B cells tell scleroderma fibroblasts to produce collagen

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Abstract

In fibrosis fibroblasts are activated and overproduce collagen in a process with unknown drivers and equally unknown brakes that recently implicated a novel and surprising player, the B cell. B cells may be crucially involved in fibrosis in several ways: B cells may produce autoantibodies that can directly stimulate fibroblasts; B cells can produce profibrotic cytokines such as IL-6 or transforming growth factor beta; and, finally, B cells could directly stimulate fibroblasts by a contact-dependent mechanism. Recent experimental evidence suggests that B cells can enhance collagen production by fibroblasts, by a contact-dependent mechanism, and therefore are profibrotic ex vivo. These data strengthen the rationale of pursuing B-cell targeting therapies in systemic sclerosis.

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In the previous issue of Arthritis Research and Therapy, Francois and colleagues provided experimental evidence of a contact-dependent crosstalk between B cells and fibroblasts; scleroderma fibroblasts exhibit a more than twofold increase in collagen production when co-cultured with B cells compared with when cultured alone [1]. This B-cell-induced collagen production is comparable with the effects of transforming growth factor beta (TGFβ), the most potent profibrotic mediator. Enhancement of collagen production was seen when fibroblasts were co-cultured with circulating B cells only but not with whole peripheral blood mononuclear cells. Genes encoding collagen I and collagen III were significantly upregulated in the B-cell/fibroblast co-culture system compared with fibroblasts cultured alone. Importantly, the α-smooth muscle actin α-SMA gene that has a key role in the differentiation of fibroblasts into myofibroblasts was also overexpressed.

When the survival factor B-cell activating factor (BAFF) was added along with B-cell receptor-initiated B-cell stimulation in the B-cell/fibroblast co-culture system, a further significant enhancement of collagen production and α-SMA expression was seen; interestingly, the fibroblasts did not express any BAFF receptors. To address the question of whether B cells interact with fibroblasts via direct cell contact or via soluble factors, the authors employed in some experiments a transwell culture system allowing for a free and bidirectional diffusion of soluble factors but prohibiting direct B-cell–fibroblast contact. In these transwell experiments the induction of fibroblast collagen production was lost, clearly indicating a contact-dependent mechanism. However, transwells only partially inhibited the effect of BAFF + B-cell receptor stimulation on fibroblasts, indicating that soluble factors also participate in the BAFF scenario. The production of IL-6, chemokine (C–C motif) ligand-2 and TGFβ was significantly increased in co-cultures and the use of transwells decreased but did not abolish their production. Finally, blocking TGFβ (but not blocking IL-6) led to a significant inhibition of the effect of B cells plus BAFF on fibroblasts, suggesting a key role for TGFβ.

There are certain issues the reader must take into account to fully interpret this study. The authors initially employed co-cultures of normal B cells with either normal or systemic sclerosis (SSc) skin fibroblasts. Interestingly, the results on collagen production were similar irrespective of the fibroblasts in the co-culture. The authors chose to continue their experiments with fibroblasts obtained from patients with scleroderma skin only, again employing normal B cells in the co-culture system. Francois and colleagues did not employ B cells...
from patients with scleroderma because they speculated that the effect of normal B cells on the fibroblasts was so strong that the activated scleroderma B cell might not be able to produce any stronger effects. This is clearly speculative; an experiment not performed leads to mis-sing potentially valuable data. Therefore, the results of the study by Francois and colleagues could apply directly to the pathogenesis of scleroderma only if we knew that the scleroderma B cell is a perfectly normal B cell. There are, however, experimental data pointing to the contrary.

B cells from tight skin mice, an animal model of SSc, exhibit enhanced CD19 signaling and are hyperresponsive. Induced CD19 deficiency in tight skin mice normalizes B-cell responses and attenuates skin fibrosis, while B-cell depletion therapy in such models is effective [2,3]. B cells in patients with SSc are present in the skin [4] and the lungs [5]; expression of CD19 is increased and the cells’ homeostasis is disturbed [6]. Microarrays from scleroderma skin disclosed fibroblast, endothelial cell and, surprisingly, B-cell genes to be upregulated compared with normal skin [7]. Finally, emerging clinical data suggest a favorable effect of the B-cell depleting agent rituximab on skin and lung fibrosis in patients with SSc [8-10].

