Articles

Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study

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Summary

Background Patients with systemic lupus erythematosus (SLE) who experience persistent multiorgan dysfunction, despite standard doses of intravenous cyclophosphamide, represent a subset of patients at high risk of early death. We investigated the safety and efficacy of immune suppression and autologous haemopoietic stem-cell infusion to treat such patients.

Methods From 1996, we selected patients with persistent SLE despite use of cyclophosphamide. Patients underwent dose-intense immune suppression and autologous haemopoietic stem-cell (CD34) infusion. Peripheral blood lymphocytes were analysed by flow cytometry, ELISA, and T-cell-receptor spectratyping before and after transplantation. We mobilised autologous haemopoietic stem cells with 2·0 g/m^2 cyclophosphamide and 10 μg/kg granulocyte colony stimulating factor daily, enriched with CD34-positive selection, and reinfused after immunosuppression with 200 mg/kg cyclophosphamide, 1 g methylprednisolone, and 90 mg/kg equine antithymocyte globulin.

Results Nine patients underwent stem-cell mobilisation but two were excluded before transplantation because of infection. The remaining seven received high-dose chemotherapy and stem-cell infusion. Median time to an absolute neutrophil count higher than 0·5×10^9/L and non-transfused platelet count higher than 20×10^9/L was 9 days (range 8–11) and 11 days (10–13), respectively. At a median follow-up of 25 months (12–40), all patients were free from signs of active lupus. Renal, cardiac, pulmonary, and serological markers, and T-cell phenotype and repertoire had normalised.

Interpretation Patients remained free from active lupus and improved continuously after tranplantation, with no immunosuppressive medication or small residual doses of prednisone. T-cell repertoire diversity and responsiveness was restored. Durability of remission remains to be established.

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Introduction

After introduction of intravenous cyclophosphamide and improved antihypertensive therapy, lupus-related mortality declined in the late 20th century.[1] Mortality does, however, remain at about 1% per year.[2–5] Although the pathogenesis of lupus is unknown, it is associated with various immune-mediated abnormalities involving T lymphocytes.[6–9] Dose intensification of cyclophosphamide, to the point of marrow suppression, with concurrent use of antithymocyte globulin, is intended to provide a window of time free of memory-T-cell influence, during which the maturation of new lymphocyte progenitors may occur without recruitment to anti-self reactivity.

The mechanism of remission induced by autologous stem-cell transplantation might be only transient dose-intense immune suppression. Alternatively, this treatment might fundamentally alter the immune system after transplant. We investigated the efficacy of this treatment in patients with persistent active systemic lupus erythematosus (SLE) despite treatment wth intravenous cyclophosphamide.

Methods

Patients

We enrolled patients who presented at Northwestern Memorial Hospital, Chicago, IL, USA. Eligible patients had WHO class III–IV glomerulonephritis unresponsive to at least six cycles of intravenous cyclophosphamide; lupus cerebritis or transverse myelitis unresponsive to at least six cycles of intravenous cyclophosphamide; lupus vasculitis, involving the heart or lung parenchyma, unresponsive to at least six cycles of intravenous cyclophosphamide; lupus-associated, life-threatening, severe haematological cytopenias unresponsive to standard-dose cyclophosphamide; or catastrophic antiphospholipid syndrome.

The protocol was approved as a US Food and Drugs Administration study under IDE 6559. Each patient or parent signed an informed consent form reviewed by the institutional review board before treatment and blood sampling.

Conditioning regimen and treatment

The mononuclear cell fraction of each pheresis product was CD34 selected by use of a CEPRATE column. An average of four 15 L leucophereses was required per patient. The mean number of leucophereses necessary for cell collection was four (range 1–10).

We gave patients 200 mg/kg cyclophosphamide intravenously in daily doses of 50 mg/kg on days −6 to −3 before transplantation. 90 mg/kg antithymocyte globulin

	Patient						
	1	2	3	4	5	6	7
Age (years)	24	15	26	17	39	16	51
Disease duration (years)	13	<1	3	5	20	2	7
Number of years corticosteroid dependent	13	<1	3	5	20	2	7
Immune suppressive medications at referral	Prednisone (80 mg) HCQ, Cyc	Prednisone (80 mg) HCQ, Cyc	Prednisone (70 mg) HCQ, Cyc, CSA	Prednisone (60 mg) HCQ, Cyc	Prednisone (40 mg) HCQ, Cyc	Prednisone (60 mg) HCQ, Cyc	Prednisone (40 mg) HCQ, Cyc
Total cyclophosphamide (g/m^2)	11·2	1·4	15·8	20	34·6	3·0	2·1
SLEDAI	37	35	17	28	18	37	12
Anti-ds DNA titre	1:1280	1:1280	1:40	1:640	1:640	1:1280	1:40
Creatine clearance	14 mL/min	46 mL/min	69 mL/min	58 mL/min	90 mL/min	22 mL/min	88 mL/min
Proteinuria/24 h	23,000 mg	5,400 mg	1,500 mg	13,000 mg	400 mg	8000 mg	200 mg
Renal pathology	Class IV	Class IIIC	Class I/II	Class IV	None	Class IV	None
Left-ventricle shortening fraction (normal 30–45%)	24%	27%	38%	46%	38%	33%	34%
FVC (normal 3·4–4·8 [L]) DLCO (normal 18–22 [min])	2·0/16	1·92/13, alveolar haemorrhage	1·98/12, hypoxia at rest	2·9/19·8	2·0/16	1·6/11	2·6/16
Central nervous system history	Chronic headache, fatigue, depression, transverse myelitis	Fatigue, depression	Chronic headache, depression, fatigue	Chronic headache, auditory hallucinations, seizure, MRI abnormalities, depression, fatigue	Chronic headache, fatigue, depression	Recurrent seizures, headache, depression, fatigue	Chronic headache, depression, fatigue
Haematocytopenias	Haemoglobin 8 g/L, platelets 60×10^9/L	Haemoglobin 7·8 g/L	Haemoglobin 10 g/L	Haemoglobin 8·6 g/L	Haemoglobin 10 g/L	Haemoglobin 7 g/L	Haemoglobin 11 g/L

