*Journal of Clinical Immunology, Vol. 20, No. 1, 2000*

# Intense Immune Suppression for Systemic Lupus—The Role of Hematopoietic Stem Cells

RICHARD K. BURT,[1,3] ALBERTO MARMONT,[2] JAMES SCHROEDER,[1] ROBERT ROSA,[1] and ANN E. TRAYNOR[1]

*Accepted: October 13, 1999*

The treatment of severe autoimmune diseases has been recently revitalized by the introduction of intense immune suppression with immune ablative intent followed by three different procedures. These are allogeneic hematopoietic stem cell transplantation (HSCT), autologous HSCT (using either marrow or peripheral blood), and intense immune suppression without stem cell support. Current trials suggest that high dose immune suppressive therapy with or without autologous hematopoietic stem cell support can induce remission of previously refractory disease. Follow-up is too brief to determine if intense immune suppression, and more specifically autologous HSCT, will ultimately cure SLE. It is conceivable that an allogeneic source of stem cells from a normal donor (e.g. HLA matched sibling) will be required to achieve a cure. It is also possible that autologous HSCT, even if not curative, may prolong the life of patients with otherwise high-risk features. In carefully selected patients, the potential benefits of this procedure may outweigh the risks.

**KEY WORDS:** Immune suppression; systemic lupus; hematopoietic stem cell transplantation.

## INTRODUCTION

Despite differences in presentation, manifestations, and course, the common factor for patients with systemic lupus erythematosus (SLE) is hyper-reactivity of T and B cells to endogenous and environmental stimuli. The ethiopathogenesis of SLE resides in a complex interplay of genetic, endogenous (including hormonal), and environmental stimuli. Because each of these factors acts both independently and concurrently, it has been equated to a Troika, the three horse Russian carriage, where each horse pulls independently but concertedly (1).

Evidence of a suspected autoimmune etiology for most diseases is indirect and based on response to immune suppression. Rose's criteria for proof that a disease is autoimmune requires transfer of disease to a normal person by transfer of antibodies or immune cells from an afflicted individual (2). The autoimmune nature of SLE is supported by several observations. Nephritis may be induced in asymptomatic mice by injection of anti-DNA antibody from mice afflicted by a lupus-like nephritis (3). In SCID mice that cannot reject xenogeneic proteins, injection of anti-DNA antibody from patients with SLE induces proteinuria but not nephritis (4). The LE cell results from phagocytosis of antibody coated nuclei. In 1959, normal individuals were transfused with 300 mls of plasma from LE positive patients with SLE (5). There was no observable clinical effect, but the patients developed transient LE cells. It is unknown if continued transfusions of SLE plasma could have precipitated clinical disease. Although not invariably true, disease activity for most patients with SLE correlates with antidouble stranded DNA (anti-ds DNA) antibody titer (6).

In general, of all autoimmune diseases, SLE is one of the most responsive to immune suppression, indirectly supporting an autoimmune pathogenesis. Mortality from SLE has markedly improved due to more aggressive immune suppression as well as improved supportive care from dialysis, apheresis, and newer antihypertensive medications. For patients with lupus nephritis, 5 year survival in the 1950s was almost zero (7). With introduction of high-dose corticosteroids in the 1960s, the 5 year survival remained a dismal 25% (8). Following addition of oral cyclophosphamide and azathioprine, 5 year survival improved to 40–70%. In the 1980s, introduction of intra-

[1] Northwestern University Medical Center, The Robert H. Lurie Cancer Center, Chicago, Illinois.
[2] The Department of Hematology, Ospedale San Martino, Genova, Italy.
[3] To whom correspondence should be addressed.



