The Role of B Cells in the Pathogenesis of Systemic Sclerosis

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ABSTRACT: Systemic sclerosis (SSc) is characterized by extensive collagen deposition, microvasculopathy and autoantibodies. All three features can be promoted by activation of T cells and B cells. T cells are of Th2 type producing profibrotic cytokines IL-4 and IL-13 and inducing dendritic cell maturation that promotes Th2 response. B cells are overactivated and promote fibrosis by autoantibodies that activate fibroblasts or inhibit the degradation of extracellular matrix. They also promote fibrosis by cell-cell contact with fibroblasts or dendritic cells. B cells, through autoantibodies, may promote vasoconstriction and obliterative vasculopathy. They may also sustain activation of T cells by functioning as antigen-presenting cells. An immunoregulatory subset of B cells, namely IL-10-producing Bregs, is decreased in SSc. Finally, B cells have a critical role in animal models of SSc. All this evidence suggests an important role for B cells in the pathogenesis of SSc and makes B cells a potential target for therapeutic intervention in this disease.

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Systemic sclerosis (SSc) is characterized by extensive fibrosis in skin and internal organs, vasospastic episodes (Raynaud's phenomenon) and fibrointimal proliferation of microvascular vessels, and autoantibodies [1]. The pathogenesis of SSc is incompletely understood. Raynaud's phenomenon and microvasculopathy may appear years before skin fibrosis [2], and endothelial cell apoptosis was shown to be an early event in an avian SSc model and was also detected in early human SSc [3]. Autoantibodies may also appear years before skin fibrosis and some of them, such as anti-topoisomerase I antibodies and anticentromere antibodies, are characteristic of SSc and are associated with the SSc skin extent [4]. These two features, Raynaud's phenomenon and autoantibodies, are now included in the new classification criteria for SSc.

B cells are hyperactivated in systemic sclerosis

B cells promote fibrosis and vasoconstriction in SSc

Activation of fibroblasts is an absolute requirement for excessive collagen deposition, and some evidence suggests that this activation may be initiated and/or propagated by T cells and B cells. The role of T cells in SSc has been outlined previously [5-7]. Briefly, T cells are detected in skin biopsies early in the disease process before histological fibrosis and there are signs of activation of T cells in the skin. In SSc skin these T cells bear markers of activation [8,9]. Sequence analysis of the β-chain of T cell antigen receptor from SSc skin biopsies revealed oligoclonal T cells, and in one patient the same clone persisted over time (in three available biopsies over 13 months). These findings indicated an antigen-driven expansion of T cells in skin lesions [10]. There are signs of T cell activation in peripheral blood as well. T cells are of Th2 type producing profibrotic IL-4 and IL-13. T cells can also induce maturation of dendritic cells that promote th2 response [7]. Deletion of the IL-4 or IL-4 receptor gene in animal models of SSc, namely TSK-positive mice and bleomycin-induced mouse model, reduced fibrosis. This finding demonstrates the importance of IL-4 in fibrosis [6,7].

B CELLS PROMOTE FIBROSIS AND VASOCONSTRICTION IN SSC

Although B cells are variably detected in SSc skin lesions, there is sufficient evidence to implicate B cells in the pathogenesis of SSc. B cells are overactivated in SSc. Firstly, SSc B cells overexpress the B cell stimulatory receptor CD19 by 30–50% [11,12]. It should be mentioned that overexpression of CD19 by 15–29% in a transgenic mouse cell line resulted in spontaneous autoantibody production [11], whereas in TSK-positive mice, an animal model of SSc with skin fibrosis and autoantibodies, CD19 depletion completely inhibited autoantibody production and reduced skin fibrosis [13]. Secondly, the inhibitory B cell receptor CD22 is inhibited in SSc. Many patients with SSc have anti-CD22 autoantibodies that are functional and inhibit the CD22 inhibitory signals [14].

B cells can promote fibrosis through cytokines, autoantibodies, and cell-cell contact with dendritic cells and fibroblasts, or through the deficiency of regulatory B cells (Bregs). They may also function as antigen-presenting cells (APC) and thus affect T cell activation. On the other hand, B cell depletion therapy with rituximab (anti-CD20 monoclonal antibody) decreases skin fibrosis.