CSA=cyclopsorine; Cye=cyclophosphamide; HCQ=hydroxychloroquine; SLEDAI=systemic lupus erythematosus disease activity index; MRI=magnetic resonance imaging; FVC=forced vital capacity; DLCO=diffusional capacity of carbon monoxide.

Table 1: **Patient profile upon referral: the 7 patients who underwent transplant**

was given in daily doses of 30 mg/kg on days −5 to −3. Patients received 1g methylprednisolone intravenously before each dose.

Patients were treated on a hepafiltered haematology oncology floor. They started a low microbial diet, 400 mg/day oral or intravenous fluconazole and 500 mg oral or intravenous valaciclovir three times daily on admission. These were discontinued when the absolute neutrophil count rebounded to $0{\cdot}5\times10^9$/L. 750 mg oral ciprofloxacin twice daily was started on admission and changed to intravenous piperacillin and tazobactam when the absolute neutrophil count fell lower than $0{\cdot}5\times10^9$/L, at which point we also started patients on liposomal amphotericin B 3 mg/kg daily. Subcutaneous granulocyte colony stimulating factor 5 μg/kg was started on the day of haemopoietic stem-cell infusion and continued until the absolute neutrophil count was higher than $1{\cdot}0\times10^9$/L for 3 consecutive days. For the first 6 months after transplantation, patients were treated with daily fluconazole or itracanozole and monthly aerosolised pentamidine.

Immune reconstitution

We analysed peripheral blood mononuclear cells before and after transplantation in the first four patients. Methods included flow cytometry for expression of intracellular interleukin 4 and for expression of CD69, a T-cell activation marker. ELISA of supernatants was used to measure secretion of interferon-gamma. In three patients, T-cell-receptor Vβ CDR3 spectratyping was done to assess diversity in T-cell repertoire. We did time-course experiments to find out the duration of phorbol 12 myristate 13 acetate (PMA) exposure relative to peak cytokine expression with use of control cells from healthy donors. Results were consistent with previously reported values. During PMA activation of patients' samples, a single sample of peripheral blood mononuclear cells obtained from a healthy control was placed in parallel incubation with the patients' samples and underwent parallel flow cytometry. The values for these control samples were similar to those obtained in the time-course experiments.

We assessed surface markers by use of antibodies conjugated with fluorescein isothiocynate to CD69, CD3, CD4, and CD8. Isolated peripheral blood mononuclear cells were suspended (2×10^6cells/mL) in RPMI 1640 (Gibco BRL Life Technologies, Grand Island, NY, USA) with 10% heat-inactivated fetal bovine serum (Gibco BRL Life Technologies) and incubated for 24 h at 37°C in 5% carbon dioxide. CD69 expression was measured after a 6·5 h incubation with PMA and ionomycin. Cells were resuspended in 0·5 mL 1% formaldehyde (10%

Patient	Absolute neurophils >5×10^9/L	Platelets ≥40×10^9/L transfusion	Infections	Fluid and electrolytes	Pulmonary effects	Central nervous system effects
SLE 1	Day 10	Day 12	Varicella zoster Day 60	Fluid overload, hyperkalaemia, hyperphosphataemia	Pulmonary oedema	None
SLE 2	Day 8	Day 10	None	Fluid overload, hyperkalaemia, hyperphosphataemia	Pulmonary oedema	None
SLE 3	Day 9	Day 11	None	Fluid overload	None	None
SLE 4	Day 11	Day 13	Varicella zoster, day 60	Oedema, fluid overload	Mechanical ventilation	Seizure between harvest and transplant
SLE 5	Day 9	Day 11	None	Oedema, fluid overload	None	None
SLE 6	Day 8	Day 10	Herpes simplex virus in cranial nerve V_2 distribution, day 50	Oedema, fluid overload, hyperkalaemia hyperphospataemia	Mechanical ventillation	Seizures when dilantin subtherapeutic until day 120
SLE 7	Day 10	Day 12	*Pneumocystis carinii*, day 60	Oedema, fluid overload	Pulmonary oedema	None