0271-9142/00/0100-0031$18.00/0 

**Table I.** Mouse Models of Lupus[a]

| Model | Onset/manifestation | Predominant sex with disease | Genetics | Result of hematopoietic stem cell transplantation |
|---|---|---|---|---|
| NZB/NZW (B/W) | Spontaneous anti-ds DNA antibody and GN | female | Polygenic- at least 8 loci on muliple chromosomes contribute | Allogeneic transplant from normal strain cures |
| NZB/SWR ($SNF_1$) | Spontaneous anti-ds DNA antibody and GN | female | Polygenic | N/A |
| MRL-1pr | Spontaneous/lymph-adenopathy, splenomegaly anti-ds DNA antibody, and GN | female | Single defect of Fas gene prevents apoptosis of autoreactive lymphocytes in polygenic susceptible strain | Syngeneic T cell depleted transplant prolongs survival |
| BXSB | Spontaneous/anti-ds DNA antibody and GN | male | In polygenic susceptible background a single Y chromosome gene (*Yaa*) accelerates disease | Allogeneic transplant from normal strain cures |
| NZW/BXSB | Spontaneous/anti-ds DNA, antiphopholipid antibody and early death from coronary artery disease | male | Polygenic | N/A |

[a] ds = double stranded, GN = glomerulonephritis, N/A = not applicable.

venous pulse cyclophosphamide (500–100 mg/$m^2$ monthly) improved 5 year survival to 80% (9).

## ANIMAL MODELS OF SLE

A spontaneous lupus-like illness occurs in many species including dogs, monkeys, and mice. Most studies have, for economic reasons, been confined to highly inbred murine strains (Table I) (10). New Zealand Black (NZB) mice develop a spontaneous hemolytic anemia. When NZB mice are mated to normal New Zealand White (NZW) mice the $F_1$ offspring (NZB $\times$ NZW) or B/W mice develop antinuclear antibodies (ANA), anti-ds DNA antibodies, and early death from glomerulonephritis (11). Disease occurs in both males and females but is more severe in females. The B/W lupus-like illness arises from polygenic loci contributed by both parents. At least 8 genes on multiple chromosomes are involved and may be inherited in a dominant or recessive manner (12). Individual loci appear to be linked to different stages of disease (e.g., antibody formation, glomerulonephritis, or mortality). Mating NZB mice to another normal mouse strain (SWR) also results in offspring (SNF1) with a lupus-like illness. SNF1 mice have disease similar to B/W mice manifest by spontaneous onset autoantibodies, glomerulonephritis, female predominance, and linkage to polygenic loci (13).

The Murthy Roth lab lymphoproliferative (MRL/lpr) mouse develops massive lymphadenothapy, glomerulonephritis, erosive arthritis, polyarteritis, hypocomplementemia, and a variety of autoantibodies including: ANA; anti-ds DNA; anti-Sm; rheumatoid factor; and antiphospholipid antibodies (14). The congenic MRL/+/+ mouse develops similar serologic abnormalities and glomerulonephritis but does not develop lymphoproliferation and disease is milder with a life span of up to 24 months. In contrast, MRL/lpr mice die within 3–6 months. As mentioned, MRL/lpr and MRL/+/+ mice are congenic which means that they have been bread for consecutive generations to differ in only one loci. This single loci difference, the lpr gene, encodes a defective Fas molecule (15). Surface Fas expression, when engaged by the Fas ligand, induces apoptosis. Therefore, a single gene defect, Fas, results in an accelerated autoimmune phenotype in a genetically susceptible strain.

The BXSB strain of mice also develops a spontaneous lupus-like illness (16). The disease is worse in males than females. Acceleration of disease phenotype in males is due to a Y chromosome gene termed Y chromosome autoimmune accelerator (*Yaa*) (17). The function of the *Yaa* gene is unknown but unless expressed in a susceptible strain is unable to cause disease. *Yaa* has been postulated to increase expression of adhesion molecules aiding T cell stimulation of B cells. Finally, male offspring of BXSB $\times$ NZW mice develop antibodies to DNA, platelets and phospholipids and die of accelerated coronary artery disease (18). This is pertinent to lupus patients since some suffer from increased vascular events including coronary artery disease.

Immune cells arise from the hematopoietic compartment. Therefore, hematopoietic (i.e., bone marrow) transplantation from a nondisease susceptible donor has been attempted to treat animal autoimmune disease (19–22). Allogeneic bone marrow transplantation (i.e., immune ablation and infusion of hematopoietic stem cells from a normal strain) prevents and/or cures lupus-

like disease in BXSB and B/W mice (19, 20). While allogeneic HSCT can cure lupus-like disease, it is generally thought that autologous or syngeneic marrow transplantation would transplant disease prone cells and be ineffective. Although not curative, lymphocyte depleted syngeneic marrow transplantation induces life-prolonging remissions in MRL/lpr mice (21, 22).

