Autologous Stem Cell Transplantation for Rheumatoid Arthritis — Interim Report of 6 Patients

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ABSTRACT. We assessed the safety and efficacy of autologous stem cell transplantation (ASCT) using T cell depleted grafts in the treatment of severe rheumatoid arthritis. Methods included mobilization 2 g/m^2 cyclophosphamide (Cy) and granulocyte-colony stimulating factor; graft manipulation of positive CD34+ and negative T cell selection; and conditioning by 200 mg/kg Cy. All 6 patients improved according to American College of Rheumatology response criteria (one patient ACR70, 2 ACR50, 3 ACR20), but relapsed at 1.5–9 months when they began cyclosporine A (CSA). Five improved (one patient ACR remission, 2 ACR70, one ACR50, one improved but did not satisfy ACR response criteria). No serious complications occurred during ASCT or up to 30 months' followup. There was prolonged reduction in CD4+ T cells. ASCT is safe and has short term efficacy. T cell purging does not prevent relapse. Five patients responded to CSA when their disease had previously been refractory, suggesting an immunomodulatory effect. No serious infectious complications occurred despite prolonged reduction in CD3+CD4+ lymphocytes. (J Rheumatol 2001;28 Suppl 64:21–4)

Key Indexing Terms:
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In recent years, autologous stem cell transplantation (ASCT) has been used as an experimental treatment for a wide range of autoimmune diseases including rheumatic conditions[1]. European guidelines for ASCT in autoimmune disease have been published[2] and all cases are being collated in a registry set up on behalf of the European Group for Blood and Marrow Transplantation (EBMT) and the European League Against Rheumatism (EULAR)[3]. The registry has recently reported 276 patients (40 with rheumatoid arthritis, RA) from 64 centers in 20 countries that have undergone SCT (almost entirely autologous) specifically for severe autoimmune disease.

RA is probably one of the commonest treatable causes of chronic disability in the industrialized countries. The economic and personal costs are considerable. There is a subset of patients in whom disease remains active and difficult to control despite trying multiple conventional disease modifying agents alone and in combination. Recently, tumor necrosis factor-α (TNF-α) antagonists have become available for severe patients; however, these agents are not effective in all cases. Therefore a significant minority of patients have disease that is unresponsive to any available medications. These patients have persistent inflammation that leads to progressive joint and end organ damage. Prognosis is poor for this unfortunate group of patients.

An Australian study using autologous blood stem cells for patients with RA showed that following a good initial response, relapses occurred[4]. Changes in protocol for ASCT may increase efficacy, but also toxicity. Purging of T cells from the graft should in principle reduce relapse after conditioning by preventing reinfusion of activated lymphocytes. In this study we aimed to show greater efficacy without toxicity by using highly selected grafts. We describe 6 patients with RA who received purified ASCT in Leeds, UK, as part of the EULAR/EBMT program. We assessed safety, efficacy, and immune reconstitution in these patients.

MATERIALS AND METHODS

Patient Selection and Preparation

Eligibility. Patients with active RA despite maximal conventional therapy and for whom there are no other reasonable treatment options were considered eligible for entry into the program. Diagnosis of RA was confirmed using American Rheumatism Association (ARA) criteria[5]. Patients were defined as having active disease by the presence of ≥ 6 swollen and ≥ 6 tender joints, greater than 1 hour of early morning stiffness, and erythrocyte sedimentation rate ≥ 28 mm/h or C-reactive protein ≥ 10 mg/dl. Patients must have failed ≥ 4 disease modifying antirheumatic drugs (DMARD), ideally including triple therapy with methotrexate (MTX), sulfasalazine, and hydroxychloroquine. When these patients were entered into the program, therapy with anti-tumor necrosis factor was not routinely available in the UK.

Exclusion criteria. Significant renal, respiratory, hepatic, cardiovascular, hematological, and neurological disease were excluded using physical examination and appropriate screening investigations. Patients were also screened

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for chronic viral infection including hepatitis B and C and human immunodeficiency viruses. Patients were ARA functional grade I–III[6].

Counselling. All patients were counselled by a consultant rheumatologist and hematologist regarding risks (including infertility, infection, disease relapse, mortality) and potential benefits. Informed consent was obtained. The local research ethics committee approved this study.

Concomitant therapy. All DMARD were discontinued 3 weeks prior to harvesting. Nonsteroidal antiinflammatory medications were stopped when the platelet count dropped during conditioning.

EULAR/EBMT/Leeds Protocol for ASCT in RA

Mobilization and harvesting. Intravenous cyclophosphamide (2 g/m^2) was given with mesna, antiemetic cover, and adequate hydration. The following day subcutaneous granulocyte colony-stimulating factor (G-CSF, granocyte 263 µg/day) was commenced and continued until the end of leukapheresis.

