Autologous stem-cell transplantation in refractory autoimmune diseases after *in vivo* immunoablation and *ex vivo* depletion of mononuclear cells

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Statement of findings

Autoimmune diseases that are resistant to conventional treatment cause severe morbidity and even mortality. In the present study we demonstrate that complete remissions can be achieved in refractory polychondritis and systemic lupus erythematosus (SLE), even at advanced stage, with the use of autologous stem-cell transplantation (SCT). Remissions persisted after reconstitution of the immune system. In the treatment of advanced systemic sclerosis (SSc), stable disease may be achieved with autologous SCT.

Keywords: autologous stem-cell transplantation, polychondritis, refractory autoimmune disease, systemic lupus erythematosus, systemic sclerosis

Synopsis

**Introduction:** Patients with persistently active autoimmune diseases are considered to be candidates for autologous SCT. We performed a phase 1/2 study in a limited number of patients who were refractory to conventional immunosuppressive treatment. Following a period of uncontrolled disease activity for at least 6 months, autologous SCT was performed, after *in vivo* immunoablation and *ex vivo* depletion of mononuclear cells.

**Aims:** To investigate feasibility, toxicity and efficacy of the treatment, and the incidence of emergent infections.

**Methods:** Seven patients (aged between 23 and 48 years) were included in the single-centre trial: one had relapsing polychondritis, three had treatment-refractory SLE and three patients had SSc. Stem-cell mobilization was achieved by treatment with moderate-dose cyclophosphamide (2 g/m²; in terms of myelotoxic side effects or myelosuppression) and granulocyte colony-stimulating factor (G-CSF). CD34⁺ cells of the leukapheresis products were removed by high-gradient magnetic cell sorting. After stem-cell collection, immunoablation was performed with high-dose cyclophosphamide (200 mg/kg body weight) and antithymocyte globulin (ATG; 90 mg/kg body weight). Autologous SCT was followed by reconstitution of the immune system, which was monitored by six-parameter flow cytometry and standard serology. The trial fulfilled the European League Against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT) guidelines for blood and bone marrow stem-cell transplants in autoimmune disease.
Results: Among the seven patients studied, the patient with relapsing polychondritis and the patients with SLE were successfully treated and remained in complete remission during a follow up of 10–21 months. Remission persisted despite reconstitution of the immune system, resulting in high numbers of effector/memory-type T-helper lymphocytes and increasing populations in the naïve T-cell compartment. Before autologous SCT, one of the patients with SLE had a long-lasting secondary antiphospholipid syndrome, with high anticardiolipin antibodies and thromboembolic events. After autologous SCT the antiphospholipid antibodies became negative, and no thrombosis occurred during follow up. Two of the patients with SSc were unaffected by treatment with autologous SCT for 6 or 13 months. The other patient with SSc died 2 days after autologous SCT because of cardiac failure.

During stem-cell mobilization with G-CSF, flares of autoimmune disease were seen in the patient with polychondritis and in one patient with SLE. The strategy utilized for depletion of CD34− cells led to a reduction by 4.5–5 log of contaminating CD3+ cells in the transplant. T-cell add-back was required in the patient with polychondritis and in one patient with SLE to provide a dose of 1 × 10^6 CD3+ cells/kg body weight for the transplant.

Discussion: In vivo immunoablation in combination with autologous SCT after ex vivo depletion of CD34− cells can block the autoimmune process in relapsing polychondritis or SLE without incidence of severe infections. The remissions were achieved in patients with advanced disease that was refractory to previous intensive immunosuppressive therapy. The present results do not indicate that large-scale contamination of the stem-cell transplant with autoreactive cells after selection for CD34+ cells occurred. After the preparative regimen, the application of G-CSF was avoided, because induction of flares of the autoimmune disease were noticed during the mobilization of stem cells. In SSc patients, distinct remissions were not observable after autologous SCT; the serological and clinical status did not improve. Follow-up periods of more than 12 months may be required to identify successful treatment with autologous SCT in SSc patients. Among the various autoimmune diseases the efficacy of autologous SCT appears to be dependent on the underlying pathophysiology. The results of the present phase 1/2 study suggest that patients with advanced stage SSc should not be treated with autologous SCT, until the reasons for the lack of response and the possible mortality due to cardiac complications are identified. The observation of flares of autoimmune disease after application of G-CSF emphasizes the need for critical evaluation of the role of G-CSF in immunoablative regimens.