Application of these murine lupus-like models to SLE must be done with caution since the mice originate from highly inbred strains. Murine models are important to identify genetic loci mediating autoimmune phenomena. However, in outbred human populations, environmental exposure and regulatory cells and other suppressor mechanisms may have significant roles in controlling disease-associated genes. In fact, unlike murine models of lupus, patients with SLE can enter spontaneous remissions off therapy. Therefore, the potential for autologous HSCT to cure SLE remains possible but uncertain.

## SLE ETIOLOGY

SLE is associated with certain HLA genotypes and deficiencies of early complement components which also map to the MHC. Monozygotic twin studies suggest an approximate 1/3 concordance for clinical disease (23). This suggests a strong nongerm line component for clinical SLE. However, most twin studies do not follow twin pairs for extended intervals to determine if disease will eventually manifest in the discordant twin. Also, concordance for ANA among identical twins is generally 70% (24).

SLE is probably a genetically heterogeneous disease influenced by multiple loci. The common thread is T and B cell hyperreactivity. T cells from patients with SLE provide help to B cells to produce autoantibodies (25). We have, therefore, focused on analysis on pre- and posttransplant T cells. A normal person's peripheral T cells are less than 1% positive for CD69 (a marker of activation). Upon *ex vivo* exposure to a mitogen such as PMA more than 80% of the T cells will present CD69 within 4–6 hours. Pretransplant T cells from patients with lupus display the activation marker CD69 in 10–54% of the unstimulated cells and fail to further upregulate CD69 expression in response to PMA (submitted Traynor *et al*.). Fresh T cells and T cell lines from patients with lupus display enhanced TCR-mediated protein tyrosine phosphorylation and intracellular free calcium concentrations (26, 27). Therefore, T cells from patients with lupus may "overshoot" surface mediated signalling events. T cells from patients with active lupus also display a Th2 cytokine skewing (28).

In general, Th2 cytokines promote B cell antibody production. The pretransplant T cell receptor CDR3 repertoires are also skewed indicating expansion of reactive T cells (Traynor *et al*., submitted). After transplant, these pretransplant T cell hyperactivation abnormalities normalize (Traynor *et al*., submitted). Although encouraging, the posttransplant duration of a normalized T cell phenotype is unknown. Analysis of B cell activation, signal transduction and immunoglobulin receptor skewing is yet to be performed on the pre and posttransplant samples.

## SEROLOGY

SLE offers an opportunity to follow pre- and posttransplant serology as a marker for disease. The presence of antinuclear antibody (ANA) is determined by an immunofluorescence capture of serum immunoglobulins that bind to a cell's nucleus. The target cells may be monolayers of rodent (usually liver or kidney) or human cell lines. Depending on the target cells, false positives of 1/40 titer may occur in 10–15% of normal subjects. The microscopic pattern of immunofluorescence is usually reported. Antibodies to RNA proteins (Sm, Ro/SSA, La/SSB) cause a speckled pattern (29). Antibodies to chromatin and DNA cause either a homogeneous or rim pattern (29). A patient with SLE may have a homogeneous or rim pattern at low titers but at higher dilutions convert to a speckled pattern if the concentrations of anti-RNA antibodies are higher than anti-DNA antibodies. Patients with SLE who enter remission often maintain high ANA titers. This may be because ANA is not specific and reflects all antibodies that bind to the nucleus even if nonpathogenic. Therefore, ANA titer does not dictate therapy or disease activity.

Depending upon the method of detection, anti ds-DNA antibodies (unlike ANA) may correlate with disease activity. Methods such as ELISA, *Crithidia luciliae* immunofluorescence, hemagglutination, and PEG assay detect low and high avidity anti-DNA. Although sensitive, titers from these assays do not equate with disease activity. High avidity anti-DNA antibody is detected by precipitation, complement fixation, and the Farr assay. Anti-DNA titers from these assays are less sensitive but correlate with disease activity. Rising titers may herald a flare and may dictate need to start therapy (6).