Table 2: **Toxic effects**

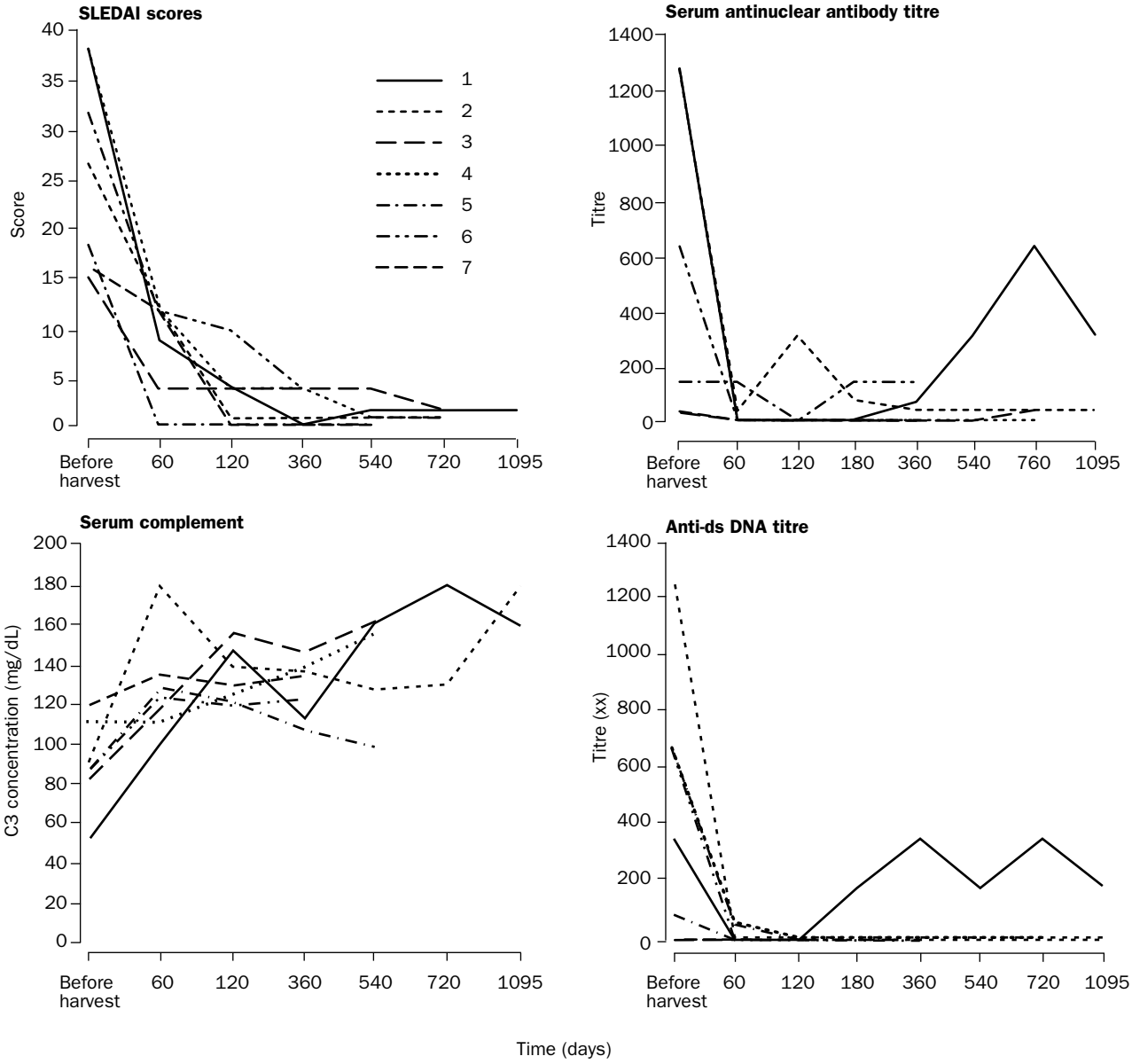

Figure 1: **Systemic lupus erythematosus disease activity index scores and pertinent serological values for each patient before and after transplantation**

formaldehyde, methanol free, Polysciences, Warrington, PA, USA) for flow cytometry (Coulter XL-MCL, Beckman-Coulter, Miami, FL, USA). Results were expressed as a percentage of CD3-positive cells (light-scatter-derived lymphocyte population) expressing the measured antigen. For assessment of intracellular interleukin 4, we incubated T lymphocytes with 3 mmol/L monoesin, a secretion inhibitor (controls), or 3 mmol/L monoesin alone followed by addition of activation agents, 5 nmol/L PMA, and 1 mmol ionomycin (Sigma, St Louis, MO, USA). Positive or negative fluorescence discrimination was based on the use of isotype controls, identically stained and unstimulated control sample, or both. The monoclonal antibody panel consisted of CD3 peridinin chlorophyll protein (PerCp), CD69 phycoerythrin (PE), interleukin 4 PE, and the isotype controls IgG1 and IgG2b, and rat IgG2a.

