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A Pilot Randomized Trial Comparing CD34-Selected Versus Unmanipulated Hemopoietic Stem Cell Transplantation for Severe, Refractory Rheumatoid Arthritis

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Objective. **Evidence from animal studies, case reports, and phase I studies suggests that hemopoietic stem cell transplantation (HSCT) can be effective in the treatment of rheumatoid arthritis (RA). It is unclear, however, if depletion of T cells in the stem cell product infused after high-dose chemotherapy is beneficial in prolonging responses by reducing the number of infused autoreactive T cells. This pilot multicenter, randomized trial was undertaken to obtain feasibility data on whether CD34 selection (as a form of T cell depletion) of an autologous stem cell graft is of benefit in the HSCT procedure in patients with severe, refractory RA.**

Methods. **Thirty-three patients with severe RA who had been treated unsuccessfully with methotrexate and at least 1 other disease-modifying agent were enrolled in the trial. The patients received high-dose immunosuppressive treatment with 200 mg/kg cyclophosphamide followed by an infusion of autologous stem cells that were CD34 selected or unmanipulated. Safety, efficacy (based on American College of Rheumatology [ACR] response criteria), and time to recurrence of disease were assessed on a monthly basis for up to 12 months.**

Results. **All patients were living at the end of the study, with no major unexpected toxicities. Overall, on an intent-to-treat basis, ACR 20% response (ACR20) was achieved in 70% of the patients. An ACR70 response was attained in 27.7% of the 18 patients who had received CD34-selected cells and 53.3% of the 15 who had received unmanipulated cells ($P = 0.20$). The median time to disease recurrence was 147 days in the CD34-selected cell group and 201 days in the unmanipulated cell group ($P = 0.28$). There was no relationship between CD4 lymphopenia and response, but 72% of rheumatoid factor (RF)–positive patients had an increase in RF titer prior to recurrence of disease.**

Conclusion. **HSCT can be performed safely in patients with RA, and initial results indicate significant responses in patients with severe, treatment-resistant disease. Similar outcomes were observed in patients undergoing HSCT with unmanipulated cells and those receiving CD34-selected cells. Larger studies are needed to confirm these findings.**

Patients with severe rheumatoid arthritis (RA) often have significant disability and may experience premature mortality (1). This has led to a change in the management of the disease, with an emphasis on the early use of disease-modifying antirheumatic drugs (DMARDs). Despite this more aggressive approach to the control of RA, however, a significant proportion of patients still experience severe, treatment-resistant dis-

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ease. Hemopoietic stem cell transplantation (HSCT) after high-dose chemotherapy has been suggested as a possible treatment approach for patients with severe disease, based on encouraging data in animal models (2) and the observation that patients who underwent HSCT for malignancy often had significant remissions of coexistent autoimmune diseases (3). Phase I studies using HSCT for severe autoimmune disease have now commenced, with some early encouraging results (4).

The use of an autologous stem cell product in HSCT for diseases such as RA raises the theoretical possibility of reinfusing pathogenetic autoreactive T cells, which some investigators have suggested is the cause of recurrence of disease post-HSCT (5). As a result, T cell depletion of the graft has been recommended (6), despite the fact that there are no data to support this approach and some investigators have questioned the role of T cells in the pathogenesis of RA (7). We have previously shown that treatment with cyclophosphamide (CYC; 200 mg/kg) followed by an unmanipulated stem cell product is a safe and effective regimen in patients with RA (8). In order to obtain preliminary data on whether T cell depletion confers an additional benefit in autologous HSCT for RA, we conducted a pilot multicenter, randomized trial of CD34-selected HSCT (as a form of T cell depletion) compared with unmanipulated HSCT in patients with severe RA who had been treated unsuccessfully with multiple DMARDs including methotrexate (MTX).

PATIENTS AND METHODS

Patients. Patients were eligible for the study if they met the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 criteria for classification of RA (9), were between 18 and 65 years old, and had active disease (defined as the presence of $\geq$6 swollen and tender joints plus at least 2 of the following: $\geq$9 joints capable of response that were tender on pressure or motion, morning stiffness lasting $\geq$1 hour, and an erythrocyte sedimentation rate [ESR] $>$28 mm/hour). Disease duration must have been 1–20 years and patients must have been treated previously with MTX and at least 1 other DMARD, with adequate disease control not achieved. Exclusion criteria included left ventricular ejection fraction $<$50%, carbon monoxide diffusing capacity $<$70% of predicted, human immunodeficiency virus infection, hepatitis B or C infection, an abnormal bone marrow aspirate, or severe psychiatric illness compromising the patient's ability to provide informed consent. The study was approved by the institutional ethics committees of the 5 hospitals participating in the trial and was conducted in conformity with the declaration of Helsinki and the National Health and Medical Research Council Statement on Human Experimentation. Patients were randomized from September 1998 to December 1999, with transplantation performed in all patients by March 2000.

