

Interleukin 6 in Systemic Sclerosis and Potential Implications for Targeted Therapy

CHAYAWEE MUANGCHAN and JANET E. POPE

ABSTRACT. Objective. The purpose of this study was to review the potential importance of interleukin 6 (IL-6) in systemic sclerosis (SSc).

Methods. PubMed and Scopus databases and American College of Rheumatology (from 2009-10) and European League Against Rheumatism abstracts (2009-11) were searched using keywords “scleroderma; SSc; cytokines; interleukins; interleukin 6” and publications were excluded if not pertaining to IL-6 in SSc. Data were extracted from selected articles to construct a cell interaction model of the effects of IL-6 in SSc.

Results. A total of 416 reports were found (PubMed, n = 82; Scopus, n = 331; 3 abstracts); 372 were excluded (irrelevant) leaving 41 publications and 3 abstracts (39 from PubMed, 18 from Scopus; but 16 were repeated from PubMed search), where 40 suggested IL-6 was important in SSc and 4 did not. Effects of IL-6 in SSc were summarized schematically.

Conclusion. Of the 44 publications, 40 suggested that IL-6 may be important in SSc, allowing for a conceptual framework within SSc including effects on macrophages, fibroblasts, plasma cells, monocytes, and extracellular matrix. (J Rheumatol First Release April 15 2012; doi:10.3899/jrheum.111423)

Key Indexing Terms:

SYSTEMIC SCLEROSIS
SCLERODERMA

INTERLEUKIN 6

TARGETED THERAPY
CYTOKINES

Systemic sclerosis (SSc or scleroderma) affects more women than men, with peak onset between age 40 and 55 years¹. The consequences may be devastating, with increased mortality especially in those with inflammatory markers such as the erythrocyte sedimentation rate (ESR)^{2,3,4,5}. To date, altering cytokines such as interferon have not been proven to be of benefit in SSc⁶.

Interleukin 6 (IL-6) is a pleiotropic proinflammatory multifunctional cytokine produced by many cells such as lymphocytes, fibroblasts, and monocytes⁷. The functions of IL-6 include T cell activation, initiation of acute-phase reactants (e.g., C-reactive protein), and stimulation of hematopoietic precursor cell growth, causing maturation of B cells into antibody-producing cells and cell differentia-

tion. Many autoimmune diseases have upregulation of IL-6 including SSc, rheumatoid arthritis, juvenile idiopathic arthritis, and psoriasis^{8,9}. Basic and clinical research suggests that IL-6 has an important role in the pathobiology of SSc. Thus a systematic review of the literature was performed to determine whether mechanisms influenced by IL-6 within SSc could be identified.

Ethics approval was not required for our study as it is a review using previously published data.

MATERIALS AND METHODS

PubMed and Scopus databases were searched by 2 reviewers from the inception until May 2011 for English-language original articles, and meeting abstracts of both the American College of Rheumatology 2009-10 and European League Against Rheumatism (EULAR) 2009-11 were searched. The key words searched were “scleroderma; systemic sclerosis; SSc; cytokines; interleukin; and IL-6.” Data were extracted from articles (involving studies of any of “in vivo, in vitro, human, animal models”) to construct a cell interaction schematic model of the effects of IL-6 in SSc where each pathway was from 1 or more publications. Negative articles were included in the literature review but not highlighted within the schematic. Data were excluded if they were found to be irrelevant to the objective, i.e., determining the mechanisms of IL-6 in SSc.

RESULTS

Four hundred sixteen publications were found (PubMed, n = 82; Scopus, n = 331; 3 abstracts); 372 were excluded (as irrelevant), leaving 41 publications and 3 abstracts (39 from

From the Division of Rheumatology, Department of Medicine, Faculty of Medicine, Mahidol University, Siriraj Hospital, Bangkok, Thailand; and Division of Rheumatology, St. Joseph's Health Care, University of Western Ontario, Schulich School of Medicine and Dentistry, London, Ontario, Canada.