In our eight patients who underwent autologous HSCT, anti-ds DNA antibody gradually became negative in posttransplant samples while ANA titer fell but persisted and in some cases transiently rose then fell again without therapeutic intervention. The gradual decline of autoreactive antibody titers may be secondary to

the long half-life of immunoglobulins, to the persistence of damaged pretransplant autoreactive cells that gradually die, or the gradual generation of posttransplant autoregulatory cells or circuits. However, in the Genova case, following autologous HSCT a borderline speckled ANA converted to homogeneous ANA (1:160) after 42 months posttransplant (30). This patient has maintained clinical remission (30).

## DISEASE INDICES

There is no established definition of disease remission for SLE. However, multiple indices exist to measure disease activity. Activity instruments include the British Isles Lupus Assessment Group scale (BILAG) (31), Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (32), Systemic Lupus Activity Measure (SLAM) (33), and the Lupus Activity Index (LAI) (34). The index employed depends on institutional and investigator familiarity. We used the SLEDAI to measure disease activity on all transplant patients. Patients are scored for 24 lupus related manifestations. For example, seizures are given a score of 8 points, hematuria 4 points, and fever 1 point. SLEDAI scores vary from 0 to a maximum of 105. Any score over 20 is considered active disease. Disease flare is defined as a change in the SLEDAI by 3 points or more. In our patients, the pretransplant SLEDAI was generally $>30$ despite conventional therapy and declined to $<$ five and in most cases to zero during the posttransplant period.

In our initial SLE transplant trial, we did not include a damage index. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for SLE was designed to measure accumulated organ damage from any cause: disease; treatment; or sequelae (e.g., hypertension) (35). The transplant conditioning regimen is composed of very high doses of chemotherapy which could damage already impaired organ systems. However, we have found that following transplant end organ function, especially creatinine and creatinine clearance, slowly improve over an interval of approximately one year. Hypertension gradually resolves over the same time interval. Multiple antihypertensive agents have to be gradually withdrawn to prevent hypotension. It is, therefore, appropriate to include a Damage Index in pre- and posttransplant assessment of these patients.

## HEMATOPOITIC STEM CELL TRANSPLANT FOR SLE

In general, for all patients with SLE, current mortality is one percent per year. Multiple variables predict for higher mortality including disease activity, visceral organ involvement, and age. Younger age at onset, vital organ involvement (nephritis, cerebritis, pneumonitis, serositis thrombocytopenia and leukopenia), and high disease activity indices diminish five year survival (36–38). We, therefore, reasoned that patients with active visceral SLE who failed the most intense standard treatment available (monthly pulse cyclophosphamide (pCy) given at a dosage of 500–100 mg/m$^2$) would respond to transplant doses of cyclophosphamide (TDCy) (200 mg/kg/IV divided over four days = 6–7 g/m$^2$). We modeled our conditioning regimen after a standard transplant regimen developed by Drs. Storb and Deeg at the Fred Hutchinson Cancer Center for aplastic anemia (cyclophosphamide 200 mg/kg and antithymocyte globulin (ATG) 90 mg/kg). The autologous stem cells were mobilized into the peripheral blood with cyclophosphamide (2g/m$^2$) and G-CSF (10 $\mu$g/kg/day). We chose this regimen because it is relatively well tolerated, and SLE has historically responded to increased cyclophosphamide dosing. Experience in transplant of hematologic malignancies had already defined 200 mg/kg of cyclophosphamide as the maximally tolerated dose. Higher doses would be complicated by hemorrhagic myocarditis. In other centers, however, mobilization of stem cells was performed with higher cyclophosphamide doses (4.0–4.5 grams/m$^2$) plus G-CSF. In these cases, in order to avoid exceeding cardiotoxic doses of cyclophosphamide, conditioning was with combination of thiotepa and cyclophosphamide (39) or BEAM (carmustine, etoposide, cytosine arabinoside, melphalan) (40).