Graft manipulation. Cells underwent CD34 positive and CD3 negative purging using the Isolex 300i system (Baxter) in order to achieve greater than 5 log reduction in T cells. A product containing > 2 × 10^6 CD34+ cells/kg was obtained for reinfusion. An unmanipulated backup graft was also stored.

Conditioning. Intravenous cyclophosphamide 200 mg/kg was administered over 4 days (–5 to –2) with mesna, antiemetic cover, allopurinol, and adequate hydration. Stem cells were reinfused on Day 0. Prophylactic acyclovir, fluconazole, and trimethoprim were given for 6 weeks.

Patient Assessment

Patients were assessed for safety and efficacy. All adverse events including infections were documented. ARA remission status and American College of Rheumatology (ACR) response was calculated at 1 and 3 months and then at 3-monthly intervals post-ASCT.

Immune Reconstitution

FACS analysis of peripheral blood lymphocytes was performed at baseline, 1 and 3 months and then at 3-monthly intervals post-ASCT. The following lymphocyte subpopulations were assessed: CD3+, CD3+CD4+, CD3+CD8+, and CD19+.

RESULTS

Patient data. Patient details are summarized in Table 1. Six patients with RA (1987 ACR criteria) have been transplanted. All patients were Caucasian, 3 female, 3 male, age 24–55 years, disease duration 3–17 years, number of failed DMARD 4–8. Three were seropositive, 3 seronegative. Patients 1, 2, and 4 were taking 10 mg prednisolone throughout.

Toxicity. Three patients had episodes of neutropenic sepsis during conditioning, which were mild and responded to intravenous antibiotics within 24 hours. One patient had an infection at a long-line insertion site that responded to 2 courses of intravenous antibiotics. Patient 5 developed bilateral pleural infusions and a pyrexia of unknown origin. His temperatures spiked to > 40°C for several days and did not respond to antibiotics. No organisms grew from blood, urine, or pleural aspirate. Antibiotics were stopped and his temperatures settled. Seven weeks after ASCT he was readmitted with pancytopenia. Bone marrow aspiration revealed hypocellularity. All drugs were discontinued including diclofenac, acyclovir, trimethoprim, and fluconazole. He was give subcutaneous G-CSF and intravenous methylprednisolone (1.5 g) and his count responded over 4 days. Total white cell counts reached > 1.0 × 10^9 cells/l on Day 12–13 in all patients. Median length of admission was 21 days. No longterm toxicity including infection was seen. All female patients have resumed their menses.

Efficacy. Dramatic reductions in disease activity were seen in all patients following ASCT: one patient achieved ACR70, 2 ACR50, and 3 ACR20 without DMARD. All patients relapsed

Table 1. Patient characteristics, progress during autologous stem cell transplant (ASCT), and outcome after ASCT.

	Patient					
	1	2	3	4	5	6
Patient characteristics						
Age, yrs	39	24	42	25	55	39
Sex	M	F	M	F	M	F
Disease duration, yrs	10	3	5	17	2	4
RF positive	No	No	Yes	No	Yes	Yes
Number of failed DMARD	8	5	4	5	4	4
Progress during ASCT						
Complications	NPS	NPS	Cannula site infection	NPS	PUO, graft failure	None
Days until WCC > 1.0 × 10^9/l	12	12	13	13	12	12
Days in hospital	21	24	21	21	39	21
Response to ASCT						
Length of followup, mo	33	27	18	15	15	12
Best ACR response before DMARD	20	50	70	50	20	20
Relapse, mo	4	9	2	8	3.5	1
DMARD commenced on relapse	CYA	CSA, MTX, INF, LEF	CSA, INF	CSA, LEF	CSA, LEF	CSA
Best ACR response to DMARD	Remission	0	50	70	Improved	70

NPS: neutropenic sepsis, all mild and responded to intravenous antibiotics; PUO: pyrexia of unknown origin; CSA: cyclosporine; MTX: methotrexate; INF: infliximab; LEF: leflunomide; WCC: total white blood cell count.

at 1–9 months. Five of these patients subsequently responded to cyclosporine A (CSA); one complete remission, 2 achieved ACR70, one ACR50, and one improved but did not reach ACR20. One patient failed to respond to CSA, MTX, infliximab, or leflunomide. All patients have enjoyed a remarkable improvement in quality of life; mean RA Quality of Life score dropped from 25 at baseline to 16 at 1 year.