C. Muangchan, MD, Division of Rheumatology, Department of Medicine, Faculty of Medicine, Mahidol University, Siriraj Hospital; Research Fellow, Rheumatology, University of Western Ontario; J.E. Pope, MD, MPH, FRCPC, Division of Rheumatology, St. Joseph's Health Care, University of Western Ontario, Schulich School of Medicine and Dentistry.

Address correspondence to Dr. J.E. Pope, St. Joseph's Health Care, 268 Grosvenor St., London, Ontario N6A 4V2, Canada.

E-mail: janet.pope@sjhc.london.on.ca

Accepted for publication January 26, 2012.

Table 1. Importance of interleukin 6 (IL-6) in serum of patients with systemic sclerosis (SSc).

1. IL-6 is elevated in serum and skin biopsies from SSc patients^{10,11}. Study 11 demonstrated elevated local dermal expression of IL-6 in established, but not early, diffuse SSc but numbers were small.
2. Serum IL-6 is significantly elevated in patients with early dcSSc¹²⁻¹⁸.
3. Serum IL-6 is related to disease activity of SSc¹²⁻¹⁸.
4. Serum IL-6 is correlated with total skin score and the extent of skin thickening^{13,21}.
5. Serum IL-6 is negatively associated with lung function; lower IL-6 levels if better percentage of forced vital capacity predicted^{13,15} and digital ulcer healing¹⁹.
6. Serum IL-6 is related to erythrocyte sedimentation rate, C-reactive protein, and immunoglobulin levels in SSc^{13,17,20}.
7. SSc serum may have anti-IL-6 antibodies²², which can bind to IL-6 receptors with high affinity²³.

dcSSc: diffuse cutaneous SSc.

PubMed, 18 from Scopus; but 16 were repeated from the PubMed search), where 40 suggested IL-6 was important in SSc and 4 did not. Table 1 summarizes findings for IL-6 in serum of patients with SSc with various disease manifestations. Mechanisms of fibroblast cell-cytokine interactions in SSc and IL-6 are illustrated in Figure 1.

SSc skin fibroblasts produce high levels of IL-6²⁴ and activation of the IL-6 gene²⁵. Expression of IL-6 gene is correlated with collagen production²⁶. IL-1, platelet-derived growth factor (PDGF), and tumor necrosis factor- α (TNF- α) induce fibroblasts to produce IL-6^{27,28,29,30}. SSc fibroblasts produce IL-1 that feeds back to fibroblasts to produce IL-6, PDGF, and procollagen type I^{31,32}. T cells, B cells, mast cells, basophils, and eosinophils stimulate fibroblasts through CD154/CD40 interaction³³, resulting in production of IL-6, IL-8, intercellular adhesion molecule 1,

