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Progressive Systemic Sclerosis- from the molecular background to innovative therapies

Peter Szodoray[1], Emese Kiss[2]

[1]Immunobiological Laboratory, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway, [2]National Institute of Rheumatology and Physiotherapy, Budapest, Hungary

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1. ABSTRACT

Systemic sclerosis (SSc) is a complex autoimmune disorder. The cornerstones of the pathogenesis are vascular damage, fibrogenesis and various cellular and humoral autoimmune processes. The aim of the present review is to describe pathogenic steps, leading to the hallmark clinical picture of SSc. Indeed, numerous therapeutical approaches have been tested/are in use, directed towards vascular damage, fibrogenesis, as well as autoimmune processes in order to decelerate the progression of the disease. These therapies are also discussed in the review. Finally, we described certain novel immune-modulating possibilities, namely autologous stem-cell transplantation and extracorporeal photochemotherapy.

2. INTRODUCTION

Systemic sclerosis (SSc) is a prototypical systemic autoimmune disease, characterized by a wide spectrum of clinical manifestations, driven by disproportional collagen deposition in the blood vessels, skin and internal organs (lungs, heart, gastrointestinal tract, kidneys). Probably, the earliest pathological changes in patients with SSc are in the endothelial cell function leading to the damage of the microvasculature (1,2). This initial damage is subsequently followed by inflammatory cell-migration, leading to a wide array of endothelial injuries, namely arterial intimal fibrosis and extensive functional loss of these blood vessels (3).

The pathology behind the uncontrolled fibrotic

processes are partly driven by cytokines and growth factors, such as transforming growth factor-beta (TGF-β), connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), as well as and endothelin-1 (ET-1), secreted in the skin and lungs, leading to fibroblast activation, promoting accumulation of collagen, proteoglycans, fibronectin, elastin, as well as tenascin (4). Besides these factors, other cytokines e.g. interleukin (IL)-13, IL-21, chemokines, including CCL2 (monocyte chemoattractant protein-1 (MCP-1)/(MCAF) and macrophage inflammatory protein 1-beta (MIP-1β), angiogenic factors, namely vascular endothelial growth factor (VEGF), peroxisome proliferator-activated receptors (PPARs), acute phase proteins, as serum amyloid P protein (SAP), caspases, and components of the renin-angiotensin-aldosterone system (ANG II) have been identified as important regulators of fibrosis (5), therefore may also be relevant in the development of fibrosis in SSc.

Bone marrow-derived mesenchymal progenitor cells, denoted as fibrocytes maintain a constant re-load for the expanding fibroblast population within the fibrotic lesional tissue in SSc, therefore further contribute to the connective tissue accumulation (6). The aforementioned locally produced chemoattractant factors drive this migration, leading to fibrosis and eventual organ damage. Moreover, the biosynthesis of collagen molecules involves several intracellular post-translational modifications, followed by excretion and extracellular aggregation of the collagen molecules into fibrils, which are subsequently stabilized by intermolecular cross-links (7,8).

The importance of post-translational collagen cross-linking is in the pathogenesis of SSc is inevitable. The increase in pyridinoline cross-links is likely to be the result of increased activity of the enzyme responsible for the hydroxylation of the telopeptides, namely telopeptide lysyl hydroxylase (TLH). The highly increased expression of TLH in fibroblasts cultured from the fibrotic skin of SSc patients further reinforces the pathogenetic importance of the irreversible accumulation of cross-linked collagen in fibrotic tissues in these patients (9).

The microarray analysis of SSc revealed that these patients can be distinguished by unique gene expression signatures and besides the fibrotic program, the results were indicative of cell proliferation, and immunological alterations (10).'

Although, the pathogenesis of the disease is still unclear, the immunological damage can be explained by the increased T-cell activation. Activated T-helper lymphocytes secrete various fibrosis-related cytokines, such as IL-1, IL-4, IL-13, TGF-β, as well as interferon-gamma (IFN-γ), leading to fibrocyte migration, and eventual collagen deposition. In parallel, activated T-cells interact with B-lymphocytes, leading to B-cell proliferation and differentiation into plasma cells (11).