Immune reconstitution. Four patients continued to have low CD3+ peripheral blood lymphocytes (PBL) at 12 months; all had low CD4+ PBL but only one had low CD8+ PBL (Figure 1A-C). All patients relapsed despite peripheral lymphocyte depletion. CD19+ B cells were reduced in all patients but recovered faster in Patient 2 (poor responder). CD19+ B cells remained low in Patient 1 (best responder) until his most recent assessment (Figure 1D). The differences in CD19+ B cell counts were not reflected in the level of IgM rheumatoid factor (RF) (Figure 1E). Both Patients 1 and 2 remained seronegative throughout.

DISCUSSION

These cases highlight the potential benefits of SCT in autoimmune disease, and this phase I/II study assessed the safety and efficacy of ASCT for treatment of resistant RA. Previous studies have shown that CD34+ selection was efficacious and safe, but disease relapse occurred. Our patients received highly selected grafts where CD34+ selection was combined with T cell depletion to achieve a 5-log reduction of T cells within the graft. In this cohort the treatment was safe and had short term efficacy; but despite selection, all 6 patients relapsed.

It is the general consensus that T cells play a pivotal role in the pathogenesis of autoimmune diseases such as RA. Therefore it seems logical to maximally decrease the number of autoreactive and memory lymphocytes during ASCT in order to prevent disease relapse. Patients who received allogeneic grafts during SCT for coincidental malignancy have entered complete remission of their autoimmune disease for many years[7]. During allogeneic transplant, more severe conditioning regimes are used and autoreactive lymphocytes are

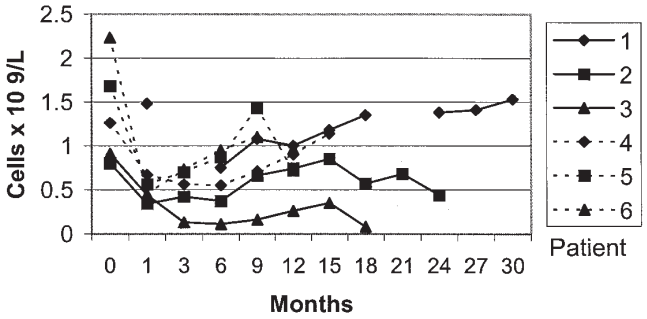

Figure 1A. Peripheral blood CD3+ lymphocyte reconstitution. Normal range CD3+: 0.77–2.68 × 10^9/l.

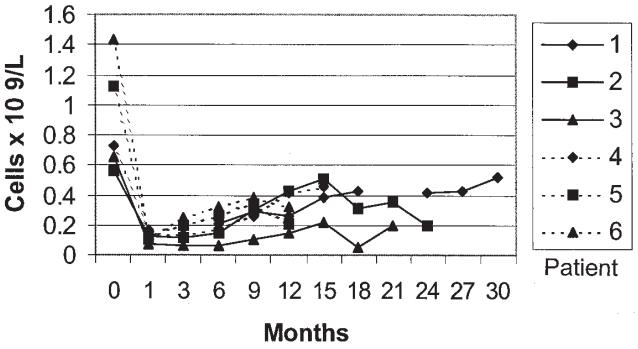

Figure 1B. Peripheral blood CD3+CD4+ lymphocyte reconstitution. Normal range, CD4+: 0.53–1.76 × 10^9/l.

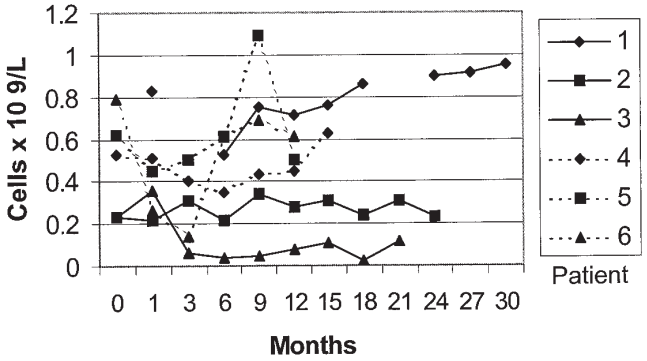

Figure 1C. Peripheral blood CD3+CD8+ lymphocyte reconstitution. Normal range, CD8+: 0.30–1.03 × 10^9/l.

