Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through de novo generation of a juvenile and tolerant immune system

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Clinical trials have indicated that immunoablation followed by autologous hematopoietic stem cell transplantation (ASCT) has the potential to induce clinical remission in patients with refractory systemic lupus erythematosus (SLE), but the mechanisms have remained unclear. We now report the results of a single-center prospective study of long-term immune reconstitution after ASCT in 7 patients with SLE. The clinical remissions observed in these patients are accompanied by the depletion of autoreactive immunologic memory, reflected by the disappearance of pathogenic anti–double-stranded DNA (dsDNA) antibodies and protective antibodies in serum and a fundamental resetting of the adaptive immune system. The latter comprises recurrence of CD3+CD45RA+CD4+ T cells (recent thymic emigrants) with a doubling in absolute numbers compared with age-matched healthy controls at the 3-year follow-up \( (P = .016) \), the regeneration of thymic- derived FoxP3+ regulatory T cells, and normalization of peripheral T-cell receptor (TCR) repertoire usage. Likewise, responders exhibited normalization of the previously disturbed B-cell homeostasis with numeric recovery of the naive B-cell compartment within 1 year after ASCT. These data are the first to demonstrate that both depletion of the autoreactive immunologic memory and a profound resetting of the adaptive immune system are required to reestablish self-tolerance in SLE. This trial was registered at www.clinicaltrials.gov as #NCT00742300. (Blood. 2009;113:214-223)

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with heterogeneous clinical manifestations. It is characterized by the generation of pathogenic antibodies directed against a variety of autoantigens, including nuclear and cytoplasmic antigens, such as double-stranded DNA (dsDNA), nucleosomes, and by complement activation.1 It is thought that, in genetically susceptible persons, an initial breakdown of peripheral tolerance permits the activation of autoreactive lymphocytes, which then propagate autoimmune responses in a self-perpetuating process.2,3 We recently demonstrated that autoimmune reactions in lupus-prone mice (NZB/W) result in the generation of long-lived plasma cells, which secrete pathogenic autoantibodies and are resistant to conventional immunosuppression and B-cell depletion therapy.4,5

Whereas glucocorticoids and immunosuppressants ameliorate manifestations of SLE in many patients, current therapies are insufficient to control the disease in a subset of patients, and their clinical prognosis remains poor because of the development of vital organ failure, cumulative drug toxicity, and the increased risk of cardiovascular disease and malignancy.6 Immunoablative chemotherapy followed by autologous hematopoietic stem cell transplantation (ASCT) has recently emerged as a promising experimental therapy for severely affected patients, providing them the potential to achieve treatment-free, long-term remission.7,8 The rationale for applying ASCT to autoimmune diseases has been the hope that immunoablation could eliminate inflammation-driving pathogenic cells from the immune system and that regeneration of the patients’ immune system from hematopoietic precursors could reestablish immunologic tolerance.9,10 So far, direct evidence for that is lacking, and no study has determined whether immunoablation and ASCT can actually “reset the immunologic clock” in SLE.

Here we describe the long-term reconstitution of T- and B-cell subsets and serologic changes in 7 patients with SLE for up to 8 years after receiving immunoablation and ASCT, and show that immunoablation with high-dose chemotherapy, methylprednisolone, and antithymocyte globulin (ATG) efficiently depletes naive and memory T and B cells and long-lived plasma cells, including those that are autoreactive. In addition, ASCT reactivated the thymus, leading to the development of a tolerant, “juvenile” adaptive immune system, which is reflected by long-term, treatment-free, clinical remissions.
blood stem cells were collected by leukapheresis after infusion of 2.0 g/m² cyclophosphamide infusion. The graft was enriched for CD34+ cyclophosphamide followed by daily granulocyte colony-stimulating factor criteria11 and failure of remission despite treatment with 2 different standard therapies for SLE according to American College of Rheumatology classification criteria of SLE. Clinical trial protocol