Full article

Introduction

Refractory autoimmune diseases cause a high degree of morbidity and even mortality, although they are not considered to be malignant diseases. During treatment with conventional and experimental immunosuppression, patients can experience treatment-related morbidity without significant gain in quality of life. Autologous SCT is a novel experimental approach for treating patients with refractory autoimmune diseases [1]. Worldwide, 74 patients with severe autoimmune disease have thus far been treated in 22 centres [2]. Of these 74 patients, 38 received autologous SCT for treatment of rheumatic autoimmune diseases.

In the present study one patient with therapy-resistant polychondritis, three patients with advanced SLE and three patients with SSc qualified for an aggressive experimental therapy. After stem-cell mobilization all patients were treated with a rigorous immunosuppressive regimen including cyclophosphamide and ATG to achieve in vivo depletion of T cells and other mononuclear cells. The preparative regimen was followed by autologous SCT of CD34+ cells after an effective ex vivo depletion of mononuclear cells by high-gradient magnetic cell sorting in order to exclude contamination of the transplant with CD34− cells. The present phase 1/2 trial was aimed at investigating the toxicity of this protocol and the incidence of infections. In addition, the efficacy of autologous SCT with respect to clinical and serological remissions and their duration was evaluated.

Patients and methods

Patients

All patients had long-lasting histories of severe and progressive disease without any signs of improvement under conventional immunosuppressive treatment. Inclusion criteria were defined as persistently active disease with poor prognosis and inadequate response to standard protocols (glucocorticoids and at least two different regimens of immunosuppressive drugs, such as intravenous cyclophosphamide 800–1000 mg/application). Furthermore, the patients needed to have adequate function of all major organs in order to tolerate conditioning and transplantation. The exclusion criteria were infections and uncontrolled arrhythmia or congestive heart failure. Further exclusion criteria were as follows: ejection fraction below 50% determined by echocardiogram; lung function test (LFT; transfer factor for carbon monoxide [TLCO] <45%); glomerular filtration rate below 40 ml/min or serum creatinine greater than 2.0 mg/dl; hyperalimentation; and age greater than 59 years. The patients were included in the...
trial only after written consent had been obtained. The present study on autologous SCT for refractory autoimmune diseases was approved by the state ethics committee.

**Patient 1**
A 41-year-old female was admitted with relapsing polychondritis, which was first diagnosed in 1985. The disease was manifested by severe arthralgias, costosternal pain, vasculitis, scleritis, saddle nose and tracheal involvement; the patient had also sustained a life-threatening episode of pyoderma gangrenosum. Despite continuous and intensive conventional therapy for several years, no remission was achieved. During disease progression there was a risk of developing a tracheo-oesophageal fistula. The previous therapy regimens had included intravenous Ig, high-dose methylprednisolone, methotrexate, anti-CD4 antibody and intravenous cyclophosphamide (cumulative dose 6.0 g/m² per month) with concomitant application of steroids. At admission, the daily dose of methylprednisolone was 30 mg. Her Karnofsky score was 60%.