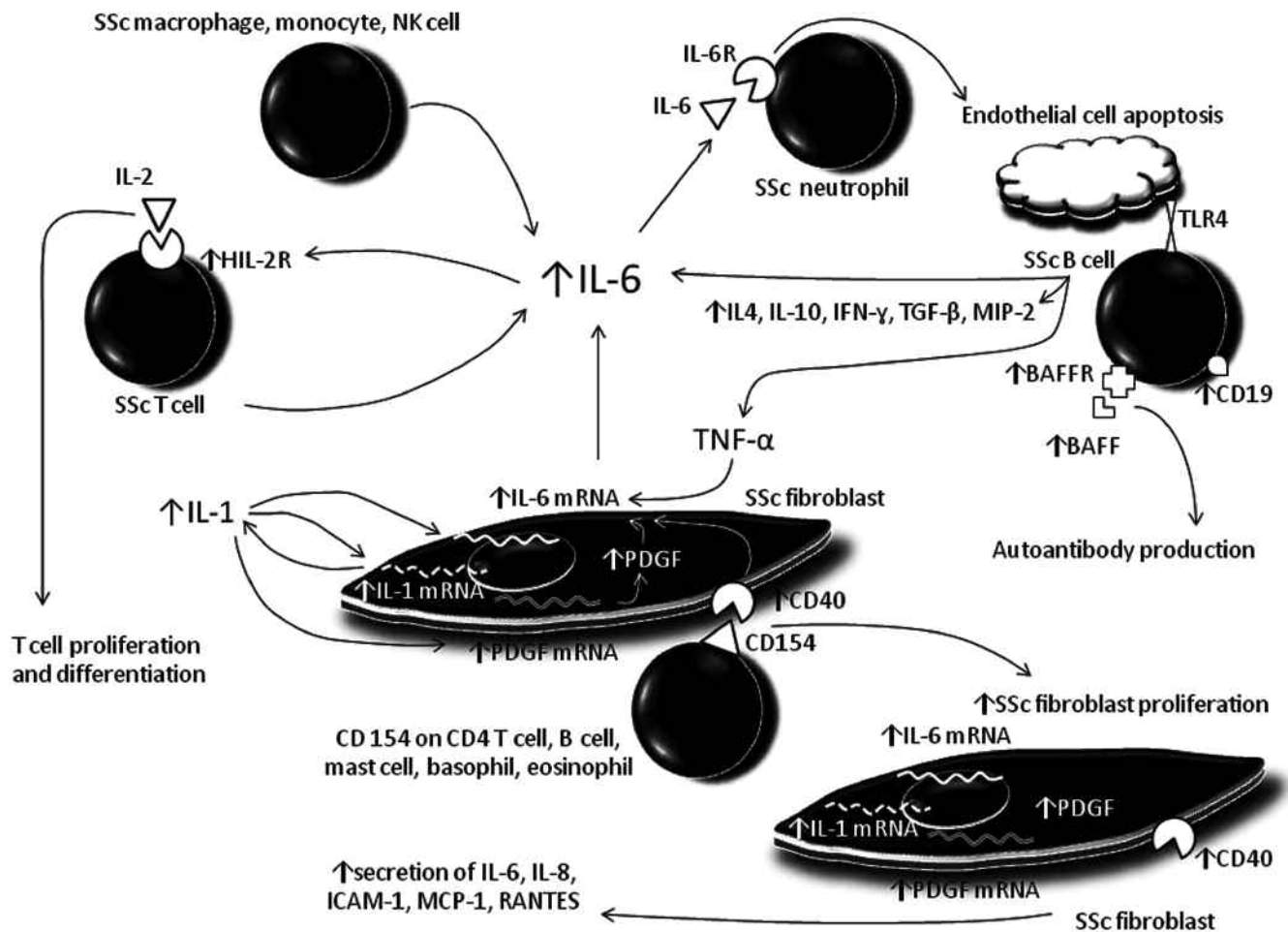


Figure 1. Fibroblast cell-cytokine interactions in systemic sclerosis (SSc) and interleukin 6 (IL-6). BAFF: B cell-activating factor; BAFFR: B cell-activating factor receptor; ICAM-1: intercellular adhesion molecule 1; IFN- γ : interferon- γ ; HIL-2R: high affinity IL-2 receptor; MIP-2: macrophage inflammatory protein 2; MCP-1: monocyte chemoattractant protein 1; PDGF: platelet-derived growth factor; RANTES: regulated upon activation normal T cell expressed and presumably secreted; TLR4: Toll-like receptor 4; TGF- β 1: transforming growth factor- β 1; TNF- α : tumor necrosis factor- α ; mRNA: messenger RNA.