The overt humoral immune responses generate autoantibodies, including anti-topoizomerase I, as well as further pro-inflammatory/fibrogenic cytokines, resulting in, again, fibroblast activation and vascular damage (11).

Unfortunately, SSc is still non-curable, and in many instances even to delay the disease progression is a challenge. Life-threatening complications may develop due to acute renal insufficiency (scleroderma's renal crisis) or pulmonary hypertension.

3. THERAPEUTIC APPROACHES IN SYSTEMIC SCLEROSIS

Generally, the treatment modalities in SSc are targeting the following four components in the pathogenesis: immune-modulating agents aiming to decelerate damaging, autoimmune processes; treatment, improving rheological parameters; anti-fibrotic agents; also various symptomatic therapeutical possibilities. Recently, novel treatment alternatives have been introduced, namely autologous stem-cell transplantation and extracorporeal photopheresis, which may open new avenues and improve the quality of life of SSc patients.

3.1. Immune-modulating treatment

Since various immune-competent cell types are involved in the pathogenesis of SSc, therapies have been developed, targeting a wide array of cellular and humoral immune responses. Corticosteroids are still the basis of treatment for most autoimmune diseases and in SSc, the administration of the medication has been shown to improve both the skin fibrosis or organ involvement (12). In contrast, it has been described that high-dose corticosteroid application (≥ 15 mg/day) may contribute to the development of renal crisis (13). It is of utmost importance to emphasize that in general, corticosteroid treatment is a double-edged sword; although theoretically it is beneficial in SSc, its therapeutical use is limited, since it can induce renal crisis. In SSc treatment, glucocorticosteroids can be used in patients with alveolitis, pericarditis, or associated myositis.

Besides glucocorticosteroids, methotrexate, the alkylating agent, cyclophosphamide, as well as cyclosporine A/tacrolimus, antagonizing calcineurin, an important enzyme in T-cell receptor signaling have been introduced (14). On the other hand, although Cyclosporine A via the reduction of IL-2 secretion can diminish T-cell activation and proliferation, it can cause glomerular sclerosis. This phenomenon raises the possibility that Cyclosporine A can induce fibrosis, presumably not just in the glomerular apparatus, but in other organs, organ-systems as well. Consequently, Cyclosporine A is used with limitations in the treatment of SSc, due to its dangerous side effects. Sirolimus, also known as rapamycin is used in patients with SSc and inhibits the response to interleukin-2 (IL-2) and thereby blocks activation of T- and B-cells. In contrast, Tacrolimus inhibits the production of IL-2 (Table 1).

3.2. Medications, affecting rheological parameters

Another therapeutical intervention in SSc is to improve rheological parameters and therefore increase circulation both in the skin and internal organs. The periodic vasospasm episodes finally lead to irreversible morphological changes and vascular damage. Accordingly,

Table 1. Immune-suppressive/modulating treatment in SSc

Name	Description/Function
Glucocorticosteroid	Besides general anti-inflammatory properties, suppresses cell-mediated immunity. Inhibits genes that code for the cytokines IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8 (CXCL8) and IFN-γ. Reduces T cell proliferation, increases T-cell apoptosis. Suppresses humoral immune responses, reduces IL-2 and IL-2R expression of B-cells. Reduces both B cell clonal expansion and antibody synthesis. Down-regulates the expression of Fc receptors on macrophages, leading to decreased phagocytosis of opsonised cells.
Methotrexate	Antimetabolite and antifolate drug, acts by inhibiting the metabolism of folic acid. Competitively and reversibly inhibits dihydrofolate reductase (DHFR), also enzymes involved in purine metabolism, leading to accumulation of adenosine, or the inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells. Suppresses IL-1, IL-2 and IL-6.
Cyclophosphamide	Nitrogen mustard alkylating agent, cross-links DNA, reduces the function and number of T-helper and activated T cells, reduces the number of B cells.
Cyclosporine-A/ tacrolimus	Binds to the cytosolic protein, cyclophilin of T-cells. The cyclosporine-cyclophilin complex inhibits calcineurin, which, is responsible for activating the transcription of IL-2. It also inhibits interleukin release and reduced function of effector T-cells. Reduces IL-2 synthesis and release, T-cell responses and interaction, reduces IFN-γ production, B and natural killer (NK) cell function, macrophage and T-cell interaction.
Sirolimus	Inhibits responses to IL-2 and thereby blocks activation of T- and B-cells. The mode of action of Sirolimus is to bind the cytosolic protein FK-binding protein 12 (FKBP12) in a manner similar to tacrolimus.