Protocol design. The study was a pilot open-label, prospective, randomized trial comparing unmanipulated HSCT with CD34-selected HSCT after high-dose CYC in patients with severe, refractory RA. The following ACR outcome criteria were determined at baseline: swollen and tender joint counts, pain score, patient and physician global assessment on a visual analog scale (VAS), Health Assessment Questionnaire (HAQ) score (10), serum rheumatoid factor (RF) level, C-reactive protein (CRP) level, and ESR. After screening, patients were randomized to be administered autologous stem cell transplants either without manipulation or after CD34 cell selection of the collected peripheral blood stem cell product.

Stem cell collection. Filgrastim (10 μg/kg; Amgen, Thousand Oaks, CA) was administered subcutaneously on each of 4 days (designated days 1–4 of this portion of the study), and leukapheresis was performed on day 4 or day 5 if the peripheral CD34 cell count was adequate. The minimum target for collection was 2×10^6 CD34 cells/kg. After the first 14 patients the dose of filgrastim administered to patients was increased to 12 μg/kg twice daily because of a larger-than-expected number of patients requiring more than 1 leukapheresis cycle. Thus, the remaining 18 patients received 12 μg/kg twice daily. The patient's usual corticosteroid dosage was maintained throughout the period of stem cell collection, but MTX was ceased. Swollen and tender joint counts as well as a pain VAS score were recorded before administration of filgrastim (day 1) and on day 5. CD34 cell selection was performed using the Ceprate (Cellpro, Boston, MA) or the Isolex 300I Magnetic cell separator (Nexell Therapeutics, Irvine, CA). The post-selection target of 2×10^6 CD34 cells/kg and a 3-log depletion of T cells was attained with both the Isolex and the Ceprate devices.

Hospital admission. Patients were admitted to the hospital for the HSCT procedure within 2 months of stem cell collection. All DMARDs, including MTX, were discontinued except corticosteroids, which were maintained at the patient's usual dosage until 6 weeks post-HSCT, at which time they could be reduced. CYC (50 mg/kg of ideal body weight) was administered intravenously 5 days, 4 days, 3 days, and 2 days prior to stem cell infusion (designated days -5, -4, -3, and -2 of this portion of the study), for a total dose of 200 mg/kg. Intravenous mesna was given each day at a dose of at least 60% of the CYC dose for that day. Stem cells were infused on day 0 and standard supportive care was given to all patients until engraftment, according to individual hospital protocols. All patients received standard antibiotic prophylaxis during the hospitalization, and beginning at the time of discharge, all received cotrimoxazole (160 mg trimethoprim/800 mg sulfamethoxazole) 2 days per week (twice on each of the 2 days) until 3 months post-HSCT.

Followup phase. Patients were evaluated at monthly intervals post-HSCT until recurrence of disease or for a maximum of 12 months, whichever came first. Following recurrence of disease, patients were not formally followed up but were given the option of being evaluated on a 3-monthly basis, after DMARD treatment was reinstituted. At each followup visit, the parameters in the core set of outcome criteria (as outlined above) were assessed and information on

Table 1. Characteristics of the patients, by treatment group*

	HSCT with unmanipulated cells (n = 15)	HSCT with CD34-selected cells (n = 18)
Age, years	42 (23–63)	35 (22–61)
No. female/no. male	12/3	12/6
RA duration, years	10 (2–19)	10 (3–15)
Previous DMARDs	5 (2–7)	4 (3–6)
% taking prednisone	73	72
Daily prednisone dosage, mg	12 (5–25)	14 (5–25)
% RF positive	80	89
RF level, IU/ml	95 (20–1,300)	170 (20–1,100)
Swollen joint count	19 (10–59)	12.5 (6–34)
Tender joint count	31 (9–66)	32 (9–50)
ESR, mm/hour	35 (9–95)	35 (10–97)
CRP, mg/liter	18.5 (6–141)	25 (5–148)
Pain, 10-cm VAS	6.5 (1.6–9.1)	4.2 (2.5–8.3)
Physician global assessment, 10-cm VAS	6.4 (0.6–8.7)	6.5 (0.3–9.4)
Patient global assessment, 10-cm VAS	6.4 (1.6–9.1)	5.5 (2.2–9.7)
HAQ score	1.2 (0.4–2.1)	1.1 (0.7–2.0)

