Paediatric Rheumatology Workshop/Series Editor: P. Woo

Haematopoietic stem cell transplantation for active systemic lupus erythematosus

A. Traynor and R. K. Burt

Northwestern University School of Medicine, Division of Hematology Oncology and the Robert H. Lurie Cancer Center, 250 East Superior, Wesley Pavilion, Room 1456, Chicago, IL 60611, USA

Abstract

Objective. For patients with systemic lupus erythematosus (SLE) who are at risk of disease-related mortality, we have initiated a protocol of intensive immunosuppression and haematopoietic stem cell support. The first patient enrolled in this study was in the midst of a lupus flare manifest by nephritis and rapidly declining renal function, uncontrolled hypertension, immune-mediated cytopenias, and serositis characterized by a large pericardial effusion and abdominal pain. Antinuclear antibody (ANA), anti-double-stranded (ds) DNA and complement were abnormal. This patient is now more than 1 yr post-stem cell transplant and is taking no immunosuppressive medication. Her serologies are normal, effusions have resolved, blood pressure is normal and renal function is markedly improved. The clinical and serological course of this patient is summarized here.

Methods. Autologous haematopoietic stem cells (HSC) were mobilized with cyclophosphamide (2.0 g/m2) and granulocyte colony-stimulating factor (G-CSF) (10 µg/kg/day). Stem cells were enriched ex vivo using CD34-positive immunoselection and reinfused after immunosuppression with cyclophosphamide (200 mg/kg) and antithymocyte globulin (ATG) (90 mg/kg).

Results. White blood cell engraftment with an absolute neutrophil count (ANC) of >500/µl (0.5 × 109/l) and platelet engraftment with a non-transfused platelet count of >20 000/µl (20 × 109/l) occurred on day 10 and 14, respectively. Therapy was complicated by a cell lysis-like effect with hyperphosphataemia, hyperuricaemia, normal anion gap metabolic acidosis and transient exacerbation of renal insufficiency.

Conclusion. This is the first autologous T-cell-depleted haematopoietic stem cell transplantation performed to treat lupus in an active flare. This patient has, for the first time since disease onset (13 yr ago), entered a complete clinical and serological remission which persists at >1 yr of follow-up. The durability of this remission is unknown.

Key words: Haematopoietic stem cell transplantation, Autologous, Systemic lupus erythematosus.

Accepted 15 March 1999.
Correspondence to: A. Traynor.
Methods

Patient selection

Eligibility criteria were approved by the Northwestern University Investigational Review Board and by the US Food and Drug Administration, and required that patients be <55 yr of age at the time of pre-transplant evaluation, have a clinical diagnosis of SLE, and have at least one of the following adverse prognostic features.

1. Biopsy-proven WHO class II, III or IV glomerulonephritis which has failed NIH short-course cyclophosphamide therapy (500–1000 mg/m² monthly for at least 6 months), with treatment failure defined as failure of serum creatinine to return to normal or pre-exacerbation level.

2. Vasculitis and/or immune complex deposition causing end-organ signs or symptoms, that are life threatening or organ threatening, e.g. cerebritis, transverse myelitis, pulmonary haemorrhage, or cardiac failure that remains active despite corticosteroid and cyclophosphamide therapy.

3. Transfusion-dependent cytopenias that are immune mediated and not controlled with danazol, prednisone and at least one alkylating agent.

4. Catastrophic antiphospholipid syndrome, defined as an antiphospholipid titre >5 s.p. above the mean and two or more antiphospholipid-related manifestations including either severe cytopenias that have failed corticosteroid therapy or vascular occlusions that have failed anticoagulant therapy.

5. Serositis, associated with shrinking lung syndrome or cardiac compromise, which has failed to remit with corticosteroid and cyclophosphamide therapy.

Stem cell procurement (Table 1a)

Haematopoietic stem cells are mobilized with cyclophosphamide and granulocyte colony-stimulating factor (G-CSF) (Amgen, Thousand Oaks, CA, USA).

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>2.0 g/m²</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Rest X X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-CSF*</td>
<td>10.0 µg/kg/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukapheresis</td>
<td>10–20 l leukapheresis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*G-CSF is given s.c. daily until leukapheresis is completed.