monocyte chemoattractant protein 1, and RANTES (regulated upon activation normal T-cell expressed and presumably secreted)^{34,35,36}. Peripheral blood mononuclear cells (PBMC) are another source of IL-6^{15,37,38,39}. PBMC also produce oncostatin M and soluble IL-6 receptor (sIL-6R), which modify IL-6 transsignaling⁴⁰. Elevation of sIL-6R is correlated with decreased forced vital capacity (FVC) and DLCO⁴⁰. Soluble gp130 (a blocker of IL-6/sIL-6R that interacts with membrane gp130) is not elevated in SSc serum, thus allowing activation of cells through the IL-6 complex and membrane gp130⁴⁰. SSc T cells and natural killer cells overproduce IL-6^{15,41}. Through IL-6 stimulation, SSc T cells will overexpress IL-2 using high-affinity IL-2R⁴². Overexpression of CD19 caused SSc B cells to lose peripheral tolerance. Reduction of CD19 can decrease skin and lung fibrosis and lower IL-6, with subsequent suppression of serum levels of IL-4, IL-6, IL-10, interferon- γ (IFN- γ), TNF- α , transforming growth factor β 1, and macrophage inflammatory protein 2 (MIP-2) and decreased production of autoantibodies^{43,44,45,46}. B cell-activating factor (BAFF) produced by macrophages is overexpressed in SSc skin and BAFF receptor is increased in B cells from patients with SSc. In serum from patients with diffuse cutaneous SSc (dcSSc), increased BAFF levels are correlated with IL-6 levels, skin fibrosis, decreased FVC, arthritis, myositis, high ESR, and immunoglobulin production. Decreased BAFF levels correlate with regression of skin fibrosis, whereas increased levels are associated with new onset of organ involvement⁴⁷. Increased levels of IL-6 in SSc serum interact with IL-6R expressed by neutrophils, which caused endothelial cell apoptosis and breakdown of extracellular matrix^{46,48}. Hyaluronan (a product of extracellular matrix breakdown) stimulates B cells through Toll-like receptor 4 (TLR-4) to produce IL-4, IL-6, IL-10, IFN- γ , TNF- α , TGF- β 1, and MIP-2⁴⁶.

To date, there are some case reports of use of an anti-IL-6 antibody (tocilizumab 8 mg/kg once a month) for 6 months to treat dcSSc. Two patients with dcSSc had improved skin scores during treatment with tocilizumab⁴⁹. Another study reported 5 cases of dcSSc with active polyarthritis and insufficient response to conventional disease-modifying antirheumatic drugs. Tocilizumab treatment was found to decrease the swollen and tender joint counts (measured by the Disease Activity Score-28), where 3 of 5 patients achieved a EULAR good response. However, the skin score, quality of life, and lung function did not change⁵⁰. In another report, IL-6 decreased in patients with SSc treated with rituximab (a chimeric monoclonal antibody to CD20, 1 g given and repeated 14 days later) in 9 patients with dcSSc. After 6 months patients presented a median decrease of skin score of 43% (range 21%–64%) and in all patients levels of IL-6 were still suppressed at 6 months⁵¹. Levels of IL-6 may be abnormally high in pulmonary arterial hypertension (PAH) or other vascular abnormalities;

but data on IL-6 and SSc-associated PAH were not found in the literature review, with the exception of a recent report of a scleroderma cohort that described a polymorphism in the gene for TLR-2 that was associated with increased levels of IL-6 and PAH⁵².

Not all of the literature supported that IL-6 is important in SSc. Three negative studies were found. One study showed increased *in situ* localization of IL-6 in skin biopsies from atrophic epidermal diseases, including localized scleroderma, SSc, and other diseases, but no elevation of IL-6 in serum from patients with SSc (the study had positive data on skin biopsy but negative in SSc serum)⁵³. A different study also did not find elevations of IL-6 in serum of subjects with SSc compared to controls⁵⁴. Another report did not find significant differences in IL-6 polymorphisms in subjects with SSc compared to controls⁵⁵.

DISCUSSION

Potential explanation of pathophysiology in SSc related to IL-6. Aberrations of genes⁵⁶, changes of the immune system, and environmental triggers cause abnormal immune responses. Increased levels of serum IL-6 secreted from many cells interact with IL-6R, enhancing activation of endothelial cells, expression of adhesion molecules, and apoptosis, which seem to occur early in the pathogenesis of SSc. Various nuclear antigens induce and activate antigen-presenting cells to secrete many cytokines and chemokines, including IL-6, recruiting more changes. T cells are activated, differentiated, and proliferate, and this is partly influenced by IL-6. Activated T cells stimulate SSc fibroblasts, which are overexpressed in CD40 through interactions of CD40/CD154, resulting in proliferation and upregulation of inflammatory cytokines/chemokines, including IL-6. PBMC are another source of IL-6 overproduction in SSc. BAFF is oversecreted from SSc monocytes, macrophages, and other cell types. CD19 B cells in SSc are increased, with more BAFF receptors, which results in loss of peripheral tolerance and immune reaction to self-extracellular matrix breakdown products, which are stimulated through interactions of TLR-4 and oversecretion of IL-6.