Methods

Clinical trial protocol

Enrollment in the monocentric phase 1/2 clinical trial included a diagnosis of SLE according to American College of Rheumatology classification criteria1 and failure of remission despite treatment with 2 different standard immunosuppressive therapies, including at least 6 cycles of intravenous cyclophosphamide at doses of 500 to 1000 mg/m². A detailed description of the patients and trial design has been published previously.8 Peripheral blood stem cells were collected by leukapheresis after infusion of 2.0 g/m² cyclophosphamide followed by daily granulocyte colony-stimulating factor (10 µg/kg; Amgen, Thousand Oaks, CA) beginning 72 hours after cyclophosphamide infusion. The graft was enriched for CD34+ cells by CliniMACS (Miltenyi Biotec, Bergisch Gladbach, Germany). Data on the purity of CD34+ -enriched hematopoietic stem cells are reported in Table 1. Immune ablation was achieved by 50 mg/kg per day of cyclophosphamide intravenously for 4 days (days 1-4). ATG (Fresenius, Bad Homburg, Germany) intravenously for 3 days (days 1-3) and 20 mg/kg per day of rabbit anti-thymocyte globulin (Thymoglobulin, Genzyme, Cambridge, MA) intravenously for 3 days (days 1-3). A detailed description of immunosuppressive therapies, including at least 6 cycles of intravenous mycophenolate mofetil; and —, not applicable.

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<th>Patient no.</th>
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<th>No. of infused CD34+ /kg</th>
<th>Percentage of CD34+</th>
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No. of infused CD34+ -enriched hematopoietic stem cell grafts

Clinical responses to ASCT

All 7 patients successfully completed the protocol treatment. Clinical remission, defined as a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of less than 3 without immunosuppressive treatment or use of antimalarials and with less than 7.5 mg of prednisolone daily, was achieved in all 7 patients. One patient relapsed after being free of clinical symptoms for 18 months after transplantation, as described earlier12; he died of SLE-related pulmonary embolism 38 months after ASCT. Another patient died because of uncontrolled invasive central nervous system aspergillosis 3 months after ASCT. The remaining 5 patients showed no clinical or serologic evidence of SLE activity during a median follow-up of 60 months (range, 24-96 months).

Blood samples and cell preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (GE Healthcare, Little Chalfont, United Kingdom).

Flow cytometry

Absolute CD4+ and CD19+ lymphocyte numbers were calculated based on the total lymphocyte count and the percentage of CD4+ and CD19+ cells, as identified by flow cytometry using the BD Multitest panel (BD Biosciences, San Jose, CA). The following monoclonal antibodies (mAbs) were used for phenotypic analyses: anti-CD19-peridinin chlorophyll protein (PCH1, 525C11), anti-CD20-fluorescein isothiocyanate (FITC, IA6-2), anti-CD4-phycoerythrin (PE, 2H4), and anti-CD8-PE-Cy7 (BD Pharmingen, San Diego, CA). A detailed description of the antibodies and mAbs used for flow cytometry is provided in the Supplementary Methods. Immunoassays were performed using freshly isolated PBMCs in the presence of mAbs in 1% bovine serum albumin in phosphate-buffered saline on ice for 10 minutes after blocking with 10 µg of human IgG for 10 minutes. Cells were washed before analysis on a FACScalibur flow cytometer (BD Biosciences).

Table 2. Demographic data and clinical features of patients

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<th>Patient no.</th>
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<th>Clinical manifestation</th>
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<th>Therapy pretransplantation</th>
<th>Follow-up, mo</th>
<th>Clinical outcome</th>
<th>Current therapy</th>
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<td>CY, AZA, MTX</td>
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<td>Exitus letalis 3+ mo</td>
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</table>

APS indicates antiphospholipid syndrome; CY, cyclophosphamide; AZA, azathioprine; HCO, hydroxychloroquine; CSA, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil; and —, not applicable.
Perturbations of Vβ families were considered to be significant in patients when they were outside of these normal intervals.