(b) Conditioning regimen

<table>
<thead>
<tr>
<th>Day</th>
<th>−6</th>
<th>−5</th>
<th>−4</th>
<th>−3</th>
<th>−2</th>
<th>−1</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide (mg/kg)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antithymocyte globulin (mg/kg)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Rest X X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem cell reinfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
her diagnosis, her haemoglobin generally had ranged from 6.5 to 7.2 mg/dl (4.03–4.46 mmol/l), her WBC was 2000–2400/μl (2–2.4 × 10⁹/l) and the platelet count had remained at ~100 000/μl (100 × 10⁹/l). Serum C3, antinuclear antibody (ANA) and anti-double-stranded (ds) DNA never achieved normal values, even during periods of clinical remission. At 14 yr of age, the patient had been hospitalized with pneumonitis, nephritis and bleeding secondary to immune-mediated thrombocytopenia. She required dialysis and mechanical ventilation, and was treated with plasmapheresis, corticosteroids and cyclophosphamide. Since diagnosis, active disease had prevented 50%. The electrolyte abnormalities gradually corrected.

Abdominal pain and several episodes of lupus nephritis, abdominal pain and tenderness resolved. Reimaging of the pericardial effusion showed a decrease of roughly 50%. The electrolyte abnormalities gradually corrected. Ten days after cyclophosphamide infusion, the WBC rose to >1000/μl (1.0 × 10⁹/l). Beginning that day, a 20 l leukapheresis was performed daily for 3 consecutive days. The collected peripheral blood stem cells (PBSC) contained 10⁸ T cells/kg patient weight. After CD34⁺ cell selection, the number of T cells was diminished by almost two logs (3.1 × 10⁶ T cells/kg). The patient was discharged home on day 14.

After a 6 week interval, the serum creatinine stabilized at 2.5 mg/dl (221 μmol/l). The patient was readmitted and high-dose cyclophosphamide (200 mg/kg over 4 days) and ATG (90 mg/kg over 3 days) were administered followed by a 3 day rest before reinfusion of CD34⁺-enriched stem cells. G-CSF (5 μg/kg/day) was begun the following day. Following cyclophosphamide infusion, the patient again experienced hyperphosphataemia, hyperuricaemia, normal anion gap metabolic acidosis, and a rising serum creatinine despite prophylaxis with allopurinol and alkaline hydration. Owing to hyperphosphataemia, dialysis was performed on two occasions 8 days apart. Neutrophil recovery, defined as an absolute neutrophil count >500/μl (0.5 × 10⁹/l), occurred on day 10. The platelet count rose to >20 000/μl (20 × 10⁹/l) without transfusion by day 14. During the period of neutropenia, low-grade fever (100.5°F) developed. Blood cultures grew Staphylococcus epidermidis. The empirical broad-spectrum antibiotic coverage begun for neutropenic fever included piperacillin/tazobactam, ciprofloxacin, vancomycin and lipid-formulation amphotericin. The patient was discharged home on day 14.

Laboratory values by day 60 after stem cell reinfusion had improved or normalized (Table 2), and the patient had no evidence of active disease with an SLE Disease Activity Index (SLEDAI) score of zero. The rash, arthralgias, abdominal pain, and pericardial and pleural effusions fully resolved. At 1 yr follow-up, this patient remained in clinical remission (Table 2). She is no longer receiving immunosuppressive or antihypertensive medications. Her only post-treatment infectious complication was reactivation of herpes zoster which resolved with acyclovir.

Fig. 1. Percutaneous kidney biopsy prior to protocol enrolment. Diffuse proliferative glomerulonephritis with evidence of both acute inflammatory infiltrate and fibrotic scarring.
Table 2. Clinical outcome after haematopoietic stem cell transplantation for systemic lupus erythematosus. Patient SLE #1