B CELL CYTOKINES PROMOTE FIBROSIS

B cells can produce transforming growth factor-beta (TGFβ) [15] and IL-6 which are increased in SSc [16]. TGFβ is a powerful profibrotic cytokine, and IL-6 is a growth factor for fibroblasts, increasing collagen production [17]. On the other hand, anti-IL-6 receptor monoclonal antibody decreased skin thickness in patients with SSc [18] and in a bleomycin-induced mouse model of SSc [16].

AUTOANTIBODIES PROMOTE FIBROSIS

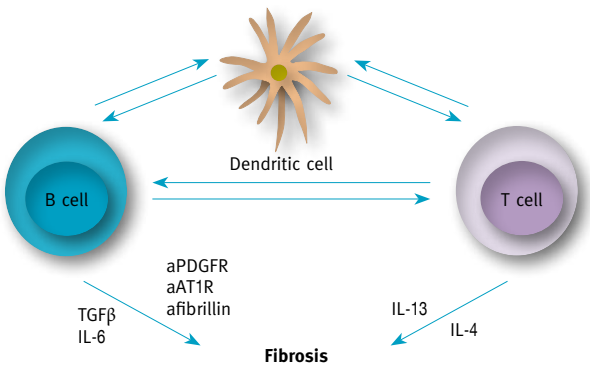
Some autoantibodies detected in patients with SSc promote fibrosis, either by activating fibroblasts or by inhibiting matrix metalloproteinases (MMPs). These autoantibodies act against various antigens including constituents of fibroblasts, and endothelial cells. Autoantibodies may also target platelet-derived growth factor receptor (PDGFR) [19], fibrillin, matrix metalloproteinase (MMP)-1 [20], MMP-6, endothelin type A receptor (ETAR), and angiotensin type 1 receptor (AT1R) [6,21]. Anti-ETAR are agonists that activate fibroblasts, cause vasoconstriction and activate T cells [21-23]. Anti-AT1R autoantibodies are also agonists causing vasoconstriction and fibroblast activation [21,23,24]. Furthermore, anti-ETAR and AT1R antibodies injected into mice induced obliterative pulmonary arteriopathy [25].

IL-10-producing Bregs are reduced in SSc, particularly in SSc-associated interstitial lung disease

B CELLS PROMOTE FIBROSIS BY CELL-CELL CONTACT

B cells can promote fibrosis by cell-cell contact with fibroblasts or dendritic cells [Figure 1]. Co-culture of B cells with fibroblasts induced contact-dependent fibroblast collagen production [26]. Also, culture of B cells with dendritic cells induces dendritic cell maturation that favors Th2 over Th1 response [27].

Figure 1. In systemic sclerosis, interaction among B cells, T cells and dendritic cells results in autoantibodies and cytokines that activate fibroblasts and promote fibrosis



TGFβ = antibodies against transforming growth factor-beta, IL = interleukin, aPDGFR = antibodies against platelet-derived growth factor receptor, aAT1R = antibodies against angiotensin type 1 receptor, aETAR = antibodies against endothelin type A receptor

DEFICIENCY OF BREGS EXPECTED TO FACILITATE FIBROSIS

Regulatory B cells (Bregs) are a small subset of B cells and have a very important function [28]. Bregs inhibit Th1 and Th17 differentiation and expand/maintain T regulatory cells (Tregs), thus ameliorating autoimmune diseases [29-32]. IL-10-producing Bregs were found reduced in SSc, particularly in SSc-associated interstitial lung disease [12,33]. Furthermore, Bregs numbers were negatively correlated with SSc-specific autoantibodies, anti-topoisomerase I and anti-centromere antibodies [33]. IL-10-producing Bregs were also decreased in human chronic graft-versus-host disease, a model for SSc [34].

B CELLS MAY FUNCTION AS APC AND REDUCE T CELL ACTIVATION

B cells can be antigen-presenting cells and by B cell/T cell talk sustain T cell activation in certain environments [35]. It is likely that this may operate in SSc, given the therapeutic efficacy of rituximab in SSc [36]. In patients with IgG4-related disease, a fibro-inflammatory condition characterized by fibrosis and lymphoplasmacytic infiltration, rituximab administration reduced activated fibroblasts, B lymphoplasmacytic and T cell infiltrates in lesions [37].

B CELL DEPLETION THERAPY DECREASES FIBROSIS

In a small series of patients with SSc, B cell depletion with anti-CD20 monoclonal antibody (rituximab) decreased skin thickness and stabilized lung fibrosis [36], thus reinforcing the importance of B cells in SSc.