Peripheral blood mononucluear cells were adjusted to a concentration of 10^6/mL in the plate, stimulated with 5 nmol/L PMA and 1 μmol/L ionomycin for 15 h at 37°C. Supernatant was stored at −80°C. Interferon-gamma was measured by ELISA (R&D Systems, Minneapolis, MN, USA).

RNA was prepared from 10 million lymphocytes by a variation of the Chomcyznski and Sacchi method,[10] with Triazol (Bethesda Research Laboratories, Gaithersberg, MD, USA). DNA carry-over was prevented by incubation with RNase-free DNase I, (Sigma) 2 units of DNase per mg RNA for 1 h at 37°C. RNA integrity was checked by electrophoresis on agarose gel. cDNA was generated from 0·25–0·5 mg total RNA with the Gene Amp RNA PCR Kit (Perkin Elmer Cetus, Norwalk, CT, USA). For Vβ-receptor expression, we amplified cDNA with a C-region primer and relevant single-family Vβ-region primers by incubation with 10 mL cDNA in a 50 mL reaction mixture containing magnesium chloride (1·5 mmol/L), dXTPs (200 mmol/L), 1·25 U Gold Taq polymerase in standard-reaction buffer (Perkin Elmer Cetus). Amplification steps were 94°C for 1 min followed by 35–40 cycles of 94°C for 25 s, 55°C for 35 s, and 72°C for

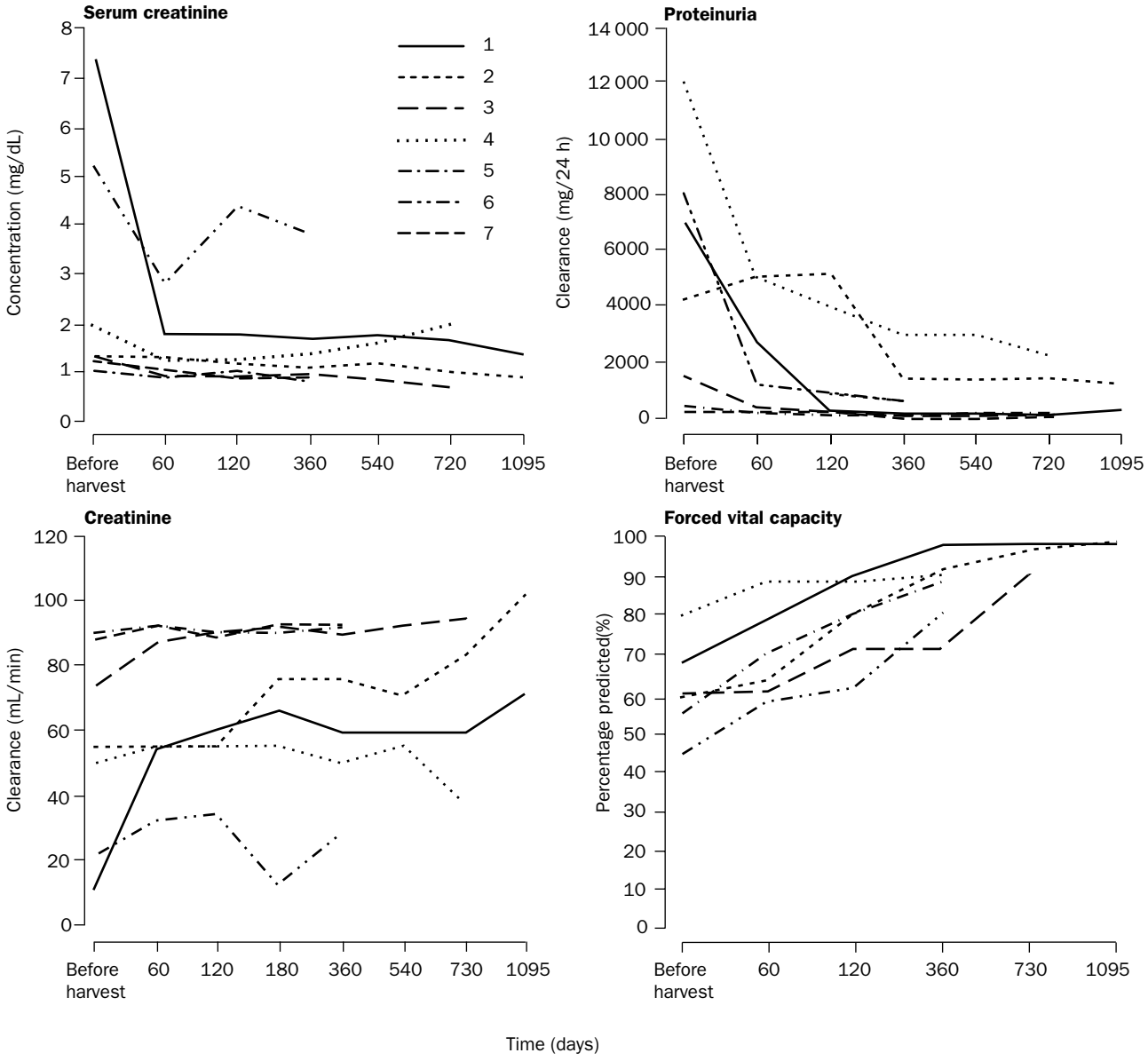

Figure 2: **Kidney and lung function before and after transplantation**

30 s. The final step was 72°C for 10 min. After amplification, the PCR product was separated on a 50 cm 5% polyacrylamide sequencing gel, and we analysed radioactive bands.

