Stem Cell Transplantation in Systemic Sclerosis: Rationale and Status Report

Daniel E. Furst MD[1,2] and Alan Tyndall MD[3]

[1]Virginia Mason Research Center and [2]University of Washington, Seattle, WA, USA; and 3Rheumatologische Universitatklinik, Basel, Switzerland

Key words: stem cell transplantation, systemic sclerosis, immunosuppression, bone marrow transplantation, scleroderma

IMAJ 2000;2:935–937

A hypothesis has been posited that the pathogenesis of systemic sclerosis is, in part, immune mediated and, in part, mediated through abnormalities in the vascular system. The combination of these two aspects of SSc pathogenesis, stimulated by external stimuli and against the background of a genetic susceptibility, results in fibroblast proliferation, collagen deposition and further immune stimulation [Figure 1] [1]. If this hypothesis is valid, interruption of the immune-mediated portion of the pathogenetic cycle should result in improvement in systemic sclerosis.

In this paradigm, immune effector cells would be eradicated or substantially reduced in number. Eradication of the effector arm of the immune system would thereafter control the disease. In fact there have been a number of immunosuppressive therapies attempted for systemic sclerosis. Chlorambucil was tested in a double-blind trial of systemic sclerosis but appeared to be ineffective [2]. However, in that trial disease duration was long and it is possible that the immune system was no longer of primary importance. The results of a 6 month double-blind trial of 5-fluorouracil versus placebo in a small number of patients (n=20 and 26 per group) showed greater improvement in skin score and global response in the treated than in the placebo group ($P<0.05$) [3]. However, most patients developed some gastrointestinal toxicity (96%) or leukopenia (42%), and the authors felt that the therapeutic ratio was so poor that they judged the therapy ineffective at the time [3]. Uncontrolled trials of cyclophosphamide for SSc lung disease indicated the possibility that cyclophosphamide might be effective in SSc patients [4]. An open trial of cyclosporine for SSc compared to historical controls seemed to indicate that this drug improved the skin score in 8 of 10 patients. Once more, toxicity (in this case, sometimes irreversible changes in creatinine) mitigated against the use of this compound [5].

A more definitive test of this hypothesis would require nearly complete eradication of the immune system. In the context of an essentially eradicated immune response arm, a new non-autoreactive immune system could be recapitulated so that the original disease triggers would no longer be operational in this newly constituted immune system and the disease would cease progressing. Healing, as much as possible, could then commence. Stem cell transplantation would be an effective way by which this experiment could be done.

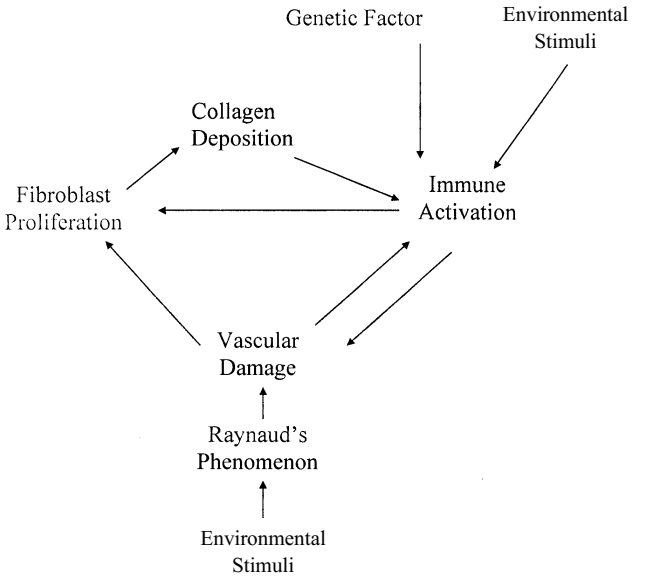


Figure 1. Hypothesis for the pathogenesis of systemic sclerosis (modified from Clements PJ, Furst DE, eds, *Systemic Sclerosis*, 1996).