Bone marrow mononuclear cells (BM-MNCs) were collected by bone marrow aspiration in 1 patient after ASCT and in 1 healthy volunteer from the femur after joint surgery. BM-MNCs were isolated by Ficoll-Hypaque density gradient centrifugation (GE Healthcare) and stained with anti–CD38-PE (B-B4; Chemicon International, Temecula, CA). Cells were washed before acquisition (LSR II flow cytometer; BD Biosciences) and analysis (FlowJo Software; TreeStar, San Carlos, CA).

**Stimulation assays**

For in vitro lymphocyte stimulation assays, 1 mL freshly collected heparinized peripheral blood was stimulated in the presence of 1 μg/mL αCD28 (clone 28.2; BD PharMingen) for 6 hours at 37°C with the following antigens: 1 μg/mL *Staphylococcus aureus* enterotoxin B as the positive control (Sigma Chemie, Deisenhofen, Germany), 20 μg/mL nucleosomes, as described previously,15 10 μg/mL SmD1 peptide, as described previously,16 varicella zoster virus lysate and cytomegalovirus (CMV) lysate (both Biodesign International, Kennebunk, ME). Brefeldin A (Sigma Chemie) was added for the last 4 hours of stimulation. Erythrocytes were lysed with FACS lysing solution and permeabilized with FACS-Perm2 (Sigma Chemie) was added for the last 4 hours of stimulation. Erythrocytes were stained for 30 minutes at room temperature with the following antibodies: anti–CD154-PE (TRAP1) or anti–CD69-PE (FN-50), anti–CD4-PerCp Cy5.5 (RPA-T4), anti–CD14-FITC (M5E2) and anti–interferon gamma (IFN-γ)-APC (B27) (all purchased from BD PharMingen). At least 2 × 10⁵ CD4⁺ T cells were analyzed.

**Serologic analysis**

Antinuclear antibodies (ANAs) were assessed by indirect immunofluorescence on HEp-2 cells. Anti-dsDNA antibodies were detected by *Crithidia luciliae* immunofluorescence and commercial enzyme-linked immunosorbent assay (ELISA).

**Statistical analysis**

T- and B-lymphocyte subpopulation frequencies were calculated using CellQuest software (BD Biosciences). A paired t test was used to compare (per patient and immune parameter) pretransplantation and posttransplantation data using Graph Pad Prism 4 software (version 4.03; Graph Pad Software, San Diego, CA). Based on distributional assumptions, the Mann-Whitney U test was used to compare data from patients treated by ASCT with those from healthy controls and conventionally treated SLE patients. All P values were 2-sided; statistical significance was set at α = 0.05.

**Results**

**Engraftment and leukocyte recovery**

The median time for recovery to 0.5 × 10⁹/L neutrophils and 10⁹/L leukocytes in peripheral blood was 14 days. Platelets recovered to more than 20 × 10⁹/L by a median of 12 days. No patient received unselected backup stem cell support. All patients had significantly reduced baseline lymphocyte counts (mean, 0.33 ± 0.14 × 10⁹ cells/L), reflecting lupus activity or side effects of immunosuppressive therapy. As in patients with hematologic diseases treated with a similar regimen,17 peripheral lymphocyte counts reconstituted slowly after ASCT and were still slightly reduced at the 6-month follow-up (0.94 ± 0.32 × 10⁹ cells/L). Mean absolute lymphocyte counts were back to normal at the 1-year follow-up (1.11 ± 0.29 × 10⁹ cells/L) and remained stable thereafter in responding patients.

**Increased naive T cells after posttransplantation immune reconstitution**

In SLE, pathogenic T-cell functions are thought to be mediated by autoreactive memory or memory effector CD4⁺ T cells. Elimination of such cells in vivo by immunooablation is therefore presumed to ameliorate autoimmune inflammation. Conversely, thymic reactivation is presumably required to reestablish central tolerance and to generate natural FoxP3⁺ regulatory T cells. To assess the effect of immunooablation and ASCT on the CD4⁺ T-cell compartment, we first used the phenotypic markers CD45RA and CD45RO to

![Figure 1. Recovery of CD4⁺ T-cell subsets over time in SLE patients treated by immunooablation and ASCT versus levels in age-matched healthy controls.](image-url)
discriminate between (CD4RA− CD4RO−) naive and (CD4RA+ CD4RO−) memory CD4+ T cells.