* Except where indicated otherwise, values are the median (range). HSCT = hemopoietic stem cell transplantation; RA = rheumatoid arthritis; DMARDs = disease-modifying antirheumatic drugs; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; VAS = visual analog scale; HAQ = Health Assessment Questionnaire.

antirheumatic and analgesic medication was recorded. Immune reconstitution studies were performed while patients remained in the study. CD4 (T helper cell), CD8 (T cytotoxic cell), and CD19 (B cell) numbers were calculated using flow cytometric analysis and the peripheral blood lymphocyte count pre-HSCT and 1.5, 3, 6, 9, and 12 months post-HSCT. The end of study was defined as 1 year post-HSCT, the day of withdrawal from the study, the day of recurrence of disease, or death. The maximum followup was 1 year post-HSCT.

End points. The primary end points were related to the efficacy of the high-dose CYC and were defined according to the ACR improvement/response criteria (11). Patients were prospectively categorized as having attained a maximal response over the followup period (improvement from baseline) of 20% (ACR20), 50% (ACR50), or 70% (ACR70), or as being nonresponders (not attaining ACR20 by 90 days). Another primary end point was time to recurrence of disease (defined as >20% increase in the corticosteroid dosage, the reintroduction of DMARDs, or a return to <20% improvement from baseline in the ACR response criteria at any time point during the 12-month period. If patients fulfilled any of these latter end points they were withdrawn from the study. The secondary end point was the comparison between the CD34-selected cell treatment group and the unmanipulated cell treatment group in terms of maximal ACR response rates, individual parameters, and time to recurrence. Safety end points were the incidence of filgrastim-induced flares (as defined by Snowden et al [12]) during stem cell collection and the comparative incidence of all adverse events between the CD34-selected cell treatment group and the unmanipulated cell treatment group during followup.

Statistical analysis. The initial plan for this pilot trial was to recruit 20–35 patients. Thirty-three patients were enrolled. Because HSCT is a new therapy with very little outcome data, it was thought that inclusion of 12–17 patients in each treatment arm would be adequate to help establish feasibility and provide data for the design of subsequent studies. The time of screening for stem cell collection was considered to be baseline. The intent-to-treat principle was applied, with unevaluable patients being recorded as nonresponders. Statistical tests were 2-tailed, and the nominal significance level of 0.05 was assumed for analyses. Significance levels reported were not adjusted for multiplicity. Maximum ACR response was compared between treatment arms using the Cochran-Mantel-Haenszel chi-square statistic for an ordinal response, with levels of none, ACR20, ACR50, and ACR70. Time to recurrence was analyzed via the Kaplan-Meier method, applying the log rank test, with a time-to-recurrence of 0 recorded for nonresponders. Patient characteristics were assessed in relation to prognostic value for ACR response (none versus minimum ACR20) and time to recurrence. Screening statistical tests included Pearson's chi-square and Wilcoxon's rank sum tests for ACR response and Wald's chi-square test for time to recurrence. Multivariate analyses were not necessary following the results of the screening analyses. All data manipulations and statistical analyses were performed using SAS version 6.12.

RESULTS

Patient characteristics. The baseline characteristics of the patients randomized to the 2 treatment arms are summarized in Table 1. The 2 groups were well matched in terms of both demographic characteristics and disease activity. DMARDs that had been previously taken without success included MTX (33 patients [100%]), sulfasalazine (28 patients [85%]), azathioprine (10 patients [30%]), cyclosporine (19 patients [58%]),

gold (26 patients [79%]), hydroxychloroquine (20 patients [61%]), D-penicillamine (14 patients [42%]), and oral CYC (5 patients [15%]). Two of the 33 patients initially randomized (1 in each treatment arm) did not complete the study. The patient in the CD34-selected cell arm was withdrawn because of failure to collect adequate stem cells after 5 leukaphereses. The patient in the unmanipulated cell arm was withdrawn because of the diagnosis of MTX pneumonitis before the stem cell collection phase. Both patients were included in the analysis on an intent-to-treat basis, as nonresponders.