endothelin receptor antagonists, phosphodiestrase-inhibitors, pentoxifyllin, prostaglandin I2 (PGI2/ prostacyclin), calcium-channel blockers, as well as A1 adrenergic receptor antagonists (Prazosin) and 5-hydroxytryptamine (5-HT2A) (serotonin) receptor antagonists (Ketanserine) have been shown to be effective in this group (14). These agents improve rheological parameters by increasing vasodilation, also can act through the improvement of erythrocyte-flexibility. Recently, novel drug developments are targeting endogenous nitrate release, which does not lead to quick vasodilation and subsequent hypotonia, but initiate a continuous, modified nitric oxide (NO) release and stable vasodilation. This effect can prevent the aforementioned periodic vasospasm and vascular damage in the capillaries. The other problem with the system is that although high VEGF levels have been shown in patients with SSc, angiogenesis is severely impaired, since the number of endothelial progenitor cells is reduced, and impaired expression of vascular endothelial growth factor receptor 1 has been described in the disease (15).

3.3. Anti-fibrosis agents

Since disproportional, increased fibrosis is a key feature of patients with SSc, an important direction in current therapeutical developments is to decelerate these fibrotic processes. Imatinib is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes. Imatinib mesylate and related tyrosine kinase inhibitors also block TGF-β pathways and have the ability to slow down fibrotic responses. Microarray- gene expression profile analyses, as well as studies of genetic polymorphisms in TGF-β signaling could aid in identifying patients who are most likely to respond to anti-TGF-β treatment (16). Besides imatinib, anti-TGF-β monoclonal antibody (mAb), as well as matrix metalloproteinases (MMPs) have the potential to decelerate accelerated fibrosis in SSc.

3.1.4. Symptomatic treatment

Several associated symptoms are common in patients with SSc. The disease affects the gastrointestinal system in more than 80% of patients. Changes in the neuromuscular function with progressive fibrosis of smooth muscle within the muscularis propria impair normal motor function, which may secondarily alter transit and nutrient absorption. The gastrointestinal symptoms include gastro-esophageal reflux and dysphagia, therefore antacids are of pivotal importance in the disease management, namely histamine 2 receptor antagonists (H2RA) and proton pump inhibitors (PPI). Gastroparesis, can be found in approximately 50% of patients. Severe small bowel disease can present as chronic intestinal pseudo-obstruction with distended loops of the small intestine, bacterial overgrowth, impaired absorption and progressive development of nutritional deficiencies (17). Accordingly, a great variety of prokinetic agents are used in SSc.

4. NOVEL IMMUNE-MODULATING APPROACHES

4.1. Autologous stem-cell transplantation (ASCT) in systemic sclerosis (SSc)

Patients, who are candidates for autologous stem-cell transplantation (ASCT) have rapid disease progression, including significant deterioration of skin symptoms and incipient kidney and/or lung involvement. Until now, approximately one hundred patients with SSc have gone through the transplant procedures in the main European and North American centers. The most frequently used conditioning protocol was high-dose cyclophosphamide (HDC) (200 mg/kg), along with anti-thymocyte globulin (ATG) and/or total body irradiation. T-cell ablation by CD34+-selection seemed to have an advantage in the treatment results. Previously, transplant-related mortality was over 10%, fortunately it has been improved to 2.5% in the recent years, due to stringent patient selection. An improvement of 25% or more in the skin score (measured by the modified Rodnan method) was reported in 70% of the patients following transplantation. Furthermore, lung function stabilized in most of the cases and renal function generally remained stable. After a follow-up period up to 60 months, 35% of the patients showed sustained improvement of the disease, while 25% progressed only after a transitional remission. Based on the favorable results of phase I/II studies (18-20), randomized, controlled studies were launched. The ASTIS trial is still running and tries to determine the superiority of ASCT over pulse cyclophosphamide treatment alone (control arm) (21-25).