It is clear, however, that patient selection for immunosuppression or immunoablation is extremely important. The ideal patient would be one in whom the immune system is active, in whom the disease is progressing rapidly, who has significantly reversible disease, and whose prognosis is predictably poor. While data that the immune system is active in systemic sclerosis are not incontrovertible, antibody-dependent cellular cytotoxicity is apparent in SSc, as shown by increased endothelial and fibroblast cytotoxicity in response to SSc mononuclear cells [6]. Increased CD4 positive, activated mononuclear cells are found in the skin of SSc patients [7]. Further, increased CD8 positive lymphocytes are found in the bronchoalveolar lavage of SSc patients

SSc = systemic sclerosis

[8]. Studies indicated that the immune system is active early in the disease, as demonstrated by the presence of lymphocyte and plasmacyte infiltrates found in early skin biopsies in SSc patients [9].

The most rapid change in patients with diffuse cutaneous SSc occurs in the first few years and it is during that time, too, that most internal organ involvement becomes manifest [10]. A good example of the rapidity of change in the internal organs is seen when measuring pulmonary function tests during the first few years of disease. The same group of authors found that during years 0–2, vital capacity changed 32% per year in one group of patients; during years 2–4 the average percent decrement was 12% and during years 4–6 only 3% [11]. Thus, it appears that patients who would be suitable for stem cell transplantation would be those with disease of less than 4 years duration. However, since SCT is known to be an aggressive and toxic therapy (5–10% mortality after SCT for malignancy) it would be appropriate to use SCT only in those patients whose prognosis is predictably poor.

Prognostic predictors are definable in systemic sclerosis. Patients with skin scores higher than 14 on a scale of 0–31 (UCLA Skin Score) a 5 year survival of approximately 50% compared to those whose skin score is less than 14, where survival approaches 90% at 5 years [12]. Cardiovascular survival is predictable based on the presence or absence of moderate to large pericardial effusions, left ventricular hypertrophy or left ventricular deviation on electrocardiogram, presence of congestive heart failure or presence of a moderate to large effusion plus left axis deviation [13]. Pulmonary function abnormalities predict 8 year survival [14]. Renal crisis, too, can be predicted, at least partially [15].

Based on data such as these, inclusion and exclusion criteria were developed for a pilot study of SCT. Entrance criteria included the diagnosis of diffuse systemic sclerosis of ≤3 years duration with a modified Rodnan Skin Score of >20 (max=51). In addition, patients had to have organ dysfunction as defined by at least one of the following:

- pulmonary involvement: forced vital capacity <80%, single-breath diffusion capacity <70% or active alveolitis by BAL.
- renal involvement, creatinine >1.3 (above the upper limit of normal of the test) or 24 hour urine protein >500 mg.
- cardiovascular involvement as defined by arrhythmia requiring therapy, cardiomegaly on X-ray, moderate or large pericardial effusion, and left axis deviation on echocardiogram.

Exclusion criteria were developed such that patients could survive the rigors of SCT. These included age under 62, pregnancy, positive human immunodeficiency virus test, DLCO <45%, creatinine >2.0 at the time of SCT, glomerular filtration rate <40 ml/min at time of SCT, uncontrolled malignant arrhythmia or congestive heart failure, ejection fraction <50%, aspartate aminotransferase >2.5 times the upper limit of normal, bilirubin <2.0 but not related to hemolysis, severe marrow hypoplasia or myelodysplasia, or use of alkylating agents for >12 months.

Thomas Medsger and Virginia Steen, at the University of Pittsburgh at the time, analyzed their prospective cohort of more than a thousand SSc patients. They examined patients selected according to the above-mentioned entry criteria with no exclusion criteria for our protocol and compared them to a control group of early diffuse SSc patients without visceral involvement. The 5 year survival was approximately 85% in the control group compared to approximately 50% in the experimental group (unpublished). It appeared, therefore, that our inclusion and exclusion criteria were appropriate for selecting a group of patients with severe disease who had a poor prognosis.