**Patient 2**
A 27-year-old female was diagnosed as having severe SLE at the age of 16 years. During the course of disease, erythema, arthralgia, myalgia, abdominal vasculitis, polyserositis, nephrotic syndrome and pericardial effusions had been observed. Despite consecutive treatments with high-dose methylprednisolone, hydroxychloroquine, azathioprine, intravenous cyclophosphamide (cumulative dose 2.8 g/m² per month), cyclosporine A, mycophenolate mofetile and daily doses of prednisolone of at least 30 mg, the disease activity remained uncontrolled for 1.5 years before stem-cell therapy. The patient had been hospitalized for the 15 months before autologous SCT. Her Karnofsky score was 40% and her European Consensus on Lupus Activity measurement (ECLAM) score was 6.5. This patient had serum antibodies against double-stranded DNA (Table 1); she fulfilled the classification criteria of the American College of Rheumatology [3].

**Patient 3**
A 48-year-old female had had severe SLE since 1993. The disease manifested as polyserositis, arthralgias, peripheral neuropathy, nephrotic syndrome, pericardial effusions and ventricular tachycardia (the latter was treated with propanolol). Treatment had included high-dose methylprednisolone, hydroxychloroquine, azathioprine, methotrexate, intravenous Ig, monthly intravenous cyclophosphamide (cumulative dose 2.7 g/m² per month) and mycophenolate mofetile. At admission, the patient was under treatment with prednisolone (20 mg/day) and oral morphium sulphate (120 mg/day). Her Karnofsky score was 60% and her ECLAM score was 6. This patient had serum antibodies against double-stranded DNA (Table 1); she fulfilled the classification criteria of the American College of Rheumatology [3].

**Patient 4**
A 37-year-old male had been diagnosed with SLE in 1989, with a nephrotic syndrome and oral lesions, erythema, arthralgias, and cardiac and pulmonary involvement. Despite treatment with prednisolone, azathioprine, intravenous cyclophosphamide (cumulative dose 7.3 g/m²) and high-dose methylprednisolone, the nephrotic syndrome (histology indicated lupus nephritis of World Health Organization grade IV) and other manifestations had not improved, and the ventricular arrhythmia (multiple couples, one triplet, multiple bigemini) remained uncontrolled. At admission, the dose of prednisolone was 100 mg/day. His Karnofsky score was 70% and his ECLAM score was 10. This patient had serum antibodies against double-stranded DNA (Table 1); he fulfilled the classification criteria of the American College of Rheumatology [3].

**Patient 5**
A 23-year-old female was first diagnosed as having diffuse SSc at age 12 years. During the course of disease, microstomia, xerostomia, arthralgias, dysphagia, cutaneous necrosis with Raynaud’s phenomenon and the onset of lung fibrosis (by high-resolution computed tomography [HRCT] scan; LFTs – total lung capacity [TLC] 72.6%, residual volume [as percentage of TLC] 127%, single breath (SB) TLCO 61.8%) had occurred. Progression of disease was observed under consecutive treatment periods with d-penicillamine, prednisolone, azathioprine, cyclosporine A, oral cyclophosphamide for 12 months (cumulative dose 3.8 g/m²) and dapsone. Treatment at admission was only symptomatic and without steroids. Her Karnofsky score was 60% and her skin score was 19.

**Patient 6**
A 25-year-old male was diagnosed with diffuse SSc in 1995 with microstomia, arthralgias, dysphagia, cutaneous necrosis with Raynaud’s phenomenon, and onset of lung fibrosis (by HRCT scan; LFTs – TLC 93.2%, residual volume [in percentage of TLC] 159%, TLCO-SB 86.2%). His finger mobility was severely limited, and he had lost 10 kg in weight since 1997. Treatment had included prednisolone, azathioprine and symptomatic therapy. At admission, the daily dose of prednisolone was 5 mg. His Karnofsky score was 60%, and his skin score was 30. In this patient steroids were applied due to the rapid progression of the disease.