Stem cell mobilization. Thirty-two patients received filgrastim for mobilization of stem cells. Filgrastim had little effect on disease activity, with only 3 of the 32 patients (9.4%) experiencing a flare of disease; 2 of these 3 patients subsequently attained an ACR70 remission after the HSCT. More patients in the CD34-selected cell arm required >1 leukapheresis (14 of 18) compared with the unmanipulated cell arm (4 of 14), which resulted in a higher cumulative dose of filgrastim in the former group. This, however, had no overall effect on disease activity in the CD34-selected cell group, as reflected by the swollen/tender joint counts between baseline (the time of initial screening) and day −6 (just prior to CYC administration). In fact, both the CD34-selected cell and the unmanipulated cell treatment groups had a nonsignificant increase in swollen/tender joint counts between these 2 time points (median swollen joint count 12.5 at screening versus 16 on day −6 [$P = 0.23$] in the CD34-selected cell group patients, and 19 at screening versus 20.5 on day −6 in the unmanipulated cell group patients [$P = 0.27$]).

With the use of filgrastim, adequate numbers of stem cells were collected for transplantation. The median CD34 count ($\times 10^6$/kg) infused on day 0 was higher in the unmanipulated cell group than in the CD34-selected cell group, reflecting the expected loss of stem cells in the selection process (3.40 versus 2.35 [$P = 0.03$]). The differences between the 2 groups in the median numbers of CD4, CD8, and CD19 cells infused reflect the planned 3-log depletion: 149.8, 73.95, and 33.0×10^6/kg, respectively, in the unmanipulated cell group, compared with 0.05, 0.03, and 0.04×10^6/kg in the CD34-selected cell group.

High-dose CYC. Thirty-one patients (14 in the unmanipulated cell group, 17 in the CD34-selected cell group) underwent the high-dose CYC therapy and stem cell infusion. The time of neutrophil engraftment (neutrophil count $>0.5 \times 10^9$/liter) was similar in the 2 groups (median 13.5 days [range 9–21 days] in the unmanipulated cell group and 14 days [range 11–24 days] in the CD34-selected cell group). Likewise, the time of platelet engraftment (platelet count $>20 \times 10^9$/liter unsupported by transfusions) was similar (median 11.5 days [range 0–21 days] in the unmanipulated cell group and 11 days [range 0–16 days] in the CD34-selected cell group). The mean number of red blood cell transfusions per patient was 2.6 in the unmanipulated cell group and 3.2 in the CD34-selected cell group. The mean number of platelet transfusions was also similar (2.3 in the unmanipulated cell group and 2 in the CD34-selected cell group). The procedure was well tolerated by both groups of patients, with a similar number of days of intravenous antibiotic use (9 days in the unmanipulated cell group and 10 days in the CD34-selected cell group), and both groups had a median hospital admission of 22 days. No patient was readmitted for complications related to the procedure.

Efficacy. HSCT proved efficacious for RA as judged by ACR response criteria. On an intent-to-treat basis, the percentage of patients attaining ACR20, ACR50, and ACR70 was 69.7% (23 of 33 patients), 45.4% (15 patients), and 39.4% (13 patients), respectively, over a median followup of 167 days (range 45–374 days). Ten patients (30.3%) were classified as nonresponders (including the 2 who did not progress to the high-dose CYC portion of the study). There was no significant difference with respect to individual ACR responses between the unmanipulated cell and CD34-selected cell groups (ACR70 53.3% versus 27.7%, respectively, ACR50 60% versus 33.3%, ACR20 73.3% versus 66.7% [$P = 0.20$ by Cochran-Mantel-Haenszel test]). Swollen/tender joint counts were dramatically reduced in the patients whose RA responded to HSCT, as demonstrated in Figure 1. Tender joint counts were significantly lower in the unmanipulated cell group compared with the CD34-selected cell group at 3 months and 6 months post-HSCT ($P = 0.03$). There was no significant difference over time between the 2 groups in ESR, CRP level, HAQ score, pain VAS, or patient or physician assessment. The median RF level at 3 months was reduced to a greater extent in the unmanipulated cell group compared with the CD34-selected cell group (30 IU/ml versus 219 IU/ml [$P < 0.01$]).