These criteria have been applied in a series of pilot studies examining the usefulness of SCT for the treatment of diffuse cutaneous SSc with visceral involvement. A registry of such patients is maintained under the aegis of the European Bone Marrow Transplant Society and under the central guidance of Alan Tyndall at the University of Basel in Switzerland. The early combined SSc experience was recently analyzed [submitted]. In August 1999, 41 patients with SSc had undergone SCT (according to this registry) – 5 in Freiburg (Germany), 4 in Leiden (the Netherlands), 7 in London (UK), 4 in Paris (France), 8 in Seattle, USA), and 12 in other locations including Leeds, Malaga, Madrid, Los Angeles, Birmingham, Hanover and Berlin.

Mobilization regimens had included granulocyte colony-stimulating factor only (n=10), GCSF plus cyclophosphamide (n=29), and cyclophosphamide alone (n=1). One patient received stem cells from bone marrow. Purging utilized a CD34+ selection methodology plus or minus additional lymphocyte depletion in 37 cases. No purging was performed in four patients (one bone marrow and three peripheral stem cell harvests). A variety of conditioning regimens was used, although most of them employed cyclophosphamide at doses between 120 and 200 mg/kg. Anti-thymocyte glubulin (or Campath) and total body irradiation, alone or in various combinations were also used. As in the other cases, data were missing in some patients.

Among the 41 patients, approximately 10% died secondary to disease progression, while more than 15% died of procedure-related causes. Death was thought to be due to cyclophosphamide alone in some cases, and to a combination of total body irradiation and cyclophosphamide in others. There were also two deaths from bleeding complications: pulmonary hemorrhage in one and central nervous system bleed in the other. As a whole, more than two-thirds showed

SCT = stem cell transplantation
BAL – bronchoalveolar lavage
DLCO = single-breath diffusion capacity
GCSF = granulocyte colony-stimulating factor

an improvement in skin score of ≥25%, while pulmonary function tended to stabilize.

In the Seattle experience, five patients showed an excellent response, 1 patient demonstrated significant improvement but relapsed (the institution of cyclosporine in that patient seems to have regained control of the disease), and two patients died, probably due to regimen-related toxicity (the regimen has now been changed).

Summary

The rationale for stem cell transplantation is feasible and tests the hypothesis that the immune system is important in the pathogenesis of SSc in carefully selected patients. At the present time, results from pilot studies of SCT in systemic sclerosis are moderately encouraging, although further refinements in SCT treatment protocols are probably necessary. Finally, the time for a controlled study of SCT is rapidly approaching.

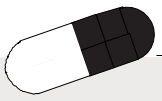
Acknowledgement. Partially supported by the Rasmuson Center for Arthritis, Orthopaedics and Musculoskeletal Diseases and the Richard Barnett Meurk Scleroderma Endowment.