The longitudinal analysis of reconstituting naive and memory CD4+ T cells is shown in Figure 1. At baseline, patients displayed a significant CD4+ T-cell lymphopenia compared with age-matched healthy controls, which was attributable to both CD4RA+ naive (median, 35/μL vs 288/μL, P < .001) and CD4RO+ memory CD4+ T cells (median, 51/μL vs 433/μL, P < .001), reflecting the disturbed T-cell homeostasis of active SLE (Figure 1B). In the regenerative phase, the memory phenotype (CD4RO−CD4RA−) was the predominant CD4+ T-cell subset; there was a significant increase in this subpopulation at 6 months after treatment compared with baseline (median, 73.4% vs 53.3%, P = .116; Figure 1A) with a doubling of absolute counts (median, 121/μL vs 51/μL, P = .027; Figure 1B). Naive CD4+ CD4RA+ T-cell counts were low or undeterminable at that time but later increased continuously, reaching complete recovery 24 months after ASCT with significant higher values than before ASCT (median, 244/μL vs 36/μL, 164/μL vs 29/μL, P = .014; Figure 1B). CD4RO+ Th counts (Figure 1B) remained significantly diminished until the 4-year follow-up.

**Increased output of RTEs**

To determine whether CD4RA+ CD4RO− CD4+ T cells in the regenerated immune system were homeostatically expanded peripheral T cells or naive T cells newly generated in the thymus, we analyzed their expression of CD31, a surrogate marker of recent thymic emigrants (RTEs).18 Six months after ASCT, 85.2% to 98.8% (median, 89.7%) of the CD4RA+ T cells in responding patients coexpressed CD31; this was significantly more than before ASCT in the same patients (median, 75.3%, P = .049) and in age-matched healthy controls (median, 64.2%, P < .001; Figure 1C). CD31 expression in CD4RA+ CD4+ T cells of the responding patients remained at these high frequencies during the entire observation period. Recovery of this T-cell subset to numbers comparable with healthy controls was completed between 12 and 24 months after ASCT (Figure 1D), reaching on average 5.2 to 12.1 times the baseline levels. Remarkably, the number of recent thymic emigrants continued to increase in responding patients; not before 36 months after ASCT, they reached a plateau at twice the level observed in age-matched healthy controls (median, 435/μL vs 164/μL, P = .016), and at 15 times the baseline levels (median, 435/μL vs 29/μL, P = .031). The number of RTEs decreased only in the patient who suffered a relapse 18 months after transplantation.

**The thymus contributes to the regeneration of FoxP3+ regulatory T cells**

Regeneration of the CD4+ T-cell (Treg) compartment was evaluated after ASCT by identifying peripheral blood CD4+ T cells containing brightly for CD25 and expressing intracellular FoxP3 (Figure 2A). Conventionally treated patients with active SLE had significantly lower frequencies of peripheral FoxP3+ Tregs than normal controls (median, 5.5% vs 8.0%, P = .001), as illustrated in Figure 2B. Those with inactive SLE had comparable, if not higher, frequencies of FoxP3+ CD4+ T cells than the controls (median, 10.5% vs 8.0%, P = .048). FoxP3+ CD4+ T-cell frequencies in regenerated immunonaive ASCT patients at time points from 2 to 7 years after ASCT (as depicted in Figure 2A) were as high as those in normal controls (median, 9.4% vs 8.0%, P = .229; Figure 2B). Overall, the absolute numbers of FoxP3+ CD4+ T cells were similar in both groups (median, 62.8/μL vs 67.2/μL, P = .963; Figure 2C). However, the patients were heterogeneous with respect to numbers of FoxP3+ CD4+ T cells. The later the follow-up date, the higher the patients’ peripheral FoxP3+ CD4+ T-cell count (161.8/μL in patient 1 at 7 years) and the lower the count in patients analyzed at earlier time points after ASCT (31.1/μL in patient 7 at 2 years).