IL-6, which is overexpressed especially in early SSc, stimulates SSc fibroblasts to differentiate and proliferate, causing collagen overproduction and fibrosis. IL-6 secreted from many sources has paracrine and autocrine effects on many cell types. This generates a cycle of chronic inflammation and fibrosis in SSc. IL-6 levels in SSc are related to disease activity (global assessments, skin scores, tendon friction rubs, some lung measures).

In addition, IL-6 and TGF- β facilitate the development of a T helper 17 (TH17) cell subpopulation⁵⁷. Circulating levels of IL-17-inducing cytokines, i.e., IL-6, IL-23, and IL-1, were increased in some patients with SSc⁵⁸. High levels of IL-17 have been reported especially in early SSc^{59,60}, and diminished over time⁶⁰. These observations

suggest that IL-17 is involved to some extent in SSc pathogenesis and support the importance of IL-6 in SSc.

Our study has limitations. Data were combined from reports of *in vitro* and *in vivo* studies and both human and animal models of SSc. Many had small sample sizes and some of the negative studies may have used samples from patients who did not have high disease activity. The clinical reports that used medications to downregulate IL-6 are few and have very small numbers of cases.

IL-6 has a role in the pathobiology of SSc. Most, but not all, studies support that IL-6 is increased in SSc in animal models, in skin and blood of patients, and especially in early dcSSc with findings within the skin and changes in the lungs. These multiple observations suggest that IL-6 may be a cytokine to target in treatment of SSc.