**Patient 7**
A 45-year-old female had diffuse SSc that was first diagnosed in 1996. During the preceding 6 months she had lost 13 kg in weight, presumably due to oesophageal involvement. Further manifestations were microstomia, xerostomia, arthralgias, Raynaud’s phenomenon, cutaneous necrosis, intermittent tachyarrhythmia and the onset of lung fibrosis (by HRCT scan; LFT – TLC 71.1%, residual volume [in percentage of TLC] 182%, steady-state (SS)
### Table 1
Clinical outcome and treatment-related morbidity of patients with polychondritis or SLE in complete remission

| Patient* | Follow up (months) | ANA† | Anti-double-stranded DNA† | Cardiolipin§ | Complement† | Steroid dosage (mg/day)¶ | Karnofsky score (%)† | ECLAM score† | Side effects during immunoblation |
|----------|--------------------|------|--------------------------|--------------|-------------|--------------------------|---------------------|-------------|----------------------------------|
| 1        | 21                 | No relevance | No relevance | No relevance | Tracheal involvement, costosternal pain, arthralgias | No relevance | 62.5 | 60 | No relevance | SIRS**: WHO grade IV Local infection |
|           |                    | PC/female/41 years/1985 | | | | | |
| 2        | 19                 | 1:5120 | 518 / 1:64 | 38 / 54 | ∅ | 82 / 10 | 35 | 40 | 6.5 | SIRS**: WHO grade IV Septicaemia and pneumonia in sepsis: WHO grade I |
|           |                    | SLE/female/27 years/1987 | | | | | |
| 3        | 16                 | 1:5120 | 5040 / 1:128 | 88 / 379 | α-Ro+ | 122 / 14 | 20 | 60 | 6 | SIRS**: WHO grade IV Septicaemia Local infection |
|           |                    | SLE/female/48 years/1993 | | | | | |
| 4        | 10                 | 1:2560 | 976 / 1:128 | 33 / 56 | α-Ro | Proteinuria | <44 / <5 | 100 | 70 | 10 | SIRS**: WHO grade IV |
|           |                    | SLE/male/37 years/1989 | | | | | |

*Characteristics: number/disease/sex/age/year of diagnosis. †Values above the arrow represent the admission values and those below the arrow represent the present status. ‡ELISA cut-off was 118 iU/ml. §The cut-offs for IgG and IgM anticardiolipin were 48 and 44 U/ml, respectively. ¶Doses corresponding to prednisolone equivalents. **SIRS due to application of cyclophosphamide and ATG. CL-IF, Crithidio luminae immunofluorescence; PC, relapsing polychondritis; DIC, disseminated intravascular coagulation; ELISA, enzyme-linked immunosorbent assay; SIRS, systemic inflammatory response syndrome; WHO, World Health Organization.
harvest of CD34+ cells. Leukapheresis was performed (10 µg/kg body weight) until a minimum number of 4.7–70.0 × 106 CD34+ cells/kg body weight (range 2.4–7.3 × 106 CD34+ cells/kg body weight). The median number of CD34+ cells collected was 14.1 × 106 CD34+ cells/kg body weight (range 4.7–70.0 × 106 CD34+ cells/kg body weight). After enrichment for CD34+ cells by using the CliniMacs™ device, the transplants contained a median of 6.1 × 106 CD34+ cells/kg body weight (range 2.4–7.3 × 106 CD34+ cells/kg body weight). The ex vivo purging procedure led to a reduction in contaminating mononuclear cells by 4.5–5 log, resulting in 1.0 × 104 CD3+ cells/kg body weight (range 0.3–1.6 × 104 CD3+ cells/kg body weight).

Preparative regimen and autologous stem-cell transplantation
The preparative regimen consisted of 200 mg cyclophosphamide/kg body weight (days –5 to –2) and ATG (rabbit; obtained from Fresenius, Bad Homburg, Germany) 90 mg/kg body weight (days –4 to –2) [8]. During ATG treatment 500 mg methylprednisone was administered twice a day. The median time interval between cyclophosphamide for mobilization of stem cells and autologous SCT was 38 days (range 29–61 days). Supportive care was provided, according to standard protocols for allogeneic bone marrow transplantation, including isolation of the patient and prophylaxis against infection. Substitution of IgGs (10 g every other week) was applied to avoid hypoimmunoglobinaemia, and was ended in all patients after 6 months.