Results

Nine patients aged 15–51 years were enrolled and underwent stem-cell harvest. Median duration of steroid dependence was 4 years (range <1–20) and median daily prednisone dose was 50 mg. Six patients had received between eight and 30 doses of monthly intravenous cyclophosphamide. One patient with recurrent pulmonary haemorrhage had received only three courses of cyclophosphamide and three episodes of plasma exchange. Two patients were diagnosed with infections after stem-cell harvest (one had transient cytomegalovirus viraemia and the other died from disseminated mucormycosis) and were excluded from analysis. All patients were seriously ill, with SLE disease activity indices of 17–37.

Of the seven patients who underwent transplantation, two were referred because of progressive lung disease and hypoxia at rest but were not ventilator dependent. One patient with alveolar haemorrhage was oxygen dependent and required intermittent ventilatory support. Four patients had WHO class III–IV glomerulonephritis and nephrotic syndrome.[11] Five patients had a creatinine clearance lower than the normal range, defined as less than 85 mL/min. All patients had uncontrolled hypertension requiring four antihypertensive agents. Two patients had myocardial hypokinesis on echocardiography. A third had experienced myocardial infarction and coronary bypass in the year before stem-cell transplant. One had echocardiographic evidence of mitral insufficiency and atrial thrombosis. Two patients had seizures immediately before transplant. One of these patients had focal abnormalities on T2 magnetic resonance imaging. A third patient had a history of transverse myelitis. All patients had been diagnosed with chronic fatigue and depression and four had severe and recurrent headaches requiring narcotic analgesia (table 1).

Patient	Percentage CD69 expressing T cells before transplantation, resting	Percentage CD69 expressing PMA-activated T cells before transplantation	Percentage CD69 expressing T cells after transplantation, resting	Percentage CD69 expressing PMA-activated T cells after transplantation
Normal control	3	87	. .	. .
SLE 1	52	54	1	75 (22 months)
SLE 2	18	42	5	60 (12 months)
SLE 3	11	44	4	85 (6 months)
SLE 4	5	30	1	78 (9 months)

Expressed as % CD3 cells with detectable CD69 expression by flow cytometry before and 6·5 h after PMA stimulation.

Table 3: **Resting and activated T cells CD69 expression before and after transplantation**

No unexpected haematological toxic effects occurred. Median time for white cell engraftment was day 9, and platelet engraftment on day 11. All transplanted patients had fever. The only positive blood culture grew *Staphlococcus epidermidis*. Two patients developed dermatomal herpes zoster 2 months after discharge. One developed *Pneumocystis carinii* pneumonia at day 60, which was treated successfully with pentamidine. Fluid retention (5–30 kg) occurred in all patients during mobilisation and conditioning. Volume overload required dialysis or continuous veno-venous hyperfiltration in three patients. Two patients required mechanical ventilation because of volume overload—both were extubated within 72 h of starting continuous veno-venous hyperfiltration. One patient, who had a history of seizure and cerebritis, had seizures 1 week after stem-cell harvest. Computed tomography and magnetic resonance imaging of the brain were unchanged. Subsequent transplantation was without central-nervous-system complications (table 2)

The median infused CD34 dose was $2{\cdot}4\times10^6$ cells/kg. Median infused T cell (CD3) and B cell (CD19) dose was $5{\cdot}4\times10^5$/kg and $5{\cdot}7\times10^4$/kg, respectively.

Lupus remained in clinical remission in all patients after transplant. Serum complements and sedimentation rates stayed normal and there was no evidence of active disease, as defined by symptoms or findings of serositis, synovitis, cerebritis, or glomerulonephritis. Persistence of some residual proteinuria, without an active urinary sediment is taken to be compatible with remission status, as is continued treatment for seizures for a previously established seizure focus. Systemic lupus erythematosus disease activity index scores after transplantation ranged from 0–5 in all patients and improved with time. No patient had a disease flare after transplantation (figure 1).

From the time of disease onset, five of the seven patients had persistently raised antinuclear antibody and anti-double stranded (anti-ds) DNA titres, and low serum complement (C3 and C4). Antinuclear antibody, anti-ds DNA, C3, and C4 normalised after transplantation. Antinuclear antibody rose transiently at 3 months after transplantation in two patients but subsequently declined without intervention and without active disease. Anti-ds DNA became positive in the first 6 months in one patient, but for the next 30 months there was no clinical evidence of disease activity.

Patient	Before transplant	After transplant
Interleukin 4*		
Normal control	1%	. .
SLE 1	5%	<1% (22 months)
SLE 2	3%	1% (12 months)
SLE 3	2%	<1% (6 months)
SLE 4	5%	<1% (9 months)
Interferon-gamma†		
Normal control	3750	. .
SLE 1	350	3400 (22 months)
SLE 2	700	4600 (12 months)
SLE 3	700	4100 (6 months)
SLE 4	1900	4100 (9 months)

*Results are expressed as % CD3 cells positive on flow cytometry of intracellular cytokines 6·5 h after exposure to PMA stimulation. †Results expressed in pg/mL, determined by ELISA in supernatant of peripheral blood mononuclear cells 15 h after PMA stimulation. Time from transplantation given in parentheses.