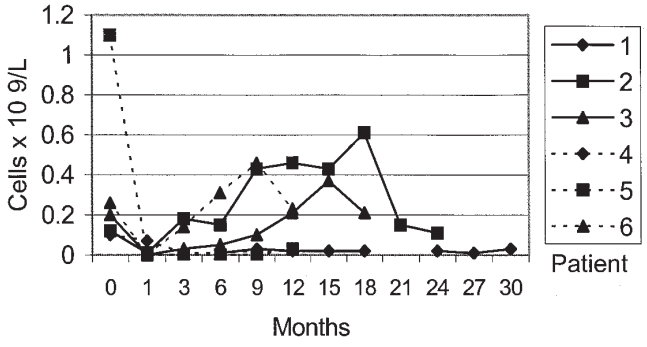

Figure 1D. Peripheral blood CD19+ lymphocyte reconstitution. Normal range CD19+: 0.06–0.66 × 10^9/l.

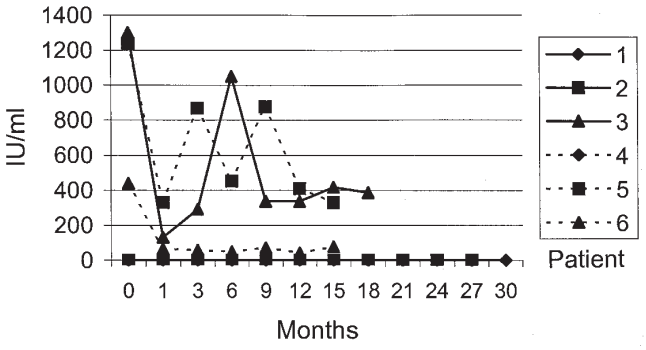

Figure 1E. Serum rheumatoid factor. Normal range < 20 IU/ml.

not reinfused. However, mortality rate from allogeneic transplant precludes its use to treat primary autoimmune diseases. During autologous SCT, the load of autoreactive lymphocytes can be reduced by myeloablative conditioning regimes and by treating the graft to remove T cells and enrich CD34+ stem cells. The patients in our cohort received selected grafts, which had been subject to CD34 positive selection and T cell purging to achieve a 5-log depletion of lymphocytes. The conditioning regimen used at our center (cyclophosphamide 200 mg/kg) was not as severe as those used by others that include total body irradiation and/or antithymocyte globulin in their protocols and may not eradicate all autoreactive T cell clones[3]. More intense conditioning may reduce relapse, but this may be at the expense of increased transplant related toxicity and delayed T cell reconstitution. In an Australian cohort, 4 patients with RA each received 100 mg/kg and 200 mg/kg cyclophosphamide during conditioning, followed by unmanipulated peripheral blood stem cell rescue[4]. Greater efficacy without increased toxicity was seen in the 200 mg/kg group. The same investigators are currently comparing outcome using unmanipulated versus manipulated grafts in ASCT for RA.

We report 6 patients with severe RA and progressive active disease with deteriorating function that had been unresponsive to previous therapies. Dramatic reductions in disease activity were seen in all patients following ASCT, but all 6 patients relapsed at 1–9 months. However, apart from Patient 2, disease was less severe and easier to control than prior to transplant, suggesting a longterm immunomodulatory effect.

There were no severe or serious complications in any of our patients either during ASCT or up to 30 months of followup. No infectious complications occurred despite all patients having prolonged reduction in CD3+CD4+ lymphocytes (Figure 1B). CD8 counts were also reduced in all patients but to a lesser extent and for a shorter period than CD4 (Figure 2C). All patients who relapsed had subnormal levels of peripheral CD4 cells at the time of relapse. We studied synovial histology at baseline, at 3 months (when the patients were well), and at relapse and found a dramatic reduction in CD4+ and CD8+ cells at 3 months, which returned in large numbers at relapse[8]. This reinfiltration despite peripheral depletion suggests that lymphocytes are preferentially recruited to or locally expanded within the synovium. Peripheral blood B cells (CD19) were below normal in all patients at one month post-ASCT, but recovered by 3 months in 2 patients (one RF positive and one negative) (Figure 2D). The patient that did the worst (Patient 2) had the most rapid recovery of B cells, whereas the patient that did the best (Patient 1) continues to have subnormal B cell levels. B cell recovery was not reflected in IgM RF levels (Figure 2E). Both Patients 1 and 2 remained seronegative throughout.

We feel that this treatment is reasonably safe and efficacious. Although our results suggest a role for T cells at relapse, double depletion of the graft does not appear to improve efficacy. The patients we selected for entry into the program were those with the most severe, aggressive, and refractory disease, for whom there was no other reasonable therapeutic options. In such patients aggressive treatments are justified to prevent disability and premature death.

Future patients entered into our program will be required to be nonresponsive to anti-tumor necrosis factor therapy. The next stage must be randomized controlled trials that would provide data regarding safety and efficacy in the treatment of this group of patients with severe and difficult disease.

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