Evaluation of response
The function of the organs involved was monitored by technical examinations. Apart from the clinical course, serological parameters were evaluated (ie ANAs, anti-double-stranded DNA, Scl-70 and other extractable nuclear antigens). Activity of SLE was determined by the ECLAM score [9]. For SSc the skin score was used [10]. Therapeutic response was defined as 50% improvement in clinical and serological parameters. Complete remission was defined as normalization without clinical symptoms of disease. The trial fulfilled the EBMT/EULAR guidelines for blood and bone marrow stem-cell transplants in autoimmune diseases [11].

Results
Preparation of stem cells
For mobilization of stem cells one leukapheresis was sufficient in five out of seven patients; only patients 2 and 3, both with SLE, needed two procedures to collect the number of CD34+ cells required for transplant engineering. The median number of CD34+ cells collected was 14.1 × 106 CD34+ cells/kg body weight (range 4.7–70.0 × 106 CD34+ cells/kg body weight). After enrichment for CD34+ cells by using the CliniMacs™ device, the transplants contained a median of 6.1 × 106 CD34+ cells/kg body weight (range 2.4–7.3 × 106 CD34+ cells/kg body weight). The ex vivo purging procedure led to a reduction in contaminating mononuclear cells by 4.5–5 log, resulting in 1.0 × 104 CD3+ cells/kg body weight (range 0.3–1.6 × 104 CD3+ cells/kg body weight). Due to this effective strategy, patients 1 and 4 needed a
T-cell add-back to provide a dose of $1 \times 10^4$ CD3+ cells/kg body weight in the transplant.

One day after the first application of G-CSF for stem-cell mobilization, severe arthralgias were observed in patient 1 (with polychondritis), suggesting a flare of the autoimmune disease. Patient 3 with SLE showed Raynaud’s phenomenon and arthralgias after the first day of G-CSF treatment. On day 7, she developed pericardial and pleural effusions, followed by ventricular arrhythmia with atrial fibrillation on day 10 of G-CSF application, presumably related to activation of the autoimmune process. The cardiac condition disappeared under digoxin. Patient 7 with SSC had a reactivation of haemorrhagic oesophagitis, which vanished upon specific treatment. Febrile periods of unknown origin were observed in patients 1 and 7. The symptoms disappeared after the application of high-dose steroids, which could be reduced gradually.

**Immunoadablation and reconstitution of the immune system**

During the immunosuppressive regimen, autologous SCT and haematological reconstitution the median period of hospitalization of the patients was 34 days (range 30–71 days). Reconstitution of granulocytes and platelets occurred rapidly within 2 weeks in all patients. After autologous SCT, the absolute number of nucleated cells reached $1.0 \times 10^9$/l on median day +14 (range day +12 to day +16). The platelet count was $50 \times 10^9$/l on median day +12 (range day +9 to day +15). At day +20 after autologous SCT, patients 5 and 6 (with SSC) showed rapid recovery of up to approximately $0.8 \times 10^9$/l T lymphocytes or natural killer cells in the peripheral blood. The median number of platelet transfusions applied during bone marrow aplasia was 5 units (range 2–10). A median of 10 units (range 6–17) of red blood cells were administered.