Disease recurrence was common in both treatment groups. The median time to recurrence was 147 days in the CD34-selected cell group and 201 days in the unmanipulated cell group ($P = 0.28$ by log rank test), giving an overall time to recurrence of 180 days (95% confidence interval [95% CI] 134–238), as demonstrated in the Kaplan-Meier curve shown in Figure 2. In 4 patients (1 in the CD34-selected cell group, 3 in the

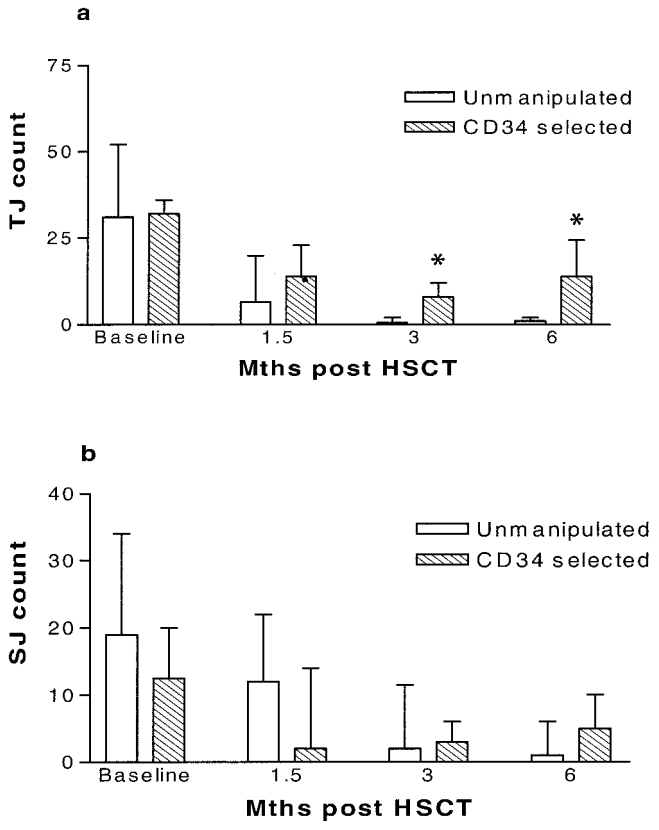

Figure 1. a, Tender joint count (TJ count) and **b,** swollen joint count (SJ count) at baseline and at 1.5, 3, and 6 months post–hemopoietic stem cell transplantation (HSCT). Values are the median and interquartile range. $* = P = 0.03$ versus unmanipulated cell treatment group.

unmanipulated cell group), RA remained in remission (ACR70 response) without reinstitution of DMARDs, with followup periods of 11, 12, 15, and 17 months, respectively. A univariate analysis of patient characteristics and disease parameters comparing responders (defined as ACR20 or better) and nonresponders showed that a high physician assessment of disease activity on VAS at baseline was associated with a better response rate and longer duration of remission (median 6.7 cm on a 10-cm VAS [range 0.8–9.4] in responders versus 5.5 cm [range 0.3–7.7] in nonresponders [$P = 0.01$]). The dose of cells infused was also analyzed for prediction of response, with no evidence of a dose effect for CD34, CD4, CD8, or CD19 cells.

Response post-recurrence. Assessment of treatment efficacy following disease recurrence was not included in the original trial design. However, formal data were available on 12 patients (36%) in 2 centers, with a median followup time of 9 months post-recurrence (range 3–18 months). One patient who was withdrawn from the study (ACR20 response not demonstrated at 90 days) subsequently attained an ACR70 response without recommencing DMARD treatment. Three patients attained ACR50 responses: 1 while taking sulfasalazine, 1 while taking MTX, and 1 while taking azathioprine and cyclosporine—medications to which their RA had previously been resistant. One patient attained an ACR70 response while taking MTX and cyclosporine (to which the disease had previously been resistant), and another while taking leflunomide. A further 2 patients attained an ACR20 response: 1 while taking MTX (previously without success) and the other while taking leflunomide. The dosages of prednisone taken by all of these patients were maintained or reduced.