Along with conventional treatment modules, ASCT by the beneficial effect of the repopulated, immunologically re-programmed, naïve stem cells could

play a significant role in the future management of SSc, leading to a better quality of life of these patients (26).

4.2. Extracorporeal photochemotherapy in systemic sclerosis (SSc)

Similar to plasmapheresis, extracorporeal photochemotherapy (photopheresis or ECP) is based on apheresis technology. The extracorporeal exposure of isolated peripheral blood mnonuclear cells (PBMCs) to 8-methoxypsoralen (8-MOP) and ultraviolet (UV)A light is followed by reinfusion of the treated cells (27). Despite the efficiency of this procedure and the fact that ECP is in the focus of researches for a long time, the biologic mechanism of its exact action, which leads to immune-suppression, is not clearly understood in detail. Approximately 2-5% of the total peripheral leukocytes during each procedure is exposed to the photoactive drug 8-MOP (28). 8-MOP, due to the UVA light covalently binds and crosslinks DNA, which damages cellular DNA, induces apoptosis in the majority of treated cells (29).

Another possible mechanism of action is that ECP induces a significant increase of CD4+CD25+ regulatory T-cell levels, presumably leading to the deceleration of autoimmune responses (30).

In addition, ECP causes a decrease in monocytoid dendritic precursors and an increase in plasmacytoid dendritic precursors. This change is appeared to be associated with a shift in the cytokine profile of cultured T cells from IL-2 and IFN-γ producing T helper (Th)1 cells to IL-10 producing Th2 cells (31).

Moreover, ECP leads to the increased expression of surface antigens by autoreactive T cells recognized by suppressor CD8+ T cells (32).

Photopheresis has been shown to induce significant improvement of skin and joint involvement in patients with scleroderma of recent onset (33), yet further multi-center studies are required to assess the real value of this procedure in SSc.

5. CONCLUDING REMARKS

In the pathogenesis of SSc several pathological processes simultaneously lead to disproportional fibrosis, vascular damage and organ failure, therefore the complex management of the disease, targeting these processes in parallel is pivotal for the more favorable disease outcome in SSc. Novel therapeutical approaches, namely ASCT and ECP can aid in the modern disease management, leading to sustained, better quality of life of these patients.

6. REFERENCES

1. R. Sgonc, M.S. Gruschwitz, G. Boeck, N. Sepp, J. Gruber and G. Wick: Endothelial cell apoptosis in systemic sclerosis is induced by antibody-dependent cell-mediated cytotoxicity via CD95. *Arthritis Rheum* 43, 2550–2562 (2000)

2. R. Sgonc, M.S. Gruschwitz, H. Dietrich, H. Recheis, M.E. Gershwin and G. Wick: Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J Clin Invest* 98, 785–792 (1996)

3. A. Kalogerou, E. Gelou, S. Mountantonakis, L. Settas, E. Zafiriou and L. Sakkas: Early T-cell activation in the skin from patients with systemic sclerosis. *Ann Rheum Dis* 64, 1233–1235 (2005)

4. J. Varga: Systemic sclerosis: an update. *Bull NYU Hosp Jt Dis* 66, 198-202 (2008)

5. T.A. Wynn: Cellular and molecular mechanisms of fibrosis. *J Pathol* 214, 199-210 (2008)

6. A.E. Postlethwaite, H. Shigemitsu and S. Kanangat: Cellular origins of fibroblasts: possible implications for organ fibrosis in systemic sclerosis. *Curr Opin Rheumatol* 16, 733-8 (2004)