In the immunoablative phase, systemic inflammatory response syndrome occurred in patients 1–6 during the first infusion of ATG. Severe arthralgias and pleural effusion were observed in patient 2; during septicemia, although she was still in aplasia after autologous SCT, arthritis and abdominal vasculitis were noted. The side effects were interpreted as flares of the autoimmune disease (Table 1). Remission was achieved by high-dose steroids. For up to 2 months after autologous SCT, the absolute counts of CD4+ cells remained below the limit of detection in all patients, and reached pretransplantation levels 4–6 months later (data not shown). Almost all CD4+ cells detectable during the second phase of reconstitution (2–6 months after autologous SCT) were CD45RA–/CD45RO+ memory/effector cells (Table 2). The activation marker HLA-DR was expressed on up to 50% of these cells in patient 1, and approximately 20% in patient 2 (data not shown). The transient appearance of activated memory/effector cells was in concurrence with viral or bacterial infections (interstitial pneumonia, localized infections of the perianal region and of the urinary tract in patient 1, and a Streptococcus mitis pneumonia in patient 2). Naïve CD4+/CD45RA+/CD45RO- cells were nearly undetectable in patients 1–4 until 6 months after autologous SCT.

For CD8+ lymphocytes, an early transient rapid expansion was observed in patients 2 and 3 within 2 months after autologous SCT. During the follow up of 10 and 6 months, respectively, the absolute numbers of CD8+ cells declined in these patients, but recovered later and remained at levels fourfold to 10-fold higher than before autologous SCT (Table 2). In patient 1 the absolute number of CD8+ cells was low during the first 7 months, but had increased fivefold at 1 year after autologous SCT in comparison with the status at admission.

**Clinical outcome after autologous stem-cell transplantation**

With a follow up between 6 and 21 months, the four patients with polychondritis or SLE were in remission, as defined by the disappearance of any clinical symptoms of disease. The physical ability of these patients had improved continuously, as shown by the Karnofsky index and the ECLAM scores (Table 1). In the SLE patients, the disease-related autoantibody titres (ANAs, anti-double-stranded DNA, cardiolipin) declined to within the normal range. In patient 3 the increased cardiolipin antibody titre was reduced to normal for the first time since August 1996 and remained low to the last date of follow up (February 2000). Despite the withdrawal of propanolol and the reduction in prednisolone dose, ventricular arrhythmias were no longer observed. In patient 4, who was suffering from nephrotic syndrome when admitted, the proteinuria improved dramatically after autologous SCT. In addition, patient 4 had a fresh deep venous thrombosis of the leg combined with symptomatic pulmonary embolism at admission, which improved after autologous SCT; anti-coagulation therapy has been continued for safety reasons. It was possible to reduce the application of corticosteroids gradually in all patients.

Patients 5 and 6 (with SSC) had neither clinical nor serological improvement (Table 3), although progression of the disease was not observable. In the LFT no major alterations were detectable, and the skin score remained stable. In both patients, Raynaud’s phenomenon improved after autologous SCT only in warm climates. Against medical advice patient 5 became pregnant during reconstitution of the immune system and gave birth to a healthy child 14 months after autologous SCT. In patient 7 (with SSC), clinical and serological progression (weight loss of 2 kg or 5% of body weight and a fourfold increase in ANA titres) was observed after moderate-dose cyclophosphamide and G-CSF for stem-cell mobilization. During immunoablation, fluid retention led to a weight gain of 7 kg within 1 week and to the occurrence of plural effusions. Transiently, she was stabilized at a significantly reduced
level of performance status before the onset of ventricular tachycardia and subsequent electromechanical uncoupling. She died on day +2 after autologous SCT due to cardiac failure, although no signs of cyclophosphamide-induced cardiotoxicity were observed during autopsy. The postmortem histology revealed an advanced stage of pulmonary fibrosis, with all the signs of cor pulmonale.

**Discussion**

The present study was conducted to evaluate the efficacy of autologous SCT in treatment-refractory autoimmune disease. After a median follow up of 14 months (range 10–21 months) four patients with polychondritis or SLE are in clinical remission. Following the immunoablative regimen, including cyclophosphamide and ATG, the disease-specific titres of autoantibodies became negative in patients 2–4, who had SLE. No symptoms of recurrent autoimmune reactivity have been detected in these patients. Obviously, the rigorous regimen of immunoablation, consisting of high-dose cyclophosphamide in combination with ATG, was successful in achieving rather complete aplasia. This combination increased the efficacy of immunoablation in comparison with that achieved by other studies [12,13], by grossly reducing the number of..
autoreactive immune cells. Plasma cells can be found resting in bone marrow for more than 90 days [14], and apparently were eliminated in the patients with SLE in the present study under treatment with ATG, presumably by the recognition of specific surface antigens by immunoglobulins of the ATG.