Immune reconstitution. Mean ± SD baseline peripheral blood lymphocyte counts were similar between the 2 groups (1,271 ± 682/μl in the CD34-selected cell group versus 1,435 ± 574/μl in the unmanipulated cell group). Figure 3 shows the CD4, CD8, CD19, and RF results from baseline to 12 months post-HSCT in the patients who remained under review and had not restarted DMARD treatment (thus reflecting a presumed effect of the HSCT procedure itself). In both treatment groups the CD4 and CD19 counts were markedly reduced post-HSCT. In contrast, the CD8 count rose post-HSCT, resulting in a reversal of the CD4:CD8 ratio that persisted throughout the observation period. There was no statistically significant difference between the 2 groups with respect to CD8 and CD19 reconstitution at any time point in the observation period. In contrast,

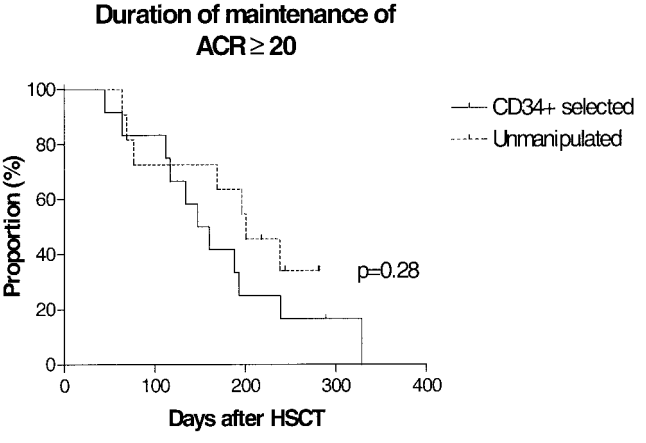

Figure 2. Kaplan-Meier curves demonstrating maintenance of American College of Rheumatology 20% response (ACR≥20) in patients receiving 200 mg/kg cyclophosphamide followed by CD34-selected or unmanipulated stem cell grafts. HSCT = hemopoietic stem cell transplantation.