7. M.E. Nimni: The molecular organization of collagen and its role in determining the biophysical properties of the connective tissues. *Biorheology* 17, 51-82 (1980)

8. B.R. Olsen. Collagen biosynthesis. In: E.D. Hay (ed). *Cell biology of extracellular matrix.* New York, Plenum Press, 139-77 (1981)

9. A.J. van der Slot, A.M. Zuurmond, A.F. Bardoel, C. Wijmenga, H.E. Pruijs, D.O. Sillence, J. Brinckmann, D.J. Abraham, C.M. Black, N. Verzijl, J. DeGroot, R. Hanemaaijer, J.M. TeKoppele, T.W. Huizinga and R.A. Bank: Identification of PLOD2 as telopeptide lysyl hydroxylase, an important enzyme in fibrosis. *J Biol Chem* 278, 40967-72 (2003)

10. A. Milano, S.A. Pendergrass, J.L. Sargent, L.K. George, T.H. McCalmont, M.K. Connolly and M.L. Whitfield: Molecular subsets in the gene expression signatures of scleroderma skin. *PLoS ONE* 3, e2696 (2008)

11. Y.S. Gu, J. Kong, G.S. Cheema, C.L. Keen, G. Wick and M.E. Gershwin: The immunobiology of systemic sclerosis. *Semin Arthritis Rheum* 38, 132-60 (2008)

12. B. White, W.C. Moore, F.M. Wigley, H.Q. Xiao and R.A. Wise: Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. *Ann Intern Med* 132, 947-954 (2000)

13. V.D. Steen and T.A. Medsger Jr: Case–control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. *Arthritis Rheum* 41, 1613-1619 (1998)

14. V.K. Bournia, P.G. Vlachoyiannopoulos, C. Selmi, H.M. Moutsopoulos and M.E. Gershwin: Recent advances in the treatment of systemic sclerosis. *Clin Rev Allergy Immunol* 36, 176-200 (2009)

15. J. Avouac, J. Wipff, O. Goldman, B. Ruiz, P.O. Couraud, G. Chiocchia, A. Kahan, C. Boileau, G. Uzan and Y. Allanore: Angiogenesis in systemic sclerosis: impaired expression of vascular endothelial growth factor receptor 1 in endothelial progenitor-derived cells under hypoxic conditions. *Arthritis Rheum* 58, 3550-61 (2008)

16. J. Varga and B. Pasche: Transforming growth factor beta as a therapeutic target in systemic sclerosis. *Nat Rev Rheumatol* 5, 200-6 (2009)

17. R. Domsic, K. Fasanella and K. Bielefeldt: Gastrointestinal manifestations of systemic sclerosis. *Dig Dis Sci* 53, 1163-74 (2008)

18. M. Sykes and B. Nikolic: Treatment of severe autoimmune disease by stem-cell transplantation. *Nature* 435, 620-7 (2005)

19. J.M. Van Laar and A. Tyndall: Adult stem cells in the treatment of autoimmune diseases. *Rheumatology (Oxford)* 45, 1187-93 (2006)

20. Farge D, Henegar C, Carmagnat M, Daneshpouy M, Marjanovic Z, Rabian C, *et al.* Analysis of immune reconstitution after autologous bone marrow transplantation in systemic sclerosis. Arthritis Rheum. 2005;52:1555-63.

21. D. Farge, C. Henegar, M. Carmagnat, M. Daneshpouy, Z. Marjanovic, C. Rabian, D. Ilie, C. Douay, N. Mounier, E. Clave, D. Bengoufa, J. Cabane, J.P. Marolleau, E. Gluckman, D. Charrona and A. Toubert: EBMT/EULAR Registry. *Ann Rheum Dis* 63, 974-981 (2004)

22. N.P. Nikolov and S.Z. Pavletic: Technology Insight: hematopoietic stem cell transplantation for systemic rheumatic disease. *Nat Clin Pract Rheumatol* 4, 184-91 (2008)