**Thymic output generates a new and diverse TCR repertoire**

CD4+ T-cell diversity in the patients’ regenerating immune systems was analyzed with a panel of TCR Vβ-specific monoclonal antibodies by flow cytometry, as recently described.19 At baseline, all patients analyzed (n = 4) showed significantly expanded TCR Vβ-expressing CD4+ T cells (Table 3) in line with previous findings on restricted TCR repertoires in active SLE.20 Early after ASCT (+3 months), CD4+ T cells in these patients still exhibited a highly restricted TCR repertoire, however, with a different TCR Vβ family usage profile (Table 3). At the time point of assessment, patients showed no clinical signs of active infection or lupus flare except for 1 patient with an acute systemic herpes infection (HHV-6, patient 4). However, all patients contracted frequent infections during the period of neutropenia shortly after ASCT. Along with the emergence of thymic naive CD31+ T cells, the CD4+ TCR repertoire gradually normalized within 1 year after ASCT (Table 3). Except for 2 patients showing transient TCR Vβ
expansion (patient 4, Vβ5.1 at 4 years and patient 6, Vβ12 at 2 years) after ASCT, the TCR Vβ profiles of CD4+ T cells remained stable and heterogeneous throughout follow-up. At the 3-year follow-up, the regenerated CD4+ T-cell TCR Vβ family usage was normal in all patients (Figure 3).

**Early expansion of memory CD4+ T cells is not driven by autoantigens**

The specificity of CD4+ memory T cells was analyzed by ex vivo short-term restimulation of whole blood with viral antigens and autoantigens, and subsequent enumeration of reactivated T cells expressing CD69 and IFN-γ (Th1 memory cells) or CD154 (all memory Th cells) during the early phase of immune reconstitution.15,16,21 Notably, these ex vivo restimulation assays were performed in patients with identified viral infections based on clinical symptoms and corresponding serologic findings (patient 1: varicella-zoster virus, patient 4: human herpes virus 6, patient 6: CMV reactivation, patient 7: herpes simplex virus 1). During viral infections, Th effector memory cells specific for VZV (patient 1) and CMV (patient 6) were readily detectable as CD69+ or CD154+ CD4+ T cells coexpressing IFN-γ. Inversely, T cells reacting to nucleosomes or SmD1 were not detectable early after ASCT (Figure 4).

### Table 3. Significantly expanded TCR Vβ-expressing CD4+ T cells at baseline and during follow-up after ASCT in patients 4 to 7

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— indicates not applicable.

*Significant expansions according to criteria described in “Methods.”

Figure 3. CD4 TCR Vβ repertoires in 5 patients after ASCT. TCR Vβ family usage in peripheral blood CD4+ T cells (%) in 5 patients 3 years after treatment. ■ represent median values in 20 healthy donors; boundaries indicate the 2.5th and 97.5th percentiles.
Normalization of disturbed B-cell homeostasis after ASCT

Active SLE is characterized by marked B lymphocytopenia, which reportedly affects CD27- naive B cells more than CD27+ memory B cells. A prominent population of peripheral plasma blasts has also been observed in active disease. To evaluate the effect of immunoablation and ASCT on these B-cell disturbances, we analyzed peripheral blood B lymphocytes from regenerating immune systems for IgD, CD27, and CD20 expression.

Before treatment, patients had significantly lower numbers of IgD+ naive B cells than normal controls (median, 4/μL vs 202/μL, \( P < .001 \); Figure 5) as well as a predominance of IgD- memory B lymphocytes (median, 67.2% vs 23.5%, \( P = .003 \)) and a prominent population of CD27high CD20+ plasma blasts (median, 10.3% vs 0.9%, \( P = .006 \)) in peripheral blood. After ASCT, B lymphocytes predominantly displayed a naive IgD+ phenotype. Complete numeric recovery of this subset was observed by 12 months after ASCT, with counts 50 times higher than at baseline (median,
196/μL vs 4/μL, P = .024). Absolute naive B-cell counts in responders were well maintained throughout follow-up.