From experience in transplant of hematologic diseases, we knew that TDCy should not cause permanent marrow failure. Without stem cell support, TDCy would result in 12–16 days of neutropenia (absolute neutrophil count $<500/\mu$l). With stem cell support, the duration of neutropenia would be decreased 4–6 days and last only 8–12 days. Autologous stem cells were, therefore, reinfused as a blood product to hasten engraftment and decrease the risk of infection.

The first transplants for SLE were published in 1997 (39, 41, 42). In the Chicago case, the conditioning regimen (as mentioned earlier) was a combination of cyclophosphamide and antithymocyte globulin. The reinfused stem cell product was lymphocyte depleted by immunoselection for CD34$^+$ cells prior to infusion. Depletion of lymphocytes was designed to avoid reinfu-

**Table II.** Published World Literature on Hematopoietic Stem Cell Transplantation of SLE[a]

| Author City (Ref No)[a] | Number of patients, conditioning | Results |
|---|---|---|
| Marmount, Genova (39) | 1, Thiotepa cyclophosphamide | Clinical remission for over 3 years |
| Burt/Traynor Chicago (41, 42) | 2, cyclophosphamide, antithymocyte, globulin, corticosteroids | Clinical remission for over 2 1/2 years |
| Fouilland, Paris (40) | 1, BEAM (carmustine, etoposide, cytosine arabinoside, melphalan) | Clinical remission for 1 year. ANA negative at 6 months but positive at 9 months |
| Musso, Palmero (43) | 1, cyclophosphamide, antithymocyte, globulin, corticosteroids | At 8 months post transplant low ANA titer and low coombs positive but anti-ds DNA negative and anti-cardiolipin antibody negative |

[a] Table includes published journal manuscripts not abstracts or papers submitted or in progress. Patients with SLE who were transplanted for another reason such as aplastic anemia or malignancy are also excluded.

sion of potential disease causing immune cells. However, the benefit, if any, of depleting the autologous graft of lymphocytes is unknown and unproven. The patient had corticosteroid dependent SLE for 11 continuous years, had failed multiple cycles of pulse cyclophosphamide, and was on dialysis with active disease including serositis, nephritis, and immune mediated thrombocytopenia, Coombs positive hemolytic anemia, and neutropenia. She is now dialysis independent more than 2 and 1/2 years posttransplant and remains in remission off all medications for over one year.

In the Genova case, the conditioning regimen was thiotepa (15 mg/kg) and cyclophosphamide (100 mg/kg). The autologous marrow was infused after a 3 log *ex vivo* lymphocyte depletion. Transplant course was uneventful and the patient achieved a clinical and serologic remission. In the Genova case, the patient has remained severely lymphopenic (depressed $CD4^+$, $CD8^+$, $CD3^+$ peripheral lymphocyte counts) for 40 months in continuous remission without infections (30). During this interval there has been a gradual slow reappearance of a homologous ANA pattern in the place of a borderline speckled ANA.

Other cases of autologous HSCT have been published from Palmero, Italy and Paris, France (Table II) (40, 43). In the Palmero case, the patient had refractory Evans syndrome secondary to SLE that resolved completely (43). Besides Europe, researchers in China have performed autologous bone marrow transplants for 6 patients with SLE (personal communication). Three of these patients were treated with cyclophosphamide and melphalan without T cell depletion of the marrow graft. All three remain in clinical remission for up to one year (Ouyang and Su, personal communication, Nanjing, China).

The response in SLE has been promising but the transplant may be complicated due to multi-organ dysfunction and a high risk of opportunistic infections secondary to chronic corticosteroid dependence and the disease itself. In the Chicago trial, preemptive therapy with lipid amphotercin, acyclovir, broad spectrum antibiotics, and aerosolized pentamidine is done during both mobilization and transplantation. End organ dysfunction appears to gradually improve over 12 months and it may take 6–12 or more months to taper and withdraw corticosteroids. Since lupus is a photosensitive disease, we advise patients to avoid direct sunlight. Nevertheless, posttransplant patients have spent hours at the beach without disease recurrence. Posttransplant titers to childhood vaccinations (tetanus, measles, etc.) have been low or nondetectable. Since lupus is characterized by hyperreactive T and B cells, we have been concerned that reimmunization may reactivate SLE. Nevertheless, we begin reimmunization at one year or when off all immune suppression. Immunization-induced recurrence of disease has not as of yet occurred.