Side effects during immunoablation may be severe, and flares of the autoimmune disease are particularly detrimental to the patient’s condition. Comparable with a previously reported observation of the induction of flares by G-CSF in a patient with rheumatoid arthritis [15], in the present trial flares of SLE were diagnosed in patient 2 who was in aplasia after the immunoablative regimen (Table 1). Flares may be attributed to ‘cytokine-primed’ clinical situations in which G-CSF is released from macrophages and residual lymphocytes during bone marrow aplasia. Flares can be controlled by high-dose steroids. After gradual reduction in the dose of steroids and without further treatment of patients 1–4, recurrence of the flares was not observed during the follow up of more than 12 months. The flares that occurred in patient 2, in aplasia and during septicaemia, may represent a ‘burn-out’ of autoreactivity in terms of an exhaustion of mediators participating in autoimmunity. In order to avoid the induction of flares, G-CSF was not applied after termination of the immunoablative regimen and during the phase of haematological reconstitution.

In a previous study in five patients with haematological malignancies or solid tumours and concomitant refractory autoimmune disease, who were treated by autologous SCT [13], the disease persisted or relapsed within 3 months. In that investigation the transplantations were performed without in vivo/ex vivo depletion of T cells, emphasizing the importance of effective immunoablation. Successful immunoablation in vivo, combined with less intensive purging of the transplant (2–3 log depletion of CD3+ cells) was reported to halt disease progression in patients with multiple sclerosis, rheumatoid arthritis or SLE [16]. The further reduction in CD3+ cells, as applied in the present study, but not in the previous investigation [16], did not lead to a higher incidence of severe infections. Thus, it is tempting to speculate that in future studies even lower amounts than 1 × 10^4 CD3+ cells/kg body weight, as applied in the present study, may be tolerated.

In the present trial, preparations with greatly reduced numbers of CD3+ cells were used for autologous SCT. By application of the recently developed CliniMACS™ device [7], a highly efficient technology was introduced for the selection of haematopoietic stem cells. High-gradient magnetic cell sorting was able to purify CD34+ cells effectively from G-CSF-mobilized peripheral blood, resulting in 4.5–5 log depletions of CD3+ T cells, thus minimizing the risk of retransplantation of autoreactive T cells. In fact, after autologous SCT no relapse of autoreactivity was observed in the patients with polychondritis or those with SLE, even though the absolute counts of CD4+, CD8+ and all other leucocytes had reached pretreatment levels. On the other hand, the large-scale depletion of T cells from the transplants did not provoke life-threatening infections before and during immunological reconstitution. The relevance of the in vivo/ex vivo depletion procedure used in our investigation may be confirmed by controlled studies.

Stem-cell support is essential in shortening the duration of aplasia and in the reconstitution of haemopoiesis after immunoablation by a regimen of cyclophosphamide and ATG. Any immunoablatative treatment without subsequent autologous SCT is associated with the risk of severe infections during neutropenia and thrombopenic bleeding. This appears to be in contrast to the results of a recent approach with high-dose cyclophosphamide followed by G-CSF but without autologous SCT [12], in which two patients with SLE had follow-up periods of 12 or 14 months with complete or partial remission. The results appear to be due to the G-CSF-induced priming after high-dose cyclophosphamide. In patients with aplastic anaemia treated with allogeneic bone marrow transplantation the advantage of a combined immunosuppression with cyclophosphamide and ATG was emphasized by a significant reduction in graft rejections [8]. The present data on patients with polychondritis or SLE are in accord with the recent results achieved by high-stringency immunoablation followed by autologous SCT in a panel of 10 patients suffering from multiple sclerosis, rheumatoid arthritis or SLE [16]. With follow-up periods of 6 and 12 months in that study the two patients with SLE were in remission at the time of publication.