IgD memory-phenotype B-cell frequencies drastically declined from a median of 67.2% at baseline to 7.0% within 6 months after ASCT (P = .002). During immune regeneration, IgD− B-cell frequencies remained lower than in healthy controls over the entire follow-up period of up to 8 years (Figure 5A). IgD memory B-cell counts did not recover until 3 years after ASCT (Figure 5B). IgD memory B lymphocytes did not detectably expand before that time, except in the patient with the lupus flare (Figure 5A). CD20−CD27high plasma blast frequencies among CD19+ B cells normalized within 6 months after ASCT in all patients analyzed, and normal levels persisted during the entire follow-up period (Figure 5C).

Autoreactive and protective antibodies in serum are largely extinguished after ASCT

All patients had ANAs and persistently high anti–double-stranded (anti-ds) DNA serum antibody titers before enrollment. After immunoablation and ASCT, anti-dsDNA antibodies disappeared in all patients within 1 month (Table 4) and recurred only in the patient with reactivated disease (patient 3). Four of 6 patients with a follow-up of at least 6 months after transplantation showed a decrease in ANA titers to negative or 1:80, which is regarded as clinically not significant (Table 4). From these 4 patients, only 1 (patient 4) showed relevant ANA recurrence, which persisted from the 3-year follow-up onward without clinical symptoms of SLE. In 2 patients, ANA persisted, albeit in significantly reduced titers.

Not only autoantibodies but also protective serum antibodies specific for measles, mumps, tetanus, and diphtheria disappeared in the immunoablated patients. All patients had received the World Health Organization-recommended vaccination before enrollment. Even though not all patients had reached protective levels of vaccine-specific antibodies before immunoablation, significant decreases in serum antibody levels for measles (P = .043, Figure 6A), mumps (P = .028, Figure 6B), tetanus toxoid (P = .048, Figure 6C), and diphtheria (P = .049, Figure 6D) were observed when tested 1 or 2 years after ASCT (Figure 6). The serologic data point to an effective depletion of long-lived plasma cells from the bone marrow.5,26 A bone marrow aspiration sample from 1 patient (patient 7), obtained early after ASCT (1 month), exhibited almost complete depletion of CD38−CD138+ plasma cells with

### Table 4. Titer of ANAs and anti–dsDNA antibodies before and after ASCT

<table>
<thead>
<tr>
<th>Patient no./antibodies</th>
<th>Before transplantation</th>
<th>After ASCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 month</td>
<td>6 months</td>
</tr>
<tr>
<td>1 ANA 5120</td>
<td>320</td>
<td>Negative</td>
</tr>
<tr>
<td>dsDNA 8</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>2 ANA 5120</td>
<td>320</td>
<td>80</td>
</tr>
<tr>
<td>dsDNA 64</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>3 ANA 2560</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>dsDNA 64</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>4 ANA 20480</td>
<td>640</td>
<td>Negative</td>
</tr>
<tr>
<td>dsDNA 64</td>
<td>Negative</td>
<td>—</td>
</tr>
<tr>
<td>5 ANA 2560</td>
<td>320</td>
<td>—</td>
</tr>
<tr>
<td>dsDNA 32</td>
<td>Negative</td>
<td>—</td>
</tr>
<tr>
<td>6 ANA 10240</td>
<td>2560</td>
<td>2560</td>
</tr>
<tr>
<td>dsDNA 80</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7 ANA 10240</td>
<td>1280</td>
<td>1280</td>
</tr>
<tr>
<td>dsDNA 80</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

ANA indicates antinuclear antibodies, inverse titer; dsDNA, anti–double-stranded DNA antibodies (C luciliae assay), inverse titer; and —, not applicable.
only 0.03% among BM-MNC compared with a normal control bone marrow with 1.24% of such cells (Figure 5D).