Two other aspects need to be mentioned. Transplant doses of cyclophosphamide (200 mg/kg) have been used without stem cell support for treatment of autoimmune diseases including patients with SLE (44). As previously noted, this regimen is not myeloablative and neutropenia is approximately 4–6 days longer compared to infusion of mobilized autologous stem cell support. Finally, in hematologic malignancies, allogeneic transplants are the most effective method to prevent disease relapse. This is due to an immunologic graft versus leukemia (GVL) effect of the donor's immune system. It has already been speculated that a donor-mediated graft versus autoimmune (GVA) effect may be beneficial in preventing relapse of autoimmune diseases following allogeneic HSCT (30, 45).

## SUMMARY

We are learning that similar to HSCT for hematologic diseases, not all autoimmune diseases will mobilize stem cells readily, respond in the same manner to treatment, or have the same complications or risk of infection. Some centers employ transplant conditioning regimens that are myeloablative, mandating stem cell support. Other cen-

ters use intense regimens that cause prolonged neutropenia but are nonmyeloablative. In this case, autologous stem cells are infused simply to shorten the duration of neutropenia and decrease risk of infection. The role of the infused stem cells, beyond supporting marrow recovery, is unknown. Importantly, if relapse occurs, it is unclear whether disease-associated lymphocytes arise from the stem cell compartment. We will attempt to answer this question in future phase II trials by infusing gene marked autologous hematopoietic stem cells as described within another section in this journal. Finally, plans are in progress to initiate phase I allogeneic HSCT pilot studies and to begin phase III studies comparing transplant dose cyclophosphamide with and without autologous hematopoietic stem cell support as well as randomized trials of autologous HSCT versus conventional therapy.

## ACKNOWLEDGMENTS

We wish to thank Dr. Bevra Hahn (University of California Los Angeles), Dr. John Klippel (National Institutes of Health), and Dr. William Burns (Medical College of Wisconsin) for years of advice and support.