B CELLS ARE CRITICAL FOR THE INDUCTION OF EXPERIMENTAL SSC

B cells were found to be important in animal models of SSc. In TSK-positive mice and in bleomycin-induced SSc mouse model, deletion of CD19 inhibited autoantibody production and reduced fibrosis [38]. The importance of B cells in SSc is summarized in Table 1.

Table 1. Evidence for the importance of B cells in systemic sclerosis

- B cells are overactivated
- B cells promote fibrosis through autoantibodies that activate fibroblasts
- B cells, through autoantibodies, inhibit the degradation of extracellular matrix
- B cells promote fibrosis through cell-cell contact with fibroblasts
- B cells promote fibrosis through cell-cell contact with dendritic cells
- B cells, through autoantibodies, may promote vasoconstriction and obliterative vasculopathy
- B cells might function as antigen-presenting cells and thus sustain activation of T cells
- IL-10-producing regulatory B cells, which expand regulatory T cells, are decreased in SSc
- B cell depletion treatment with rituximab decreases skin fibrosis in patients with SSc
- B cell CD19 deletion in TSK-positive mice and bleomycin-induced SSc mouse model inhibits autoantibody production and decreases fibrosis

FUTURE PERSPECTIVES

Having outlined the importance of B cells in the pathogenesis of SSc, it appears we should target B cells therapeutically in this devastating disease. B cell depletion with rituximab is a promising strategy and should be tested in a proper randomized controlled trial. Expanding Bregs ex vivo and re-infusing them back to the patient might be another strategy [12]. The therapeutic efficacy of B cell depletion with rituximab does not necessarily contradict the latter strategy. At present we do not know if Bregs are resistant to rituximab or if Bregs recover early during reconstitution of B cell population after rituximab treatment.