23. J.M. van Laar, D. Farge and A. Tyndall: Autologous Stem Cell Transplantation International Scleroderma (ASTIS) trial: hope on the horizon for patients with severe systemic sclerosis. *Ann Rheum Dis* 64, 1515 (2005)

24. A. Tyndall and D.E. Furst: Adult stem cell treatment of scleroderma. *Curr Opinion Rheumatol* 19, 604-610 (2007)

25. J.M. van Laar, D. Farge and A. Tyndall: Stem cell transplantation: treatment option for severe systemic sclerosis? *Ann Rheum Dis* 67 suppl 3, iii 35-38 (2008)

26. P. Szodoray, L. Varoczy, G. Szegedi and M Zeher: Autologous stem cell transplantation in autoimmune diseases. *Scand J Rheumatol* (2009) (in press)

27. R.L. Edelson: Photopheresis: a new therapeutic concept. *Yale J Biol Med* 62, 565-77 (1989)

28. F.M. Moss: New insights into the mechanism of action of extracorporeal phototherapy. *Transfusion* 46, 6-8 (2006)

29. D. Peritt: Potential mechanisms of photopheresis in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 12, 7-12 (2006)

30. E. Biagi, I. Di Biaso, V. Leoni, G. Gaipa, V. Rossi, C. Bugarin, G. Renoldi, M. Parma, A. Balduzzi, P. Perseghin and A. Biondi: Extracorporeal photochemotherapy is accompanied by increasing levels of circulating CD4+CD25+GITR+Foxp3+CD62L+ functional regulatory T-cells in patients with graft-versus-host disease. *Transplantation* 84, 31-9 (2007)

31. G. Gorgun, K.B. Miller and F.M. Foss: Immunologic mechanisms of extracorporeal photochemotherapy in chronic graft-versus-host disease. *Blood* 100, 941-7 (2002)

32. J. Bladon and P.C. Taylor: Extracorporeal photopheresis: a focus on apoptosis and cytokines. *J Dermatol Sci* 43, 85–94 (2006)

33. R.M. Knobler, L.E. French, Y. Kim, E. Bisaccia, W. Graninger, H. Nahavandi, F.J. Strobl, E. Keystone, M. Mehlmauer, A.H. Rook and I. Braverman; Systemic Sclerosis Study Group. A randomized, double-blind, placebo-controlled trial of photopheresis in systemic sclerosis. *J Am Acad Dermatol* 54, 793–799 (2006)

Abbreviations: ANG II: renin-angiotensin-aldosterone system, ASCT: Autologous stem-cell transplantation, ATG: anti-thymocyte globulin, CTGF: connective tissue growth factor, DHFR: dihydrofolate reductase, ECP: extracorporeal photochemotherapy, ET-1: endothelin-1, FKBP12: FK-binding protein 12, HDC: high-dose cyclophosphamide, 5-HT2A: 5-hydroxytryptamine (serotonin) receptors, H2RA: histamine 2 receptor antagonists, IFN-γ: interferon-gamma, IL: interleukin, mAb: monoclonal antibody, MCP-1: monocyte chemoattractant protein-1, MIP-1β: macrophage inflammatory protein 1-beta, 8-MOP: 8-methoxypsoralen, MMP: matrix metalloproteinase, NK: natural killer cell, NO: nitric oxide, PDGF: platelet-derived growth factor, PGI2: prostaglandin I2, PBMC: peripheral blood mononuclear cells, PPARs: peroxisome proliferator-activated receptors, PPI: proton pump inhibitors, SAP: serum amyloid P protein, SSc: Systemic sclerosis, TGF-β: transforming growth factor-beta, Th: T helper cells, TLH: telopeptide lysyl hydroxylase, UVA: ultraviolet A light, VEGF: vascular endothelial growth factor

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Send correspondence to: Peter Szodoray, Immunobiological Laboratory, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway, Postal address 1105 Blindern, N-0317 Oslo, Norway, Tel: 0047-22-851155; Fax: 0047-22-851278; Email: szodoray@gmail.com

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