<table>
<thead>
<tr>
<th></th>
<th>Pre-transplant</th>
<th>2 months post-transplant</th>
<th>6 months post-transplant</th>
<th>12 months post-transplant</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEDAI score</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haemoglobin (mg/dl)</td>
<td>6.5</td>
<td>10.0</td>
<td>12.3</td>
<td>12.4</td>
</tr>
<tr>
<td>White blood cells/µl</td>
<td>2000</td>
<td>6000</td>
<td>6100</td>
<td>6100</td>
</tr>
<tr>
<td>Platelets/µl</td>
<td>100,000</td>
<td>240,000</td>
<td>215,000</td>
<td>235,000</td>
</tr>
<tr>
<td>24 urine protein (g)</td>
<td>3.6</td>
<td>0.6</td>
<td>0.87</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>5.0</td>
<td>2.6</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>C3 (68–164 mg/dl)</td>
<td>52</td>
<td>100</td>
<td>114</td>
<td>113</td>
</tr>
<tr>
<td>C4 (7–47 mg/dl)</td>
<td>6</td>
<td>40</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>ANA</td>
<td>1:320</td>
<td>1:40</td>
<td>1:40</td>
<td>1:80</td>
</tr>
<tr>
<td>Anti-ds DNA</td>
<td>1:160</td>
<td>1:60</td>
<td>1:60</td>
<td>1:60</td>
</tr>
</tbody>
</table>

SLE, systemic lupus erythematosus; SLEDAI, SLE Disease Activity Index. The SLEDAI is a measure of activity or inflammation in nine organ systems. It has a theoretical maximum of 105 points. A normal person has a SLEDAI of zero.

Discussion

The immunosuppressive regimen used for this approach is based on a standard conditioning regimen in use for allogeneic transplantation of aplastic anaemia. The pathogenesis of aplastic anaemia is often due to immune-mediated suppression of haematopoiesis and may be treated with either immunosuppression or allogeneic transplantation. Some patients with aplastic anaemia undergoing allogeneic transplantation have rejected their sibling’s graft and subsequently reconstituted normal endogenous haematopoiesis. This suggests that maximal suppression of an autoimmune disease even without a source of new stem cells may cure the disease. It also highlights that this regimen is not myeloablative. The reason why CD34+ stem cells are reinfused is to shorten the duration of severe neutropenia and thrombocytopenia in order to decrease the risk of serious infection and bleeding.

Mobilization of stem cells and immune ablation with high-dose cyclophosphamide was relatively uncomplicated. The patient did not develop a fever or require antibiotics during mobilization. Subsequent G-CSF did not exacerbate the manifestations of lupus. In fact, within days of undergoing mobilization with cyclophosphamide, and while on G-CSF, clinical symptoms subsided. A cell lysis-like effect did occur following each treatment with cyclophosphamide. Renal insufficiency is usually a contraindication to stem cell transplantation for haematological malignancies and electrolyte evidence of cell lysis in patients with haematological diseases who are in remission at the time of transplantation is unusual. In contrast, renal involvement is common in SLE and is one of our criteria for transplantation. In these patients, high-dose chemotherapy may cause a cell lysis effect and the possibility of early dialysis should be anticipated.

Another potential complication anticipated in this patient was an increased risk of fungal infections from chronic steroid dependence. We therefore started a renal-sparing lipid formulation of amphotericin with the first fever. Since lupus may include central nervous system vasculitis, and since renal involvement may be associated with hypertension, there is an increased risk of a serious intracranial bleed during the interval of thrombocytopenia. This potential complication was avoided by control of the blood pressure and prophylactic transfusion of platelets.

In this patient, high-dose cyclophosphamide and ATG resulted in clinical and serological remission of active and refractory disease for the first time since its onset 13 yr ago. Specifically, this is the first time since disease onset that complement levels are normal and anti-ds DNA is negative. Interestingly, the patient’s hypertension, which had been difficult to control, requiring four different antihypertensive drugs, gradually subsided. Although remission has persisted for longer than 1 yr, response durability is unknown. The relapsing and remitting nature of lupus makes definition of a complete remission difficult, and no agreed upon medical definition for a complete remission exists in SLE. We cannot, consequently, document ‘complete remission’. However, there has been no evidence of active disease since transplantation and anti-ds DNA and complement have returned to normal. Furthermore, since the mechanism(s) of transplant-associated remission remains unclear, it is possible that a similar long-term outcome would occur without reinfusion of haematopoietic stem cells.

Based on experience with T-cell-depleted autologous haematopoietic transplantation in patients with malignancy, the redeveloping immune system can be expected to have an immunosuppressed phenotype with diminished responses to mitogens and a low CD4+ cell count for ~12 months following transplantation [14, 15]. Recovery of immunity may be due to pre-thymic CD34+ progenitor cell differentiation resulting in recapitulation of lymphocyte ontogeny. Alternatively, immunity may arise by peripheral expansion of post-thymic T lymphocytes that either survived conditioning or were reinfused with the graft [16]. Pre-thymic vs post-thymic derivation...
of T cells following transplantation may be important for remission duration. Predominance of the post-thymic pathway may allow for expansion of pre-existing disease-mediating lymphocytes. However, it also remains uncertain whether immunity re-emerging from a pre-thymic pathway will result in self-tolerance.

