions with the reply “without any difficulty.”

**HSC procurement.** Peripheral blood stem cells were mobilized with cyclophosphamide (CYC) (2 gm/m²) followed by granulocyte colony-stimulating factor (G-CSF) (Amgen, Thousand Oaks, CA). Leukapheresis was initiated when the white blood cell count was >1,000/µl (1.0 × 10⁹/liter) and continued daily until the number of harvested progenitor cells reached a minimum of 1.4 × 10⁹ CD34+ cells/kg after CEPRATE SC Stem Cell Concentrator (CellPro, Bothell, WA) positive selection.

**Conditioning regimen.** CYC (total dose 200 mg/kg) was administered in divided doses of 50 mg/kg/day intravenously for 1–2 hours on days –7, –6, –5, and –4. Antithymocyte globulin (total dose 90 mg/kg) was infused for 10–12 hours in divided doses of 30 mg/kg/day on days –6, –5, and –4. Methylprednisolone (1 gm) was administered intravenously 30 minutes before each dose of antithymocyte globulin. Patient 4 also received 400 cGy of total body irradiation (TBI) as a single dose with 25% lung block in anteroposterior/posteroanterior position on day –6. Numbers of infused CD34+ cells as well as T (CD3+) and B (CD19+) lymphocytes are shown in Table 1. Also shown in Table 1 is the lymphocyte depletion that was achieved after CD34+ positive selection, expressed on a logarithmic scale to the base of 10 (e.g., reduction from 1 × 10⁸ to 1 × 10⁷ represents 1 logarithm [log]).

**Supportive care.** Patients were treated on a heparinized hematology/oncology floor of the hospital. Low microbiological diet, oral ciprofloxacin (500 mg twice a day), fluconazole (400 mg/day oral or intravenous), and valacyclovir (500 mg 3 times a day oral or intravenous) were started on the day of admission and discontinued when the neutrophil count reached 500/µl. Subcutaneous G-CSF (5 µg/kg) was started the day of HSC infusion and continued until the absolute neutrophil count was >1,000/µl for 3 consecutive days. For 3 months following hospital discharge (approximately day 14), patients received fluconazole (400 mg/day) and

<table>
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<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
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<td>2.3 logs</td>
<td>ND</td>
<td>2.8 logs</td>
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* ND = not done; logs = logarithms to the base of 10.

| Age, years | 46 | 42 | 48 | 49 |
| Sex | F | F | M | F |
| Duration of RA, years | 7 | 7 | 4 | 6 |
| Treatment just prior to H SCT† | HCQ + SSZ + dapsone + minocycline + MTX‡ | HCQ + SSZ + MTX‡ | HCQ + MTX‡ | HCQ + gold + MTX‡ |
| Previous treatment† | HCQ + SSZ + CSA + MTX‡ | Gold + CSA | Gold + AZA | HCQ + gold + CHL + MTX‡ |
| RF (normal values 0–11 IU/liter or titer of <1:40) | 78 IU/liter | 808 IU/liter | 1:2,560 | 5,060 IU/liter |
| Radiologic findings of RA | Yes | Yes | Yes | Yes |
| Nodules | Yes | No | No | Yes |
| Pulmonary involvement | No | No | No | Yes |
| Swollen/tender joints | 27/41 | 18/21 | 24/29 | 39/37 |
| HAQ-ADL performed “without any difficulty” (of total of 20) | 0 | 1 | 3 | 0 |

* RA = rheumatoid arthritis; HCQ = hydroxychloroquine; SSZ = sulfasalazine; MTX = methotrexate; CSA = cyclosporin A; CHL = chlorambucil; AZA = azathioprine; CYC = cyclophosphamide; RF = rheumatoid factor; HAQ-ADL = Health Assessment Questionnaire Activities of Daily Living.
† MTX dosages are the maximum used, as follows: patient 1, 25 mg/week by mouth; patient 2, 20 mg/week by mouth; patients 3 and 4, 17.5 mg/week intramuscular.
either trimethoprim/sulfamethoxazole (1 double-strength tablet 3 times a week) or monthly aerosolized pentamidine (300 mg).