References

1. Furst DE, Clements PJ. Pathogenesis, fusion (Summary). In: PJ Clements, DE Furst, eds. Systemic Sclerosis. Baltimore: Williams & Wilkins, 1996:275–86.
2. Furst DE, Clements PJ, Hillis S, Lachenbruch PA, Miller B, Sterz M, Paulus HE. Immunosuppression with chlorambucil is not better than placebo for scleroderma (PSS): results of a three year, parallel, randomized, double-blind study. *Arthritis Rheum* 1989;32:584–93.
3. Casas JA, Saway PA, Villarreal I, Nolte C, Menajovsky BL, Escudero EE, Blackburn WD, Alarcon GS, Subauste CP. 5-fluorouracil in the treatment of scleroderma: a randomised, double-blind, placebo-controlled international collaborative study. *Ann Rheum Dis* 1990;49:926–8.
4. Kahaleleh MB, Fan P-S. Monthly intravenous cyclophosphamide treatment of scleroderma. *Arthritis Rheum* 1996;39:s151 (Abstr 747).
5. Clements PJ, Lachenbruch PA, Sterz M, Danovitch G, Hawkins R, Ippoliti A, Paulus HE. Cyclosporine in systemic sclerosis. *Arthritis Rheum* 1993;36:75–9.
6. Marks RM, Czerniecki M, Andrews BS, Penny R. The effects of scleroderma serum on human microvascular endothelial cells. Induction of antibody-dependent cellular cytotoxicity. *Arthritis Rheum* 1988;31:1524–31.
7. Roumm AD, Whiteside TL, Medsger TA Jr, Rodnan GP. Lymphocytes in the skin of patients with progressive systemic sclerosis: quantification subtyping and clinical correlations. *Arthritis Rheum* 1984;27:645.
8. Atamas SP, Yurovsky VV, Wise R, Wigley FM, Goter Robinson CJ, Henry P, Alms WJ, White B. Production of type 2 cytokines by CD8+ lung cells is associated with greater decline in pulmonary function in patients with systemic sclerosis. *Arthritis Rheum* 1999;42:1168–78.
9. Prescott RJ, Freemont AJ, Jones CJ, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. *J Pathol* 1992;166(3):255–63.
10. Medsger Jr TA, Steen VD. Classification, prognosis. In: Clements PJ, Furst DE, eds. Systemic Sclerosis. Baltimore: Williams & Wilkins, 1996:51–64.
11. Steen VD, Conte C, Owens GR, Medsger Jr TA. Severe restrictive lung disease in systemic sclerosis. *Arthritis Rheum* 1994;37:1283–9.
12. Clements PJ, Lachenbruch PA, Ng SC, Simmons M, Sterz M, Furst DE. Skin score: a semi-quantitative measure of cutaneous involvement which improves prediction of prognosis in systemic sclerosis. *Arthritis Rheum* 1990; 33:1256–63.
13. Clements PJ, Lachenbruch PA, Furst DE, Paulus HE, Sterz MG. Cardiac score: a semi-quantitative measure of cardiac involvement which improves prediction of prognosis in systemic sclerosis. *Arthritis Rheum* 1991;34:1371–80.
14. Tashkin DP, Clements PJ, Wright RS, Gon H, Simmons MS, Lachenbruch PA, Furst DE. Inter-relationships between pulmonary and extra-pulmonary involvement in systemic sclerosis (SSc): a longitudinal analysis. *Chest* 1994; 105:489–95.
15. Steen VD. Organ involvement: renal. In: Clements PJ, Furst DE, eds. Systemic Sclerosis. Baltimore: Williams & Wilkins, 1996:425–39.
16. Clements PJ, Furst DE. Choosing appropriate patients with systemic sclerosis for treatment by autologous stem cell transplantation. *J Rheum* 1997;24(48):85–8.

Correspondence: Dr. D. E. Furst, Virginia Mason Research Center, 1201 Ninth Ave R1-RHE, Seattle, WA 98107, USA. Phone: (1-206) 223-6836, Fax: (1-206) 223-7554, email: crgdef@vmmc.org

Capsule



Signaling through an "AND" gate

Control of cell shape and motility requires integration of multiple signals that ultimately influence polymerization of the actin cytoskeleton. The WASP protein (for **W**iskott-**A**ldrich **s**yndrome **p**rotein – defects that cause thrombocytopenia, eczema, and immunodeficiency in humans) interacts with the small guanosine triphosphatase Cdc42 and phosphatidylinositol 4,5-bisphosphate (PIP2), which are both mediators of signaling pathways that cause alterations in the actin cytoskeleton. WASP also interacts with the actin-related protein 2/3 (Arp2/3) complex, which stimulates actin nucleation.

Prehoda et al. examined how the WASP protein processes multiple inputs to coordinate the activity of Arp2/3 and actin polymerization. Their results indicate that N-WASP (neuronal WASP) exists in a "closed" state in which Arp2/3 is bound but inactive, and the binding sites for Cdc42 and Arp2/3 are inaccessible. Binding of either Cdc42 or PIP2 appears to promote an active conformation. Activation by Cdc42 and PIP2 is highly cooperative, and thus WASP can function as a "coincidence detector" or "logical 'AND' gate" that is highly activated when it receives signals from both Cdc42 and PIP2.

Science 2000;290:801