Purging the graft of lymphocytes by CD34+ selection may increase the likelihood that post-transplant immunity will arise from pre-thymic differentiation from CD34+ stem cells. When compared to non-selected grafts, CD34-enriched grafts have a more prolonged T-cell recovery interval [17]. The CD4/CD8 ratio is inverted (<1.0) for up to 1 yr after autografting. When compared to an unselected graft, CD34-selected cells have an absolute number of CD4+ T-helper cells that remains significantly lower at 1 yr. For the first 2 months after transplantation, the CD4+ T-cell population consisted predominantly of CD45RO+ helper/memory cells. It remains unclear whether the helper memory (CD4/CD45RO+) lymphocytes survived the conditioning regimen or were reinfused with the graft. By 3 months after transplantation, the absolute number of CD4/CD45RA+ (helper/naive) cells begins to increase. It is also unknown whether CD45RA+ naive lymphocytes arise by pre-thymic maturation from CD34+ haematopoietic progenitor cells or by expansion of pre-existing or reinfused post-thymic CD45RA+ cells.

In haematological diseases, the T-cell receptor (TcR) repertoire after CD34-selected haematopoietic transplantation demonstrates diminished diversity of Vβ TcR expression [17]. Following transplantation for haematological diseases, significant post-transplant expansion of specific TcR populations was observed in all patients transplanted with a CD34-enriched graft at one institution, showing an apparently random overexpression of various Vβ TcR subsets, beginning as early as 3 weeks and persisting as late as 1 yr after bone marrow transplantation (BMT) [17]. A comparison to pre-transplant TcR subsets showed that while several Vβ TcR subsets which had been present in their patient population at high percentages before BMT were present at high frequencies a year following BMT, the pattern of TcR repertoire was not identical to the pre-transplant pattern. Recipients of T-cell-depleted haematopoietic cells have previously been shown to have an initial increase in the number of circulating natural killer (NK) cells, and a delay in the recovery of normal numbers of B cells. This also occurs after non-selected haematopoietic stem cell infusion [18, 19]. After transplantation, as B-cell numbers return to normal, a greater percentage of CD5+ B lymphocytes occurs in the CD34-selected graft recipients.

These observations in patients with haematological diseases are of interest in light of what is known regarding lymphocyte populations which characterize systemic lupus. The production of pathogenic anti-DNA autoantibodies in SLE is promoted by autoimmune T-helper cells [20]. These cells display a recurrent motif of highly charged residues in their CDR3 loops, and several independent T-helper lines derived from lupus patients have shown identical TcR α and β chain sequences. These autoimmune T-helper cells of lupus patients have been shown to respond to charged epitopes in various DNA-binding nucleoproteins [21]. Defective suppressor function may contribute to this autoreactive T-helper cell population. In human peripheral blood mononuclear cells, NK-derived transforming growth factor beta (TGF-β) induces suppressor activity in CD8+ cells in conjunction with interleukin (IL)-2 [22]. Gray et al. [23] found that the NK cells of SLE patients are defective in their production of active TGF-β and that the normal T-cell autoregulatory circuit in which CD8+ cells limit CD4+ cell expansion and activation is faulty. Evidence that a CD34-selected graft favours reconstitution of the Vβ repertoire by naive cells and that NK cells are increased in the early post-transplant period indicates that immune ablation followed by stem cell transplantation may promote an early ‘tolerogenic environment’.

Summary

Several centres in Europe and America are initiating haematopoietic transplantation protocols for autoimmune diseases. We are aware of one other centre that has performed stem cell transplantation in a patient with lupus which was quiescent at the time of transplant [24]. Enthusiasm for this approach is slowed by recognition that autoimmune diseases have diverse clinical progressions, which may include an isolated episode without residual damage, an indolent progression with or without significant disability, or a rapid progression with early mortality. Intensive immune suppression and stem cell transplantation has significant potential for infectious and regimen-related side-effects and should, therefore, be reserved for patients with severe disease who have failed conventional therapy.

References