Assessment of disease status. Tender joint count (44 joints total), swollen joint count (42 joints total; hips were not estimated for swelling), patient’s assessment of pain, patient’s and physician’s global assessment of disease (using a scale where 0% is best and 100% is worst), HAQ Activities of Daily Living (20 questions), and C-reactive protein (CRP) level and erythrocyte sedimentation rate (ESR) were used to estimate disease status. Improvement was evaluated according to the ACR preliminary definition of improvement in RA (ACR 20) (8) as a $\geq 20\%$ improvement in both tender and swollen joint count plus $\geq 20\%$ improvement in $\geq 3$ of the other 5 parameters (patient’s assessment of pain, patient’s global assessment of disease, physician’s global assessment of disease, functional disability, and CRP level or ESR). The ACR 50 and ACR 70 (9) criteria (defined in the same manner as the ACR 20 criteria (8), but with improvements of $50\%$ and $70\%$, respectively, in the various scores) were also used.

RESULTS

Patient profile. Four patients (3 women, 1 man) with RA according to the 1987 ACR criteria for the classification of RA (7) were treated (Table 2). All had severe RA and were seropositive for RF, with functional disability and characteristic radiographic findings (periarticular osteoporosis, joint space narrowing, and erosions). Two patients had rheumatoid nodules, and 1 patient had pulmonary involvement (interstitial lung infiltrates on computed tomography scan and reduced diffusing capacity for carbon monoxide). Their disease was resistant to nonsteroidal antiinflammatory drugs, oral corticosteroids, and various DMARDs. At least 5 DMARDs had failed for each patient, given alone or in combination (Table 2). All patients were markedly limited in their daily activities, answering “without any difficulty” to 0–3 of 20 HAQ questions.

Toxicity. HSCT was well tolerated. Expected toxicity symptoms (nausea, vomiting, and hair loss) occurred for all patients. Patient 2 developed an uncomplicated Streptococcus sanguis bacteremia. A transient elevation of serum creatinine was observed in patient 3 (from 0.7 mg/dl pre-HSCT to 1.6 mg/dl at 4 months post-HSCT), but this returned to normal (0.9 mg/dl) at 6 months post-HSCT. This elevation may have been due to adverse effects of the drugs used, together with patient 3’s previous history of gold-induced proteinuria and the presence of hypertension and mild hematuria at the time of HSCT.

Patient outcome. An improvement in study parameters was observed after HSCT in all patients (Figure 1). Three of the 4 patients fulfilled the ACR 70 criteria (9) at 1 month and 3 months. Patient 2 had an initial improvement at 1 month in all parameters except the tender joint count, and therefore did not fulfill the ACR 20 criteria. At 9 months, despite oral prednisone and 20 mg/week of methotrexate (MTX), she had 17 swollen and 26 tender joints, 9 hours of morning stiffness, and marked functional disability.

Patient 1 had a substantial improvement and fulfilled the ACR 70 criteria throughout the 20 months of followup. A transient mild increase in the number of tender and swollen joints was observed at 3 months, but this had improved at her 6-month evaluation. Cortico-
steroids were discontinued at 8 months, but she continued taking hydroxychloroquine (HCQ) (400 mg/day). At 20 months post-HSCT, she had 2 swollen and 4 mildly tender joints and no morning stiffness. She had gained normal life activities and her rheumatoid nodule was reduced in size.

At 3 months, patient 3 had 1 swollen and no tender joints and had improved functionally. At 5 months, he developed a mild exacerbation of the arthritis, with 5 swollen and 6 tender joints and an increased ESR (from 35 mm/hour to 100 mm/hour), while receiving low-dose prednisone. The symptoms improved with an increase of corticosteroids, except for persistent knee synovitis requiring administration of intraarticular corticosteroids. At 6 months, he fulfilled the ACR 50 but not the ACR 70 criteria. However, at 9 months he had discontinued drugs, had no evidence of active synovitis or functional disability, and had fulfilled the ACR 70 criteria.

Patient 4, who also received TBI, experienced significant improvement at 1 month and 3 months. At 3 months, she was taking 20 mg/day of prednisone and 400 mg/day of HCQ. She had 2 swollen and no tender joints and no morning stiffness, and had gained normal life activities. Her rheumatoid nodule had disappeared. At 6 months, her disease relapsed with 33 swollen and 44 tender joints, prolonged morning stiffness, and marked functional disability. HCQ was replaced with MTX, but her improvement was minimal at 9 months. She no longer meets the ACR 20 criteria.