Table 4: **Interleukin 4 and interferon-gamma expression before and after transplantation**

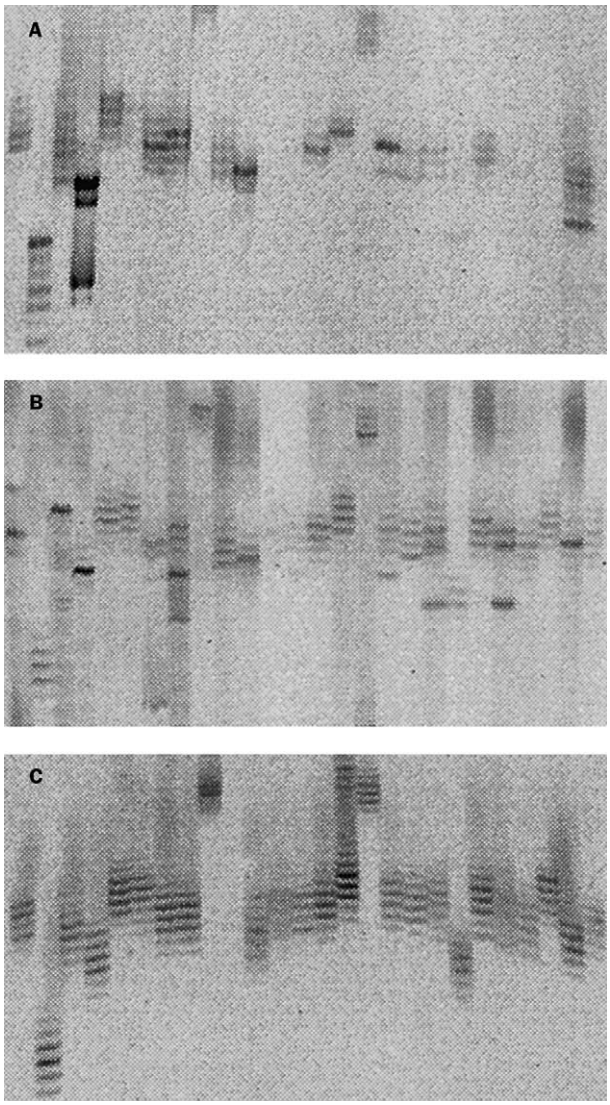

Figure 3: **Spectratyping gel electrophoretic analysis**
A=SLE patient before transplantation; B=SLE patient after transplantation; C=normal individual.

The creatinine clearance in three of the five affected patients improved substantially by 270 days after transplant and the 24 h urine protein excretion declined significantly in six patients in the first year (figure 2). Three patients normalised urinary protein excretion. Two patients with mainly pulmonary symptoms had normal lung-function tests in the 12 months after transplantation (figure 2) and remained free of supplemental oxygen.

Two patients had abnormal cardiac function before transplant. Ventricular motility and fractional shortening normalised in both after transplantation. One patient required stenting of a previous bypass graft 3 months after transplantation.

Patient four, who had active cerebritis and psychosis in the year before transplantation, had persistent depression requiring medication. Magnetic resonance imaging after transplantation was without new lesions. Residual abnormalities remained stable or decreased in size. Fatigue and headaches resolved in all but two patients, whose symptoms decreased in frequency, duration, and severity.

No patient received a cytotoxic agent or hydroxychloroquine, and corticosteroids have been discontinued in patients one, two, and five since transplantation. Patients three, four, and six were tapered to 5 mg prednisone daily. Six patients were taking one or no antihypertensive agent long-term after transplantation and one patient remained on three antihypertensive agents. Antidepressants were stopped in two patients and continued in five.

Peripheral blood lymphocytes were analysed in four patients for activation markers and cytokine profiles before and after transplantation. Before transplantation, all patients displayed spontaneous T-cell activation (CD69) higher than normal, accompanied by poor up-regulation with stimulation. After transplantation, spontaneous CD69 expression declined or normalised, and a more appropriate rise was elicited by stimulation (table 3). Cytoplasmic expression of interleukin 4, a T-helper-2 cytokine, was detected at concentrations higher than normal in T lymphocytes of each patient before transplant and at normal concentrations after transplantation. The T-helper-1 cytokine, interferon-gamma, was lower than normal in lymphocytes before transplantation and increased to normal after transplantation (table 4).

T-cell-receptor Vβ family repertoire was characterised in three patients. Repertoire compositions were skewed in samples before transplantation but had a normal distribution after transplantation. Figure 3 shows the spectratype comparison for patient two, and is representative of the comparisons before and after transplantation. The Vβ families in which oligoclonality was most evident before transplantion varied in the three patients.