## REFERENCES

1. Alarcon-Segovia D, Alarcon-Riquelme ME: Ethiopathogenesis of systemic lupus erythematosus: A tale of three troikas. *In* Systemic Lupus Erythematosus, Lahita RG (ed). San-Diego-Toronto, Academic Press, 1999, pp 55–65
2. Rose NR and Bona C: Defining criteria for autoimmune diseases (Witebsky's postulates revisited), Immunology Today 14:426–429, 1993
3. Dang H, Harbeck RJ: The in vivo and in vitro glomerular deposition of isolated anti-double-stranded DNA antibodies in NZB/W mice. Clin Immunol Immunopathol 31:265–278, 1984
4. Ehrenstein MR, Katz DR, Griffiths M, Winkler TH, Kalden JR, Isenberg DA: Human monoclonal IgG anti-DNA antibodies deposit in kidneys and induce proteinuria in SCID mice (abst). Lupus 4(Suppl 2):69, 1995
5. Marmont AM: The transfusion of active LE plasma into nonlupus recipientys, with a note on the LE-like cell. Annals New York Academy of Sciences, 124:838–851, 1965
6. Ward MM, Pisetsky DS, Christenson VD: Antidouble stranded DNA antibody assays in systemic lupus erythematosus: correlations of longitudinal antibody measurements. J Rheumatol 16: 609–613, 1989
7. Dubois EL, Commons RR, Star P, Stein CS Jr, Morrison R: Corticotropin and cortisone treatment for systemic lupus erythematosus. JAMA 149:995–1002, 1952
8. Ben-Asher S: Recurrent acute lupus erythematosus disseminatus: Report of case which has survived 23 years after onset of systemic manifestations. Ann Intern Med 34:243–248, 1951
9. Wallace DJ, Podell T, Weiner J, Klinenberg JR, Forouzesh S, Duboius EL: Systemic lupus erythematosus-survival patterns. Experience with 609 patients. JAMA 245:934–938, 1981
10. Bielschowsky M, Helyer BJ, Howie JB: Spontaneous haemolytic anaemia in mice of the NZB/Bl strain. Proc Univ Otago Med Sch (NZ) 37:9–11, 1959
11. Yoshida S, Castles JJ, Gershwin ME: The pathogenesis of autoimmunity in New Zealand mice. Semin Arthritis Rheum 19:224–242, 1990
12. Kono DH, Burlingame RW, Owens DG, Kuramochi A, Balderas RS, Balomenos D, Theofilopoulos AN: Lupus susceptibility loci in New Zealand mice. Proc Natl Acad Sci USA 91:10,168–10,172, 1994
13. Eastcott JW, Scxhwartz RS, Datta SK: Genetic analysis of the inheitance of B cell hyperactivity in relation to the development of autoantibodies and glomerulonephritis in NZB × SWR crosses. J Immunol 131:2232–2239, 1983
14. Murphy ED, Roths JB: A single gene for massive lymphoproliferation with immune complex disease in a new mouse strain MRL. *In* Proceedings of the 16th International Congress in Hematology. Amsterdam, Excerpta Medica, pp 69–80, 1976
15. Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S: Lymphoproliferative disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 356:314–317, 1992
16. Makin M, Fumiwara M, Watanabe H: Studies on the mechanisms of the development of lupus nephritis in BXSB mice. I. Analysis of immunological abnormalities at the onset period. J Clin Lab Immunol 22:127–131, 1987
17. Murphy ED, Roths JB: A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. Arthritis Rheum 22:1188–1194, 1979
18. Hashimoto Y, Kawamura M, Ichikawa K, Suzuki T, Sumida T, Toshida S, Matsuura E, Ikehara S, Koike T: Anticardiolipin antibodies in NZW × BXSB F1 mice. A model of antiphospholipid syndrome. J Immunol 149:1063–1068, 1992
19. Ikehara S, Nakamura T, Sekita K, Muso E, Hasunizu R, Ohtsuki H, Hamashima Y, Good R: Treatment of systemic and organ-specific autoimmune disease in mice by allogeneic bone marrow transplantation. Prog Clin Biol Res 229:131–146, 1987
20. Himeno K, Good RA: Marrow transplantation from tolerant donors to treat and prevent autoimmune diseases in BXSB mice. Proc Natl Acad Sci USA 85:2235–2239, 1988
21. 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 100:111–117, 1995
22. Ishida T, Inaba M, Hisha H, Sugiura K, Adachi Y, Nagata N, Ogawa R, Good RA, Ikahara S: Requirement of donor-derived stromal cells in the bone marrow for successful allogeneic bone marrow transplantation. Complete prevention of recurrence of autoimmune diseases in MRL/MP-lpr/lpr mice by transplantation of bone marrow plus bones (stromal cells) from the same donor. J Immunol 152:3119–3127, 1994
23. Deapen D, Escalante A, Weinrib L, Horowitz D, Bachman B, Roy-Burman P, Walker A, Mack TM: A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 35:311–318, 1992
24. Bock SR, Winfield JB, Lockshin MD, D'Angelo WA, Christian CL: Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. Am J Med 59:533–552, 1975
25. Rajagopalan S, Zordan T, Tsokos GC, Datta SK: Pathogenic anti-DNA autoantibody-inducing T helper lines from patients with active lupus nephritis: Isolation of CD8-T helper cell lines that