The RF level was only slightly reduced for all patients except patient 3, for whom it gradually fell and disappeared at 6 months.

**DISCUSSION**

Intense immunosuppression with subsequent autologous HSC support proved to be well tolerated and safe in these 4 patients with refractory RA and poor prognostic indicators. All patients experienced an initial improvement, although 1 patient did not fulfill the ACR 20 criteria for improvement (8) because of persistent joint tenderness, even though swelling was reduced on physical examination. All the patients had some degree of exacerbation of their arthritis in the early post-HSCT period. However, severity of these exacerbations varied. Two patients (including the patient who received TBI) developed symptoms comparable with those observed prior to HSCT, while the increased synovitis in 2 patients was mild and transient and did not cause functional disability through the followup period. The latter 2 patients fulfilled the ACR 70 criteria for improvement (9) at 9 months and 20 months, respectively.

It is not clear why 2 of 4 patients did not have sustained improvement. These patients had severe RA that was resistant to combinations of DMARDs. Disease relapses may have been due to 1) inability of the conditioning regimen to ablate disease-causing immune cells, 2) reinfusion of disease-causing immune effector cells with the autologous HSC, or 3) regeneration of disease-mediating immune cells from the autologous HSC compartment. Finally, the hypothesis may be incorrect that RA is an autoimmune disease curable by immune ablation and regeneration of normal immunity from the stem cell compartment.

It is possible that our immunosuppressive regimen was not intense enough. Despite dosages of 200 mg/kg CYC, 90 mg/kg antithymocyte globulin, and, for 1 patient, an additional 400 cGy single-fraction TBI, disease relapses occurred in our study. This conditioning regimen is relatively mild and not myeloablative. Further immunosuppression may be obtained by using a myeloablative regimen. However, the incidence of adverse effects may increase.

In our study, a reduction of the number of lymphocytes of 2.3–4.0 logs was obtained prior to graft infusion. Unmanipulated autologous stem cell transplantation has resulted in recurrence or persistence of autoimmune diseases, including RA (10). The target (or acceptable) T cell dose, reinfused with the autograft to achieve a successful HSCT, is unknown. With aggressive T cell depletion (<5 × 10⁴ T cells/kg) and even without post-HSCT immunosuppression, major HLA barriers to procedures such as haploidentical allogeneic transplantation may be overcome without graft-versus-host disease (11). Since an allograft containing <5 × 10⁴ T cells/kg can develop tolerance to foreign, major histocompatibility complex (MHC)–mismatched tissue, it remains possible that an autologous graft containing a similar reduction of T cells could regenerate tolerance to self epitopes and perhaps be more effective.

RA may be an autoimmune disease that arises from an inherited CD34+ progenitor stem cell disorder. Alternatively, RA may be immune mediated rather than autoimmune, resulting from, for example, an infectious agent. In either case, an allogeneic graft containing a new stem cell compartment may be curative by overcoming the previous stem cell predisposition for autoimmunity or immune-mediated clearance of infectious complications. Long-lasting remissions of RA after allogeneic HSCT have been reported for the treatment
of aplastic anemia (12–15). However, the development of relapse in 1 of these cases (15) despite full donor engraftment suggests that factors other than replacement of stem cells may also be involved.

Two observations support the hypothesis that mismatch between donor and recipient may be important in maintaining long-term remission. First, maternal–fetal HLA–DR and HLA–DQ disparities were associated with remission of RA during pregnancy (16). Second, immunization with allogeneic mononuclear cells resulted in remission for patients with RA (17). Although HLA-mismatched allogeneic transplantations cannot currently be considered because of high transplantation-associated mortality, mismatch of antigens other than MHC class I and II antigens may promote long-term disease remission.

In this series of patients, autologous HSCT was safe and effective in inducing major clinical response and maintained significant benefit for 2 patients at 9 months and 20 months, respectively. Despite initial improvement, response was not sustained for the other 2 patients. A regimen-related dose-response effect may exist, but the addition of low-dose TBI to the conditioning regimen did not result in sustained clinical improvement for the single patient treated this way. More aggressive T cell depletion of the autograft, use of a myeloablative regimen, or use of an allogeneic graft from a normal sibling may be necessary to decrease disease relapse rates.

REFERENCES