In the present investigation, a rapid decrease in levels of pathological autoantibodies to normal values was observed in the SLE patients responding to autologous SCT. The early phase in the reconstitution of the immune system was marked by rapid recovery of granulocytes and platelets in all patients. During the second phase of reconstitution CD4+ cells, exclusively of the antigen-experienced memory/effector type, were observed 2–5 months after autologous SCT (CD45RO+, CD45RA–; Table 2). Similar kinetics of reconstitution were described in patients after allogeneic bone marrow transplantation for haematological malignancies [17]. In the patients we studied, the activated T-helper cells may reflect the clonal expansion of persisting cells after the preparative regimen, which might have been stimulated by minor infections during reconstitution [18].

During the second phase of reconstitution, patient 2 in the present study suffered an episode of varizella-zoster infection. After in vitro incubation of mononuclear cells of that patient with varizella-zoster virus antigen, the secretion of
IFN-γ by a subpopulation of T cells was observed (data not shown). This may exclude a persisting general deficiency of the immune system due to the aggressive immunoablation. Deficiency of the immune system was considered as a basic reason for self-tolerance in autoimmune disease after immunosuppression followed by autologous SCT [18].

The two patients with SSc who were evaluable for follow up showed no clinical and serological responses at 6 or 13 months after autologous SCT. We presume that insufficient immunoablation may have a role in the treatment failures. This is supported by the persistence of Scl-70 autoantibody, and by the early recovery of lymphocytes (patient 5) and natural killer cell reconstitution (patient 6). In SSc no correlation between the activity of the disease and the presence of autoantibodies has been shown, although serum Scl-70 is associated with poor prognosis with regard to pulmonary or cardiac involvement [19]. Patients 5 and 6 were still positive for Scl-70 after autologous SCT, suggesting a need for intensification of treatment. However, the reason for insufficient immunoablation in the SSc patients is not clear, and may be related to the underlying pathophysiology that is different from that in SLE. SSc appears to be less responsive, at least at the advanced stage of tissue destruction, due to fibrotic processes that are not present in SLE.

The present results are in contrast to those of a previous report of a significant decline in ANAs in a SSc patient within 6 months after autologous SCT [20]. The stable disease in patient 5 in the present study was accompanied by a pregnancy during the follow-up period. Pregnancy in patients with autoimmune disease has been postulated to be a reason for stability in SSc and multiple sclerosis [21,22]. Patient 7 of the present trial, who had a brief history of SSc, died 2 days after autologous SCT from cardiac failure due to massive pulmonary fibrosis. After 2 g/m² cyclophosphamide was administered for stem-cell mobilization, she developed clinical and serological progress until autologous SCT. In hindsight, the advanced stage of pulmonary fibrosis was not foreseeable, and is to be considered the cause for the treatment-associated mortality.

In conclusion, the present study demonstrates that the induction of immune tolerance for disease-related antigens is feasible and can be achieved with immunoablation and subsequent autologous SCT in the case of refractory polyarthritis and SLE. Effective ex vivo depletion of CD34+ cells can be achieved with state-of-the-art technologies, and appears to be essential for sustained tolerance after immunological reconstitution. These results show unambiguously that a ‘reset’ of the immune system was brought about, which was able to deal successfully with pathogens. The treatment consisted of one admission into hospital for stem-cell mobilization, and another one for immunoablation and autologous SCT, with median durations of hospitalization of 20 and 34 days, respectively. The high costs of the complex and intensive therapy performed in the present study may be acceptable when compared with those of disease-related long-term hospitalization and invalidity.

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