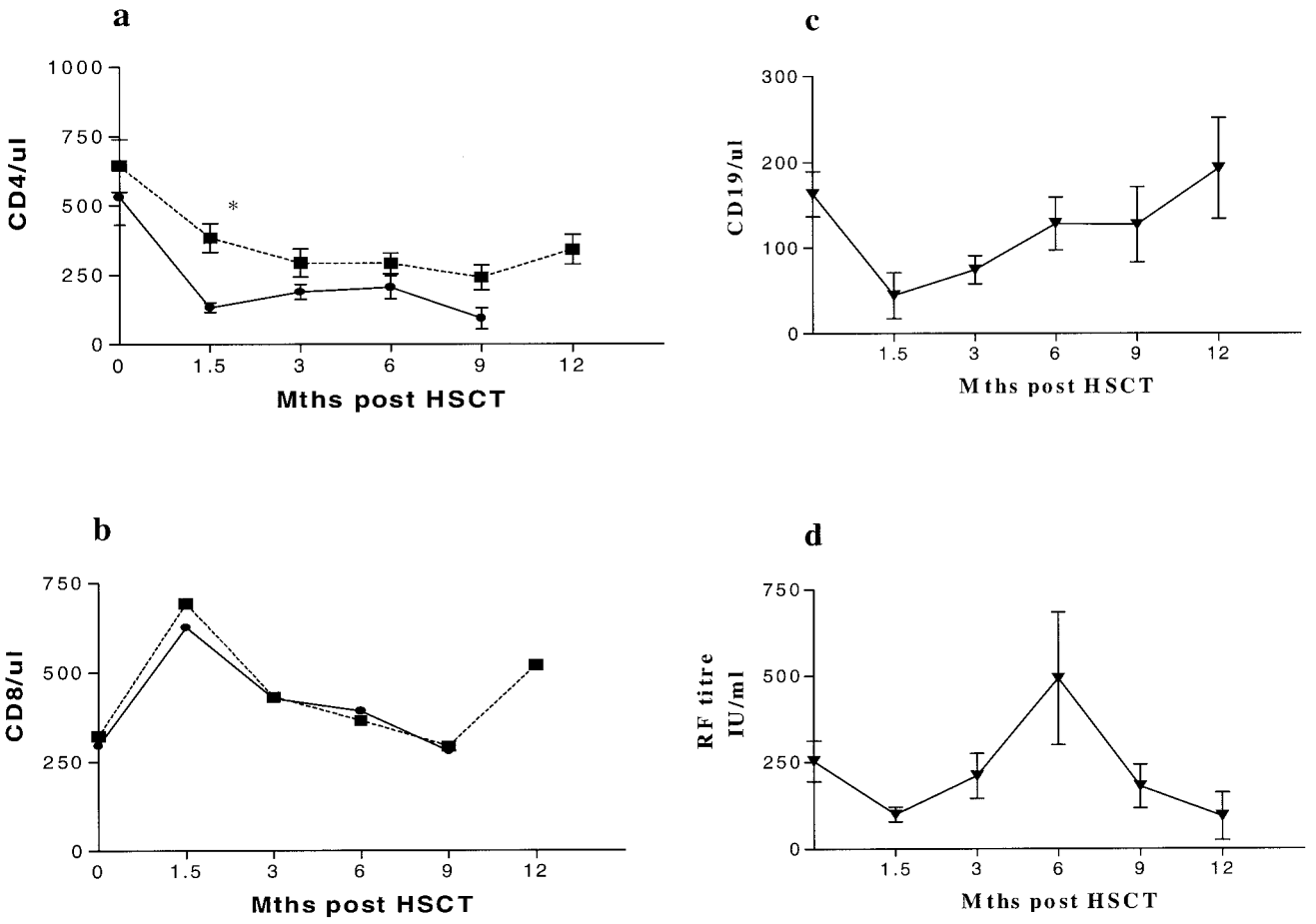

Figure 3. **a,** CD4 cell reconstitution, **b,** CD8 cell reconstitution, **c,** CD19 cell reconstitution, and **d,** rheumatoid factor (RF) levels at 1.5, 3, 6, 9, and 12 months post–hemopoietic stem cell transplantation (HSCT). ● = patients in the CD34-selected cell treatment group; ■ = patients in the unmanipulated cell treatment group; ▼ = all patients. Values are the mean ± SEM. * = $P < 0.05$ versus CD34-selected cell treatment group.

there was a greater reduction in the CD4 counts at 1.5 months post-HSCT in the CD34-selected cell group compared with the unmanipulated cell group ($P < 0.05$). The numbers of CD19 and CD8 cells returned toward baseline at 6–9 months, whereas CD4 counts remained below pre-HSCT levels for the duration of the observation period.

The pattern of B cell reconstitution was similar to the pattern of RF titer post-HSCT (Figure 3d), with a reduction at 1.5 months post-HSCT followed by a rise at 6 months. Patients in whom the RA response was maintained after 6 months continued to have recovery of B cells but had low-titer RF. Seventy-two percent of RF-positive patients (18 of 25) had a rise in RF titer prior to recurrence of disease. Baseline RF status also appeared to have an influence on disease response, with 10 of 14 patients with RF levels <100 IU/ml before HSCT attaining an ACR50–70 response, compared with 5 of 19 with RF levels >100 IU/ml.

Safety. Adverse events that occurred during hospitalization are listed in Table 2. All patients were living at the end of the followup period, with no long-term sequelae from the HSCT. There was no significant difference in the incidence of serious adverse events between the 2 groups. Adverse events during the administration of high-dose CYC and the period of hospital admission were manageable and as expected. Events shown in Table 2 predominantly reflect the pancytopenic period after CYC and did not persist after discharge. Specific infections related to the procedure included an episode of *Streptococcus viridans* sepsis in a 33-year-old woman and influenzal pneumonia in a 48-year-old man. In addition, a 48-year-old woman developed a herpes zoster infection in the T10 dermatome at 10 months

Table 2. Adverse events occurring during hospitalization, by treatment group*

Adverse event	HSCT with unmanipulated cells (n = 15)	HSCT with CD34-selected cells (n = 18)
Fever (requiring IV antibiotics)	14 (93)	15 (83)
Mucositis	7 (47)	7 (39)
Diarrhea	8 (53)	12 (67)
Nausea/vomiting	7 (47)	10 (56)
Musculoskeletal pain	11 (73)	16 (89)
Rash	11 (73)	8 (44)
Headache	10 (67)	14 (78)
Hypotension	3 (20)	4 (22)
Elevated liver enzyme levels	1 (7)	2 (11)
Anxiety/agitation	0 (0)	5 (28)

* Except where indicated otherwise, values are the number (%). HSCT = hemopoietic stem cell transfer; IV = intravenous.

post-HSCT. A 60-year-old man experienced a ruptured diverticular abscess at 2.5 months post-HSCT, which was successfully repaired at laparotomy. All infections were treated successfully, with no sequelae.