Discussion

Immunoablation followed by ASCT is an emerging treatment option for patients with severe autoimmune diseases refractory to conventional therapies, including SLE.\(^7^,\)\(^10^,\)\(^27^,\)\(^28^\) In accordance with previous reports,\(^7^,\)\(^28^\) this regimen achieved long-term (up to 8 years) clinical and serologic treatment-free remissions in our lupus patients. Notably, these patients originally had high disease activity and a poor prognosis, reflected by high SLEDAL scores and persistent anti-dsDNA antibody titers. Although the clinical efficacy of this experimental therapy is rapidly becoming evident, it remains obscure how it works. Our detailed analysis of the long-term reconstitution of the patients’ immune systems with respect to the recurrence of T- and B-lymphocyte subsets and the course of serologic changes over time demonstrated successful depletion of autoreactive immunologic memory and the regeneration of a tolerant immune system from hematopoietic stem cells. Regeneration involved reactivation of the thymus and extensive renewal of antigen-receptors, in other words, a “resetting of the immunologic clock.”

Depletion of autoreactive immunologic memory by immunoablation was most drastically reflected in the complete disappearance of autoantibodies, particularly dsDNA-specific antibodies. Depletion of immunologic memory was not restricted to autoreactive memory. In addition, pathogen-specific serum antibodies for mumps, measles, tetanus, and diphtheria were largely extinguished. This drastic ablation of humoral memory suggests that the ATG used for immunoablation directly targets the plasma cells secreting these serum antibodies. It was recently shown that the plasma cells providing humoral memory are long-lived cells that reside mostly in the bone marrow, where they dwell in specialized niches providing essential survival signals.\(^3\) Recent in vitro experiments indicated that polyclonal rabbit ATG directly targets plasma cells and B cells via complement-mediated lysis and apoptosis.\(^29^,\)\(^30^\) In line with this hypothesis, we were able to stain plasma cells ex vivo with the polyclonal rabbit ATG used for immunoablation (data not shown); moreover, plasma cells disappeared from bone marrow 1 month after immunoablation in 1 case. The depletion of long-lived plasma cells might be of particular relevance for the success of immunoablative therapy. It has been demonstrated that these plasma cells are resistant to immunosuppression by cyclophosphamide, irradiation, and CD20-mediated B-cell depletion.\(^43^,\)\(^51^\) Hence, autoreactive long-lived plasma cells represent a key component of autoreactive immunologic memory. Persistent autoantibodies secreted by long-lived plasma cells could maintain chronic inflammation and accelerate autoimmunity.\(^32^\) A retrospective survey by the European Blood and Marrow Transplant and European League Against Rheumatism Registry revealed that patients without complete loss of autoantibody responses after immunoablation and ASCT had higher rates of relapse.\(^28\) In our cohort, the only patient who relapsed became anti-dsDNA-negative after immunoablation, but anti-Ro/SSA and anti-La/SSB antibodies persisted until the relapse. From the other patients, only 1 had anti-Ro/SSA antibodies before enrollment (patient 6). Similar to the patient with the relapse, anti-Ro/SSA antibodies persisted in this patient after ASCT, albeit without evidence for SLE reactivation. So far, it is not clear why plasma cells secreting these autoantibodies seem to be more resistant to the immunoablative regimen and if their persistence characterizes patients with a higher risk for relapse.

T-cell reconstitution after immunoablation was characterized by continued generation of new naïve CD4\(^+\) T cells for up to 8 years after ASCT. In particular, naïve CD45RA\(^+\)CD44\(^-\) T cells expressing CD31 with high overall clonal diversity of the CD4\(^+\) TCR repertoire were generated. These cells have been shown to be recent thymic emigrants.\(^18\) In the regenerated patients, absolute CD45RA\(^+\)CD31\(^+\) naïve CD4\(^+\) T-cell counts continuously increased to levels twice as high as those in age-matched controls, resembling those in young children. This observation supports the notion that, after immunoablation and ASCT, the naïve CD4\(^+\) T-cell compartment is regenerated by thymic reactivation rather than by lymphopenic expansion of surviving naïve T cells, emphasized earlier for patients undergoing immunoablation and ASCT for treatment of hematologic malignancies\(^33\) and multiple sclerosis.\(^34\) In our SLE patients, the finding is even more relevant in light of the disease- and treatment-related impairment of the naïve T-cell compartment, which has been attributed to intrinsic impairment of thymic export.\(^35\) Immunoablation and ASCT obviously can correct this deficiency, rejuvenate the CD4\(^+\) T-cell compartment, and normalize naïve T-cell homeostasis.