Discussion

In mice with lupus-like manifestations, an allogeneic haemopoietic stem-cell transplant is required for sustained improvement.[11–15] Such experiments do not necessarily suggest that allogeneic transplantation will be required to cure human beings with lupus. The relative contributions of genes and environment to the development of lupus may differ between highly inbred strains of mice and highly polymorphic human populations. Around two-thirds of syngeneic human twins are discordant for this disease, which suggests that genetic penetrance is not complete in humans.[16,17]

We selected the conditioning regimen because of the established effectiveness of cyclophosphamide in the treatment of lupus. Some investigators have done immunosuppressive trials in autoimmune diseases with similar cyclophosphamide doses without stem-cell reinfusion,[18] but their patients had not been heavily pretreated and, in some cases, had never received cyclophosphamide. We reinfused stem cells to keep the duration of neutropenia to a minimum. We reasoned that eligible patients who had been heavily pretreated would be at high-risk of opportunistic infection. The role of stem-cell infusion other than shortening the duration of neutropenia to decrease risk of infection remains unclear.

Autologous haemopoietic stem-cell transplantation has been previously reported in eight patients from four different centres, including two patients reported from our centre.[19–24] The follow-up of those single cases was short and lacked determination of T-cell characteristics before and after transplantation. We report long-term follow-up in seven patients, two patients for more than 3 years and all beyond 1 year. Disease activity was stopped and organ function strikingly improved concurrent with tapering or discontinuation of immunosuppressive treatment. Improvement has in some cases been gradual over 6–18 months. Serological parameters, including antinuclear antibody, anti-ds DNA, and complement, normalised. In some cases, serology became normal for the first time since disease onset. In other cases, antinuclear antibody or anti-ds DNA titres have intermittently become raised without clinical evidence of disease.

Transient improvement and early return of disease activity suggest that underlying immune perturbations that contribute to lupus were suppressed but not eliminated by dose-intense immune suppression and autologous haemopoietic stem-cell support. Long-term normal clinical markers and serology indicates that autologous transplantation may fundamentally change the immune system. To further investigate this hypothesis, we assesssed whether T-cell abnormalities associated with lupus normalise after transplantation.

T cells were hyper-responsive before transplantation, as shown by raised resting CD69 expression. Failure to adequately up-regulate CD69 by in-vitro stimulation suggests maximum previous in-vivo activation. T-cell responsiveness, measured by resting and stimulated CD69 expression, was normal after transplantation. Before transplantation, T cells were also skewed towards a T-helper-2 profile with increased interleukin 4 and diminished interferon-gamma production. Interleukin 4 increases production of antibody, whereas diminished interferon-gamma may increase susceptibility to opportunistic pathogens. A normal T-helper-1 profile returned after transplantation. The T-cell repertoire was restricted before transplantation. This restriction could impair immune clearance of pathogens. After transplantation, the T-cell-receptor repertoire distribution became normal. Corticosteroids and cyclophosphamide may have contributed to pretransplant phenotype. However, impaired T-cell responsiveness to in-vitro stimulation and a T-helper-2 skewing has previously been shown to characterise active lupus irrespective of treatment state.[25,26]

Patients with active lupus seem to be at increased risk of infection from immune suppression and disease-associated T-cell abnormalities.[6–9,27,28] After mobilisation but before transplantation, two patients developed serious opportunistic infections (cytomegalovirus and mucormycosis). After 100 days, concordant with resolution of disease activity, no opportunistic infections have occurred in any patient who has undergone transplantation. The absence of serious infections in this period, lack of active disease for up to 3 years, and normalisation of T-cell repertoire and profile suggests that dose-intense immune suppression and autologous stem-cell support results in more than transient immune suppression. Although the durability of response and pathogenesis of lupus remains unclear, remission induced

by autologous haemopoietic stem-cell transplantation allows for a long-term analysis of abnormalities associated with an at-risk population that is not possible in cross-sectional studies.

Contributors

Ann Traynor and Richard Burt wrote the treatment protocol and managed all patients through transplantation and follow-up. They were assisted in rheumatological consultation and management by James Schroeder, renal consultation and management by Robert Rosa and Salim Mujais, and pulmonary consultation and management by Steven Baker. All researchers carefully reviewed their contributions to this paper.