REFERENCES

1. Coral-Alvarado P, Pardo AL, Castaño-Rodríguez N, Rojas-Villarraga A, Anaya JM. Systemic sclerosis: A worldwide global analysis. *Clin Rheumatol* 2009;28:757-65.
2. Joven BE, Almodovar R, Carmona L, Carreira PE. Survival, causes of death, and risk factors associated with mortality in Spanish systemic sclerosis patients: Results from a single university hospital. *Semin Arthritis Rheum* 2010;39:285-93.
3. Czirják L, Kumánovics G, Varjú C, Nagy Z, Pákozdi A, Szekanez Z, et al. Survival and causes of death in 366 Hungarian patients with systemic sclerosis. *Ann Rheum Dis* 2008;67:59-63.
4. Czirják L, Nagy Z, Szegedi G. Survival analysis of 118 patients with systemic sclerosis. *J Intern Med* 1993;234:335-7.
5. Medsger TA Jr, Masi AT, Rodnan GP, Benedek TG, Robinson H. Survival with systemic sclerosis (scleroderma). A life-table analysis of clinical and demographic factors in 309 patients. *Ann Intern Med* 1971;75:369-76.
6. Phumethum V, Jamal S, Johnson SR. Biologic therapy for systemic sclerosis: A systematic review. *J Rheumatol* 2011;38:289-96.
7. Hirano T, Akira S, Taga T, Kishimoto T. Biological and clinical aspects of interleukin 6. *Immunol Today* 1990;11:443-9.
8. Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev* 2002;13:357-68.
9. Nishimoto N. Interleukin-6 as a therapeutic target in candidate inflammatory diseases. *Clin Pharmacol Ther* 2010;87:483-7.
10. Needleman BW, Wigley FM, Stair RW. Interleukin-1, interleukin-2, interleukin-4, interleukin-6, tumor necrosis factor alpha, and interferon-gamma levels in sera from patients with scleroderma. *Arthritis Rheum* 1992;35:67-72.
11. Koch AE, Kronfeld-Harrington LB, Szekanez Z, Cho MM, Haines GK, Harlow LA, et al. In situ expression of cytokines and cellular adhesion molecules in the skin of patients with systemic sclerosis. Their role in early and late disease. *Pathobiology* 1993;61:239-46.
12. Stuart RA, Littlewood AJ, Maddison PJ, Hall ND. Elevated serum interleukin-6 levels associated with active disease in systemic connective tissue disorders. *Clin Exp Rheumatol* 1995;13:17-22.
13. Hasegawa M, Sato S, Fujimoto M, Ihn H, Kikuchi K, Takehara K. Serum levels of interleukin 6 (IL-6), oncostatin M, soluble IL-6 receptor, and soluble gp130 in patients with systemic sclerosis. *J Rheumatol* 1998;25:308-13.
14. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. *J Dermatol Sci* 2001;27:140-6.
15. Scala E, Pallotta S, Frezzolini A, Abeni D, Barbieri C, Sampogna F, et al. Cytokine and chemokine levels in systemic sclerosis: relationship with cutaneous and internal organ involvement. *Clin Exp Immunol* 2004;138:540-6.
16. Matsushita T, Hasegawa M, Hamaguchi Y, Takehara K, Sato S. Longitudinal analysis of serum cytokine concentrations in systemic sclerosis: association of interleukin 12 elevation with spontaneous regression of skin sclerosis. *J Rheumatol* 2006;33:275-84.
17. Ong V, Nihtyanova S, Black CM, Denton CP. A clinically defined subset of dcSSc is associated with elevated serum IL-6 level [abstract]. *Arthritis Rheum* 2009;60 Suppl:440.