After monitoring the TCR V\(\beta\) family repertoire of the recurring CD4\(^+\) T-cell compartment, we observed a drastic change in clonal diversity of the TCR repertoire. The originally observed clonal expansions and deletions disappeared, suggesting that treatment had led to the ablation of expanded clones and to the generation of a complete repertoire of recent thymic emigrants.\(^36\)

Among CD4\(^+\) T cells, FoxP3\(^+\) regulatory T cells regenerated to frequencies and absolute numbers comparable with those in normal controls. The fact that regeneration of the Treg compartment was accompanied by the reappearance of naïve T cells and recent thymic emigrants suggests that these regulatory T cells were generated in the thymus. Similar observations have been made in patients undergoing immunoablation and ASCT for juvenile idiopathic arthritis, suggesting a common mechanism of action of stem cell transplantation in different autoimmune diseases.\(^37\)

Whereas regeneration of thymic naïve Th cells was delayed for up to 1 year after ASCT, mature CD45RO\(^+\) memory CD4\(^+\) T cells reappeared faster with on average a doubling of absolute counts at 6 months after transplantation compared with baseline values. However, their TCR V\(\beta\) repertoires were highly restricted, reflecting responses to a limited array of available antigens during lymphopenia.\(^38^,\)\(^39\) If peripheral T-cell expansion had involved lymphopenia-driven proliferation of memory T cells in response to low-affinity self-antigens, expansion of autoreactive T-cell clones should have been observed.\(^40\) However, we found no evidence of clonal expansion of autoreactive T cells specific for SLE-associated autoantigens, such as nucleosomes or SmD1. This implies that the early expansion of memory CD4\(^+\) T cells is not driven by autoantigens and, in particular, not by those involved in SLE. Rather, we showed that clonally expanded memory T cells reacted to virus-specific antigens in patients infected with specific viruses. This implicates protective pathogen-specific immune responses as a cause of clonal expansion of memory-phenotype T cells. The expansion of protective pathogen-specific T cells in response to treatment may contribute to the control of autoimmunity by
restricting the space available in the effector-memory compartment for autoreactive T cells, the expansion of which is driven by (weak) reactions to autoantigens.

Regeneration of the B lymphocyte compartment in the treated SLE patients resembled that of patients receiving ASCT for treatment of hematologic malignancies. The majority of repopulating B cells initially showed a naive (IgD+) phenotype. Memory (IgD−) B cells did not reappear until later. In the present study, this regeneration of the B-cell compartment was remarkable in view of the significant disturbances observed in our active SLE cohort before ASCT. These patients had shown naive B-cell lymphopenia, relative predominance of phenotypically memory B cells, and expansion of CD27(high) CD20− plasma cell precursors. The complete normalization of these preexisting disturbances indicates that immunoablation had removed all autoreactive B cells. Apparently, the B-cell compartment also regenerates from stem cells after immunoablation and ASCT, and it is tolerant to self-antigens, including those that had been relevant in the patients before treatment.

In conclusion, this study provides direct evidence for a profound regeneration of the adaptive immune system in SLE patients after immunoablation and ASCT. All patients except 1 achieved long-lasting clinical and serologic remissions and are no longer reliant on immunosuppressive therapy. The 1 exception relapsed after having been in clinical remission for more than a year. The relapse might be the result of insufficient ablation of autoreactive immunologic memory, presentation of tolerance-breaking autoantigen forms to the regenerated immune system, or genetic predispositions that restart the disease in the regenerated immune system. Our findings would propose that chronic autoimmune is not an endpoint depending on continuous treatment with specific anti-inflammatory agents but may be cured by combining specific targeting of autoreactive memory and effector cells with a reactivation of thymic activity.

Acknowledgments

This work was supported by grants from the Bundesministerium fur Bildung und Forschung (01GI9944/DRFZ C4.1) and the Sonderforschungsbereiche (SFB) 650 TP12.

Authorship


Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through de novo generation of a juvenile and tolerant immune system

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