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References

1 Austin HA, Klippel JH, Barlow JE, et al. Therapy of lupus nephritis: controlled trial of prednisone and cytotoxic drugs. *N Engl J Med* 1986; **314:** 614–19.
2 Correia P, Cammeron JS, Lian JD, et al. Why do patients with lupus nephritis die? *BMJ* 1985; **290:** 126–31.
3 Abu-Shakra M, Urowitz MB Gladman DD, Gough J. Mortality studies in systemic lupus erythematosus: results from a single center, II—predictor variables for mortality. *J Rheumatol* 1995; **22:** 1265–67.
4 Urowitz MB, Gladman DD. Evolving spectrum of mortality and morbidity in SLE. *Lupus* 1999; **8:** 253–55.
5 Cervera R, Khamashta MA, Font J, et al. Morbidity and mortality in systemic lupus erythematosus during a 5-year period: a multicentre prospective study of 1,000 patients—European Working Party on Systemic Lupus Erythematosus. *Medicine* 1999; **78:** 167–75.
6 Crispin JC, Martinez A, de Pablo P, Velasquillo C, Alcocer-Varela J. Participation of the CD69 antigen in the T-cell activation process of patients with systemic lupus erythematosus. *Scand J Immunol* 1998; **48:** 196–200.
7 Hagiwara E, Gourley MF, Lee S, Klinman DK. Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10: interferon-gamma-secreting cells in the peripheral blood. *Arthritis Rheum* 1996; **39:** 379–85.
8 Rengaraju M, Gray JD, Horwitz DA, Kubin M, Trinchieri G. Increased spontaneous IL-10 production and decreased mitogen induced IFN-γ and TNF α production in untreated patients with SLE: role of CD 8+ T cell. *Lupus* 1995; **4** (Suppl 2): 82.
9 Gray JD, Hirokawa M, Horwitz DA. The role of transforming growth factor-b in the generation of suppression: an interaction between CD8+ T and NK. Cells. *J Exp Med* 1994; **180:** 1937–42.
10 Chomczynski P, Sacchi N, Single step method of RNA isolation by acid guanidium thiocyanate chloroform extraction. *Analyt Biochem* 1987; **162:** 156–59.
11 Ikeharaa S, Nakamura T, Sekita K, et al. Treatment of systemic and organ specific auto-immune disease in mice by allogeneic bone marrrow transplantation. *Prog Clin Biol Res* 1987; **229:** 131–46.
12 Wakeland EK, Morel L, Mohan C, Yui M. Genetic dissection of lupus nephritis in murine models of SLE. *J Clin Immunol* 1997; **17:** 272–81.
13 Wang B, Yamamoto Y, El-Badri NS, Good RA. Effective treatment of autoimmune disease and progressive renal disease by mixed bone-marrow transplantation that establishes a stable mixed chimerism in BXSB recipient mice. *Proc Natl Acad Sci USA* 1999; **96:** 3012–16.
14 Wang BY, Cherry, El-Badri NS, Good RA. Prevention of development of autoimmune disease in BXSB mice by mixed bone marrow transplantation. *Proc Natl Acad Sci USA* 1997; **94:** 12065–69.
15 Karussis DM, Vourka-Karussis U, Lehmann D, Abramsky O, Ben-Nun A, Slavin S. Immunomodulation of autoimmunity in MRL/lpr mice with syngeneic bone marrow transplantation (SBMT). *Clin Exp Immunol* 1995; **100:** 111–17.
16 Yocum MW Rossman J, Waterhouse C, Abraham CN, May AC, Condemi JJ. Monozygotic twins discordant for systemic lupus erythematosus. *Arthritis Rheum* 1975; **18:** 193–99.
17 Deapen D, Escalante A, Weinrib L, et al. A revised estimated of twin concordance in systemic lupus erythematosus. *Arthritis Rheum* 1992; **35:** 311–18.
18 Brodsky R, Petri M, Smith B, et al, Immunoablative high dose cyclophosphamide without stem cell rescue for refractory autoimmune disease. *Ann Intern Med* 1998; **129:** 1031–35.
19 Burt RK, Traynor AE, Ramsey-Goldman R. Hematopoietic stem-cell transplantation for systemic lupus erythematosus. *N Engl J Med* 1997; **337:** 1777–78.
20 Burt RK, Traynor AE, Pope R, et al. Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. *Blood* 1998; **92:** 3505–14.
21 Marmont AM, van Lint MT, Gualandi F, Bacigalupo A. Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. *Lupus* 1997; **6:** 545–48.
22 Fouillard L, Gorin NC, Laporte JP, Leon A, Brantus JF, Miossec P. Control of severe systemic lupus erythematosus after high dose immunosuppressive therapy and transplantation of CD34+ purified autologous stem cells from peripheral blood. *Lupus* 1999; **8:** 320–23.
23 Musso M, Poretto F, Crescimano A, et al. Autologous peripheral blood stem and progenitor (CD34+) cell transplantation for systemic lupus erythematosus complicated by Evans syndrome. *Lupus* 1998; **7:** 492–94.
24 Rosen O, Thiel A, Massenkeil G, et al. Autologous stem-cell transplantation in refractory autoimmune diseases after in vivo immunoablation and ex vivo depletion of mononuclear cells. *Arthritis Res* 2000; **2:** 327–36.
25 Horwitz DA, Gray JD, Behrendsen SC, et al. Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. *Arthritis Rheum* 1998; **41:** 838–44.
26 Su CC, Shau WY, Wang CR, Chuang CY, Chen CY. CD69 to CD3 ratio of peripheral blood mononuclear cells as a marker to monitor systemic lupus erythematosus disease activity. *Lupus* 1997; **6:** 449–54.
27 Horwitz DA. Impaired delayed hypersensitivity in systemic lupus erythematosus. *Arthritis Rheum* 1972; **15:** 353–59.
28 Hahn BH, Bagby MK, Osterland CK. Abnormalities of delayed type hypersensitivity in systemic lupus erythematosus. *Am J Med* 1973; **55:** 25–31.