18. Gourh P, Arnett FC, Assassi S, Tan FK, Huang M, Diekman L, et al. Plasma cytokine profiles in systemic sclerosis: associations with autoantibody subsets and clinical manifestations. *Arthritis Res Ther* 2009;11:R147.
19. Alivernini S, De Santis M, Toluoso B, Mannocci A, Bosello SL, Peluso G, et al. Skin ulcers in systemic sclerosis: Determinants of presence and predictive factors of healing. *J Am Acad Dermatol* 2009;60:426-35.
20. Ohtsuka T. Serum interleukin-6 level is reflected in elevated high-sensitivity C-reactive protein level in patients with systemic sclerosis. *J Dermatol* 2010;37:801-6.
21. Pope J, Harding S, Khimdas S, Bonner A, Baron M. C-reactive protein is associated with high disease activity in SSc. Results from the Canadian Scleroderma Research Group (CSR) [abstract]. *Arthritis Rheum* 2009;60 Suppl:471.
22. Takemura H, Suzuki H, Yoshizaki K, Ogata A, Yuhara T, Akama T, et al. Anti-interleukin-6 autoantibodies in rheumatic diseases. Increased frequency in the sera of patients with systemic sclerosis. *Arthritis Rheum* 1992;35:940-3.
23. Suzuki H, Takemura H, Yoshizaki K, Koishihara Y, Ohsugi Y, Okano A, et al. IL-6-anti-IL-6 autoantibody complexes with IL-6 activity in sera from some patients with systemic sclerosis. *J Immunol* 1994;152:935-42.
24. Feghali CA, Bost KL, Boulware DW, Levy LS. Mechanisms of pathogenesis in scleroderma. I. Overproduction of interleukin 6 by fibroblasts cultured from affected skin sites of patients with scleroderma. *J Rheumatol* 1992;19:1207-11.
25. Feghali CA, Bost KL, Boulware DW, Levy LS. Control of IL-6 expression and response in fibroblasts from patients with systemic sclerosis. *Autoimmunity* 1994;17:309-18.
26. Zurita-Salinas CS, Richaud-Patin Y, Kröttsch-Gómez E, Llorente L, Alcocer-Varela J, Díaz-de-León L, et al. Spontaneous cytokine gene expression by cultured skin fibroblasts of systemic sclerosis. Correlation with collagen synthesis. *Rev Invest Clin* 1998; 50:97-104.
27. Yamamoto T, Katayama I, Nishioka K. Fibroblast proliferation by bleomycin stimulated peripheral blood mononuclear cell factors. *J Rheumatol* 1999;26:609-15.
28. Kawaguchi Y, Harigai M, Suzuki K, Hara M, Kobayashi K, Ishizuka T, et al. Interleukin 1 receptor on fibroblasts from systemic sclerosis patients induces excessive functional responses to interleukin 1 beta. *Biochem Biophys Res Commun* 1993; 190:154-61.
29. Takemura H, Suzuki H, Fujisawa H, Yuhara T, Akama T, Yamane K, et al. Enhanced interleukin 6 production by cultured fibroblasts from patients with systemic sclerosis in response to platelet derived growth factor. *J Rheumatol* 1998;25:1534-9.
30. Kadono T, Kikuchi K, Ihn H, Takehara K, Tamaki K. Increased production of interleukin 6 and interleukin 8 in scleroderma fibroblasts. *J Rheumatol* 1998;25:296-301.
31. Kawaguchi Y, Hara M, Wright TM. Endogenous IL-1-alpha from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. *J Clin Invest* 1999;103:1253-60.
32. Kawaguchi Y, McCarthy SA, Watkins SC, Wright TM. Autocrine activation by interleukin 1-alpha induces the fibrogenic phenotype of systemic sclerosis fibroblasts. *J Rheumatol* 2004;31:1946-54.