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References

1. Ferri C, Sebastiani M, Lo Monaco A, et al. Systemic sclerosis evolution of disease pathomorphosis and survival. Our experience on Italian patients' population and review of the literature. *Autoimmun Rev* 2014; 13: 1026-34.
2. Prete M, Fatone MC, Favoino E, et al. Raynaud's phenomenon: from molecular pathogenesis to therapy. *Autoimmun Rev* 2014; 13: 655-67.
3. Sgonc R, Gruschwitz MS, Dietrich H, et al. Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J Clin Invest* 1996; 98: 785-92.
4. Koenig M, Joyal F, Fritzler MJ, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. *Arthritis Rheum* 2008; 58: 3902-12.
5. Sakkas LI, Platsoucas CD. Is systemic sclerosis an antigen-driven T cell disease? *Arthritis Rheum* 2004; 50: 1721-33.
6. Sakkas LI, Chikanza IC, Platsoucas CD. Mechanisms of disease: the role of immune cells in the pathogenesis of systemic sclerosis. *Nat Clin Pract Rheumatol* 2006; 2: 679-85.
7. O'Reilly S, Hugle T, van Laar JM. T cells in systemic sclerosis: a reappraisal. *Rheumatology* (Oxford) 2012; 51: 1540-9.
8. Sondergaard K, Stengaard-Pedersen K, Zachariae H, et al. Soluble intercellular adhesion molecule-1 (sICAM-1) and soluble interleukin-2 receptors (sIL-2R) in scleroderma skin. *Br J Rheumatol* 1998; 37: 304-10.
9. Kalogerou A, Gelou E, Mountantonakis S, et al. Early T cell activation in the skin from patients with systemic sclerosis. *Ann Rheum Dis* 2005; 64: 1233-5.
10. Sakkas LI, Xu B, Artlett CM et al. Oligoclonal T cell expansion in the skin of patients with systemic sclerosis. *J Immunol* 2002; 168: 3649-59.
11. Sato S, Hasegawa M, Fujimoto M, et al. Quantitative genetic variation in CD19 expression correlates with autoimmunity. *J Immunol* 2000; 165: 6635-43.
12. Mavropoulos A, Simopoulou T, Varna A, et al. B regulatory cells are decreased and functionally impaired in patients with systemic sclerosis. *Arthritis Rheum* 2016; 68: 494-504.
13. Saito E, Fujimoto M, Hasegawa M, et al. CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in the tight-skin mouse. *J Clin Invest* 2002; 109: 1453-62.
14. Odaka M, Hasegawa M, Hamaguchi Y, et al. Autoantibody-mediated regulation of B cell responses by functional anti-CD22 autoantibodies in patients with systemic sclerosis. *Clin Exp Immunol* 2010; 159: 176-84.
15. Snapper CM, Waegell W, Beernink H, et al. Transforming growth factor-beta 1 is required for secretion of IgG of all subclasses by LPS-activated murine B cells in vitro. *J Immunol* 1993; 151: 4625-36.
16. Desallais L, Avouac J, Frechet M, et al. Targeting IL-6 by both passive or active immunization strategies prevents bleomycin-induced skin fibrosis. *Arthritis Res Ther* 2014; 16: R157.
17. Duncan MR, Berman B. Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6. *J Invest Dermatol* 1991; 97: 686-92.
18. Shima Y, Kuwahara Y, Murota H, et al. The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. *Rheumatology* (Oxford) 2010; 49: 2408-12.
19. Baroni SS, Santillo M, Bevilacqua F, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 2006; 354: 2667-76.
20. Sato S, Hayakawa I, Hasegawa M, et al. Function blocking autoantibodies against matrix metalloproteinase-1 in patients with systemic sclerosis. *J Invest Dermatol* 2003; 120: 542-7.
21. Riemekasten G, Philippe A, Nather M, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis* 2011; 70: 530-6.
22. Kawamura T, Ono K, Morimoto T, et al. Endothelin-1-dependent nuclear factor of activated T lymphocyte signaling associates with transcriptional coactivator p300 in the activation of the B cell leukemia-2 promoter in cardiac myocytes. *Circ Res* 2004; 94: 1492-9.
23. Kill A, Tabeling C, Undeutsch R, et al. Autoantibodies to angiotensin and endothelin receptors in systemic sclerosis induce cellular and systemic events associated with disease pathogenesis. *Arthritis Res Ther* 2014; 16: R29.
24. Kawaguchi Y, Takagi K, Hara M, et al. Angiotensin II in the lesional skin of systemic sclerosis patients contributes to tissue fibrosis via angiotensin II type 1 receptors. *Arthritis Rheum* 2004; 50: 216-26.
25. Becker MO, Kill A, Kutsche M, et al. Vascular receptor autoantibodies in pulmonary arterial hypertension associated with systemic sclerosis. *Am J Respir Crit Care Med* 2014; 190: 808-17.
26. Francois A, Chatelus E, Wachsmann D, et al. B lymphocytes and B-cell activating factor promote collagen and profibrotic markers expression by dermal fibroblasts in systemic sclerosis. *Arthritis Res Ther* 2013; 15: R168.
27. Maddur MS, Sharma M, Hegde P, et al. Human B cells induce dendritic cell maturation and favour Th2 polarization by inducing OX-40 ligand. *Nat Commun* 2014; 5: 4092.
28. Vadasz Z, Toubi E. The many faces of B regulatory cells. *IMAJ* 2014; 16: 631-3.
29. Blair PA, Norena LY, Flores-Borja F, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 2010; 32: 129-40.
30. Iwata Y, Matsushita T, Horikawa M, et al. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 2011; 117: 530-41.
31. Wang RX, Yu CR, Dambuza IM, et al. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. *Nat Med* 2014; 20: 633-41.
32. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 2014; 507: 366-70.
33. Matsushita T, Hamaguchi Y, Hasegawa M, et al. Decreased levels of regulatory B cells in patients with systemic sclerosis: association with autoantibody production and disease activity. *Rheumatology* (Oxford) 2016; 55: 263-7.
34. Khoder A, Sarvaria A, Alsuliman A, et al. Regulatory B cells are enriched within the IgM memory and transitional subsets in healthy donors but are deficient in chronic GVHD. *Blood* 2014; 124: 2034-45.
35. Pierson ER, Stromnes IM, Goverman JM. B cells promote induction of experimental autoimmune encephalomyelitis by facilitating reactivation of T cells in the central nervous system. *J Immunol* 2014; 192: 929-39.
36. Sakkas LI, Bogdanos DP. Systemic sclerosis: new evidence re-enforces the role of B cells. *Autoimmun Rev* 2016; 15 (2): 155-61.
37. Della-Torre E, Feeney E, Deshpande V, et al. B-cell depletion attenuates serological biomarkers of fibrosis and myofibroblast activation in IgG4-related disease. *Ann Rheum Dis* 2015; 74: 2236-43.
38. Yoshizaki A, Sato S. Abnormal B lymphocyte activation and function in systemic sclerosis. *Ann Dermatol* 2015; 27: 1-9.