express the gamma delta T-cell antigen receptor. Proc Natl Acad Sci USA 87:7020, 1990
26. Vassilopoulos D, Kovacs B, Tsokos GC: TCR/CD3 complex-mediated signal transduction pathway in T cells and cell lines from patients with systemic lupus erythematosus. J Immunol 155:2269, 1995
27. Liossis SN, Ding DZ, Dennis GJ, Tsokos GC: Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. J Clin Invest 101:1448, 1998
28. Rengaraju M, Gray JD, Horwitz DA, Kubin M, Trinchierir G: Increased spontaneous IL-10 production and decreased mitogen-induced INF$\gamma$ and TNF$\alpha$ production in untreated patients with SLE: Role of CD8+ T cells. Lupus 4(suppl 2):82, 1995
29. Berstein RM, Hobbs RN, Lee DJ, Ward DJ, Hughes GRV: Patterns of antihistone antibody specificity in systemic rheumtic disease. I. Systemic lupus erythematosus, mixed connective tissue disease, primary sicca syndrome, and rheumatoid arthritis with vasculitis. Arthritis Rheum 28:285–293, 1985
30. Marmont AM: New horizons in the treatment of autoimmune diseases: Immunoablation and stem cell Transplantation. Ann Rev Med 51:115–134, 2000
31. Symmons DPM, Coopock JS, Bacon PA, Bresnihan B, Isenberg DA, Maddison P, Mchugh N, Snaith ML, Zoma AS: Development and assessment of a computerized index of clinical disease activity in systemic lupus erythematosus. QJ Med 68:927–937, 1988
32. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH: Derivation of the SLEDAI. A disease activity index for lupus patients. The committee on prognosis studies in SLE. Arthritis Rheum 35:630–640, 1992
33. Liang MH, Socher SA, Roberts WN, Esdaile JM: Measurement of systemic lupus erythematosus activity in clinical research. Arthritis Rheum 31:817–825, 1988
34. Petri M, Bochemstedt L, Colman J, Whiting-O'Keefe Q, Fitz G, Sebastin A, Hellman D: Serial assessment of glomerular filtration rate in lupus nephropathy. Kidney Int 34:832–839, 1988
35. Gladman DD, Ginzler E, Goldsmith C, Fortin P, Liang M, Urowtz M, Bacon P, Bombardi S, Hanly J, Hay E, Isenberg J, Jones J, Kalunian K, Maddison P, Nived OT, Petri M, Richter M, Sanchez-Guerrero J, Snaith M, Sturfelt G, Symmons D, Zoma A: The development and international validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for systemic lupus erythematosus. Arthritis Rheum 39:363–369, 1996
36. Ginzler EM, Diamond HS, Weiner M, Schlesinger M, Fries JF, Wasner C, Medsger TA Jr, Zieger G, Klippel JH, Hadler NM, Albert DA, Hess EV, Spencer-Green G, Grayzel A, Worth D, Hahn BH, Barnett EV: A multicenter study of outcome of systemic lupus erythematosus. I. Entry variables as predictors of progress. Arthritis Rheum 25:601–611, 1982
37. 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 22:1265–1270, 1995
38. Harisdangkul V, Nilganuwonge S, Rockhold L: Cause of death in systemic lupus erythematosus: A pattern based on age at onset. South Med J 80:1249–1253, 1987
39. Marmont AM, van Lint MT, Gualandi F, Bacigalupo A: Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. Lupus 6:545–548, 1997
40. 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 8:320–323, 1999
41. Burt RK, Traynor AE, Ramsey-Goldman R: Hematopoietic stem-cell transplantation for systemic lupus erythematosus (letter). New England Journal of Medicine 337:1777–1778, 1997
42. Burt RK, Traynor AE, Pope R, Schroeder J, Cohen B, Karlin KH, Lobeck L, Goolsby C, Rowlings P, Davis FA, Stefoski D, Terry C, Keever-Taylor C, Rosen S, Vesole D, Fishman M, Brush M, Mujias S, Villa M, Burns WH: Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. Blood 92:3505–3514, 1998
43. Musso M, Poretto F, Crescimano A, Bondi F, Polizzi V, Scalone R, Mariani G: Autologous peripheral blood stem and progenitor ($CD34^+$) cell transplantation for systemic lupus erythematosus complicated by Evans syndrome. Lupus 7:492–494, 1998
44. Brodsky RA, Petri M, Smith BD, Steifter J, Spivak JL, Styler M, Dang CV, Bridsky I, Jones R: Immunablative high dose cyclophosphamide without stem cell rescue for refractory severe autoimmune disease. Ann Intern Med 129:1031–1035, 1998
45. Burt RK, Traynor AE: Hematopoietic stem cell therapy of autoimmune disease. Current Opinion in Hematology 5:472–477, 1998