DISCUSSION

This study represents the largest reported series of patients undergoing HSCT for RA and confirms the results of previous smaller case series (8,13,14) showing that the procedure can result in substantial remissions. In our trial, an ACR70 response was achieved in 39% of the patients. We have also demonstrated that the procedure can be performed safely despite concerns about the significant toxicity associated with pancytopenia.

HSCT is an experimental procedure that is appropriately reserved for patients who have been treated unsuccessfully with all other available therapies including tumor necrosis factor (TNF) antagonists, which were not available in Australia during this study period. Nevertheless, the results in these patients, who had severe disease, compare favorably with findings in groups of patients treated with TNF antagonists (15,16), in whom ACR70 response rates of 15–18% were obtained. Current studies suggest that in 30–40% of RA patients, the disease does not respond to these agents (15,16), reinforcing the need to explore treatment alternatives. Verburg et al reported that 2 patients in their series in whom TNF antagonist treatment had failed were treated successfully with HSCT, confirming its possible role for patients with severe disease (14). More data are clearly needed, however, before definitive recommendations can be made.

The similar response rates with CD34-selected and unmanipulated HSCT found in this pilot trial suggest that T cell depletion of the stem cell graft may not provide patients with a more durable or significant response. Both the degree of ACR response and the length of remission were not significantly different between patients in the 2 treatment arms, and our data are similar to findings in previously published case series in which virtually all patients had a CD34-selected graft. Definitive conclusions cannot be drawn from this pilot study, but the findings have provided data for larger randomized studies to be performed. Based on the results obtained in this study, using a chi-square test for trend, a trial with 66 patients would provide adequate power to demonstrate whether CD34 selection is beneficial or detrimental to outcome.

Recommendations for T cell depletion have been based on case series suggesting that removal of "autoreactive T cells" will prevent recurrence of disease and enhance remissions (5). T cell depletion by CD34 selection both is an expensive procedure and may increase the potential for opportunistic infections after selected HSCT (17). It is also clear from our data that the selection procedure is nonspecific, depleting CD19 and CD8 cells and thus posing further questions concerning its utility in this setting, and challenging the current recommendations of the European League against Rheumatism/European Group for Blood and Marrow Transplantation Committee (6).

Despite the significant initial response rate, HSCT is unlikely to be curative, and disease will eventually recur. At the time of analysis, 4 of our patients remained in ACR70 remission without DMARD treatment at 11–17 months of followup, but the median time to recurrence of disease overall was 180 days (95% CI 134–238 days). Sixty-seven percent of the patients (8 of

12) who were formally followed up post-recurrence had attained ACR20–70 remissions spontaneously or in response to DMARDs. These responses, in many cases, were to agents that were previously used without success, suggesting a form of immunomodulation associated with HSCT. Although this response was seen in only a small number of patients in this study, it has previously been observed by other investigators (8,14). This observation raises the possibility that in patients with treatment-resistant RA, it may be efficacious to perform HSCT followed by early (re)introduction of therapy with DMARDs or other agents, aiming to prevent recurrence of disease.

Recurrences post-HSCT may reflect pathogenic cells in either the stem cell graft or those remaining in the host. It has been argued that eradication of memory cells in the host by intensifying the conditioning is important for response to HSCT (13). This would, however, increase the likelihood of infection, morbidity, and mortality. The immune reconstitution data reveal that CD4 T cells were profoundly depleted and this did not correlate with remissions, consistent with findings in previous studies of anti-CD4 antibody therapy (18). The reconstitution of B (CD19) cells followed a pattern similar to that of RF production, and both peaked in close proximity to the median time of disease recurrence. Furthermore, 72% of RF-positive patients (18 of 25) had a rise in RF titers immediately prior to disease recurrence, and patients with RF levels >100 IU/ml pre-HSCT appeared to have a less favorable outcome. These observations appear to add weight to the findings of others (19) that B-lineage cells and RF may have a role in recurrence of disease or, alternatively, may be a marker of recurrence.

In conclusion, HSCT following high-dose chemotherapy results in major remissions of RA, but CD34 selection of the graft does not appear to confer any benefit to the procedure when 200 mg/kg CYC is used as a conditioning regimen. Further investigation into the mechanisms of the recurrences of RA following the procedure may help to prolong the remissions attained and make HSCT a valuable treatment option for RA patients with severe disease who have been treated unsuccessfully with standard therapies.

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