33. Fukasawa C, Kawaguchi Y, Harigai M, Sugiura T, Takagi K, Kawamoto M, et al. Increased CD40 expression in skin fibroblasts from patients with systemic sclerosis (SSc): Role of CD40-CD154 in the phenotype of SSc fibroblasts. *Eur J Immunol* 2003; 33:2792-800.
34. Kawai M, Masuda A, Kuwana M. A CD40-CD154 interaction in tissue fibrosis. *Arthritis Rheum* 2008;58:3562-73.
35. Aqache I, Radoi M, Duca L. Platelet activation in patients with systemic scleroderma-pattern and significance. *Rom J Intern Med* 2007;45:183-91.
36. Kondo K, Okada T, Matsui T, Kato S, Date K, Yoshihara M, et al. Establishment and characterization of a human B cell line from the lung tissue of a patient with scleroderma; extraordinary high level of IL-6 secretion by stimulated fibroblasts. *Cytokine* 2001; 13:220-6.
37. Crestani B, Seta N, De Bandt M, Soler P, Rolland C, Dehoux M, et al. Interleukin 6 secretion by monocytes and alveolar macrophages in systemic sclerosis with lung involvement. *Am J Respir Crit Care Med* 1994;149:1260-5.
38. Giacomelli R, Cipriani P, Danese C, Pizzuto F, Lattanzio R, Parzanese I, et al. Peripheral blood mononuclear cells of patients with systemic sclerosis produce increased amounts of interleukin 6, but not transforming growth factor beta 1. *J Rheumatol* 1996;23:291-6.
39. Gurrain M, Pahwa S, Frieri M. Augmented interleukin-6 secretion in collagen-stimulated peripheral blood mononuclear cells from patients with systemic sclerosis. *Ann Allergy* 1994;73:493-6.
40. Hasegawa M, Sato S, Iln H, Takehara K. Enhanced production of interleukin-6 (IL-6), oncostatin M and soluble IL-6 receptor by cultured peripheral blood mononuclear cells from patients with systemic sclerosis. *Rheumatology* 1999;38:612-7.
41. Horikawa M, Hasegawa M, Komura K, Hayakawa I, Yanaba K, Matsushita T, et al. Abnormal natural killer cell function in systemic sclerosis: Altered cytokine production and defective killing activity. *J Invest Dermatol* 2005;125:731-7.
42. Kahaleh MB, Yin TG. Enhanced expression of high-affinity interleukin-2 receptors in scleroderma: Possible role for IL-6. *Clin Immunol Immunopathol* 1992;62:97-102.
43. Inaoki M, Sato S, Weintraub BC, Goodnow CC, Tedder TF. CD19-regulated signaling thresholds control peripheral tolerance and autoantibody production in B lymphocytes. *J Exp Med* 1997;186:1923-31.
44. Sato S, Hasegawa M, Fujimoto M, Tedder TF, Takehara K. Quantitative genetic variation in CD19 expression correlates with autoimmunity. *J Immunol* 2000;165:6635-43.
45. Saito E, Fujimoto M, Hasegawa M, Komura K, Hamaguchi Y, Kaburagi Y, et al. CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in tight-skin mouse. *J Clin Invest* 2002;109:1453-62.
46. Yoshizaki A, Iwata Y, Komura K, Ogawa F, Hara T, Muroi E, et al. CD19 regulates skin and lung fibrosis via Toll-like receptor signaling in a model of bleomycin-induced scleroderma. *Am J Pathol* 2008;172:1650-63.
47. Matsushita T, Hasegawa M, Yanaba K, Koderia M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis. Enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum* 2006;54:192-201.
48. Barnes TC, Spiller DG, Anderson ME, Edwards SW, Moots RJ. Endothelial activation and apoptosis mediated by neutrophil-dependent interleukin 6 trans-signaling: A novel target for systemic sclerosis? *Ann Rheum Dis* 2011;70:366-72.
49. Shima Y, Kuwahara Y, Murota H, Kitaba S, Kawai M, Hirano T, et al. The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. *Rheumatology* 2010;49:2408-12.
50. Meunier M, Matucci-Cerinic M, Maurer B, Riemekasten G, Bartoli F, Fiori G, et al. Outcomes of systemic sclerosis associated polyarthritis patients treated by biotherapies of tocilizumab or abatacept: A EULAR observational study [abstract]. *Ann Rheum Dis* 2011;70 Suppl:660.
51. Bosello S, Santis MD, Lama G, Spanò C, Angelucci C, Tolusso B, et al. B cell depletion in diffuse progressive systemic sclerosis: Safety, skin score modification and IL-6 modulation in an up to thirty-six months follow-up open-label trial. *Arthritis Res Ther* 2010;12:R54.
52. Broen JC, Bossini-Castillo L, van Bon L, Vonk MC, Knaapen H, Beretta L, et al. A rare polymorphism in the gene for Toll-like receptor 2 is associated with systemic sclerosis phenotype and increases the production of inflammatory mediators. *Arthritis Rheum* 2012;64:264-71.
53. Romero LI, Pincus SH. In situ localization of interleukin-6 in normal skin and atrophic cutaneous disease. *Int Arch Allergy Immunol* 1992;99:44-9.
54. Hasegawa M, Fujimoto M, Matsushita T, Hamaguchi Y, Takehara K, Sato S. Serum chemokine and cytokine levels as indicators of disease activity in patients with systemic sclerosis. *Clin Rheumatol* 2011;30:231-7.
55. Sfrent-Cornateanu R, Mihai C, Balan S, Ionescu R, Moldoveanu E. The IL-6 promoter polymorphism is associated with disease activity and disability in systemic sclerosis. *J Cell Mol Med* 2006;10:955-9.
56. Beretta L, Cappiello F, Moore JH, Barili M, Greene CS, Scorza R. Ability of epistatic interactions of cytokine single-nucleotide polymorphisms to predict susceptibility to disease subsets in systemic sclerosis patients. *Arthritis Rheum* 2008;59:974-83.
57. Deleuran B, Abraham DJ. Possible implication of the effector CD4+ T-cell subpopulation TH17 in the pathogenesis of systemic scleroderma. *Nat Clin Pract Rheumatol* 2007;3:682-3.
58. Radstake TR, van Bon L, Broen J, Hussiani A, Hesselstrand R, Wuttge DM, et al. The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular expression of TGF beta and IFN gamma distinguishes SSc phenotypes. *PLoS One* 2009;4:e5903.
59. Kurasawa K, Hirose K, Sano H, Endo H, Shinkai H, Nawata Y, et al. Increased interleukin-17 production in patients with systemic sclerosis. *Arthritis Rheum* 2000;43:2455-63.
60. Murata M, Fujimoto M, Matsushita T, Hamaguchi Y, Hasegawa M, Takehara K, et al. Clinical association of serum interleukin-17 levels in systemic sclerosis: Is systemic sclerosis a Th17 disease? *J Dermatol Sci* 2008;50:240-2.