How can B cells regulate the fibrotic process? B cells may produce autoantibodies that can directly stimulate fibroblasts to increase collagen production such as agonistic anti-platelet-derived growth factor receptor autoantibodies [11], or inhibit the function of metalloproteinases and therefore decrease collagen degradation [12]. B cells also produce profibrotic cytokines such as IL-6 or TGFβ. Finally, B cells could directly stimulate fibroblasts by a contact-dependent mechanism. Previous experiments have shown that both B cells in particular and, to a lesser extent, T cells can bind to cultured fibroblasts 	extit{in vitro} with an as yet unknown functional significance [13]. These are diagrammatically depicted in Figure 1.

This study introduces the novel concept that B cells are profibrotic 	extit{ex vivo}. Several questions remain to be answered. The first question is how B cells physically interact with fibroblasts; this should be explored in future studies. Nevertheless, the most important issue is whether B cells are profibrotic 	extit{in vivo}. The encouraging results of several studies assessing the effect of B-cell depletion in SSc may lead to the hypothesis that B cells are also profibrotic in SSc. The study of Francois and colleagues provides strong experimental evidence in favor of a crucial role of B cells in the pathophysiology of fibrosis and therefore strengthens the rationale of pursuing B-cell targeting therapies in SSc.

**Abbreviations**

BAFF: B-cell activating factor; IL: Interleukin; SSc: Systemic sclerosis; TGFβ: Transforming growth factor beta.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Francois A, Chatelus E, Wachsmann D, Sibilia J, Bahram S, Alsaleh G, Gottenberg J-E: 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.

2. Saito E, Fujimoto M, Hasegawa M, Komura K, Hamaguchi Y, Kaburagi Y, Nagaoka T, Takehara K, Tedder TF, Sato S: CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in the tight-skin mouse. 

   J Clin Invest 2002, 109:1453–1462.
3. Hasegawa M, Hamaguchi Y, Yanaba K, Bouaziz JD, Uchida J, Fujimoto M, Matsushita T, Matsushita Y, Hotakawa M, Komura K, Takehara K, Sato S, Tedder TF: B-lymphocyte depletion reduces skin fibrosis and autoimmunity in the tight-skin mouse model for systemic sclerosis. *Am J Pathol* 2006, 169:954–966.

4. Laffayris R, Kissin E, York M, Farina G, Viger K, Fritzler MJ, Merzel EA, Simms RW: B cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis. *Arthritis Rheum* 2009, 60:578–583.

5. Laffayris R, O'Hara C, Feghali-Bostwick CA, Matteson E: B cell infiltration in systemic sclerosis-associated interstitial lung disease. *Arthritis Rheum* 2007, 56:3167–3168.

6. Sato S, Fujimoto M, Hasegawa M, Takehara K: Altered blood B lymphocyte homeostasis in systemic sclerosis: expanded naive B cells and diminished but activated memory B cells. *Arthritis Rheum* 2004, 50:1918–1927.

7. Whitfield ML, Finlay DR, Murray JI, Troyanskaya OG, Chi JT, Pergamenschikov A, McGinley TJ, Brown PO, Botstein D, Connolly MK: Systemic and cell type-specific gene expression patterns in scleroderma skin. *Proc Natl Acad Sci U S A* 2003, 100:12319–12324.

8. Smith V, Van Praet JT, Vandooren B, Van der CB, Naeyaert JM, Decuman S, Elewaat D, De Keyser F: Rituximab in diffuse cutaneous systemic sclerosis: an open-label clinical and histopathological study. *Ann Rheum Dis* 2010, 69:193–197.

9. Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Kazantzis A, Sevrian C, Karampetsou M, Yiannopoulos G, Andonopoulos AP: Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. *Rheumatology (Oxford)* 2010, 49:271–280.

10. Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Kazantzis A, Korfitis P, Yiannopoulos G, Andonopoulos AP: Is there a role for B-cell depletion as therapy for scleroderma? A case report and review of the literature. *Semin Arthritis Rheum* 2010, 40:127–136.

11. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, Fraticelli P, Sambo P, Funaro A, Kazlauskas A, Awedimento EV, Gabrielli A: Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med* 2006, 354:2667–2676.

12. Sato S, Hayakawa I, Hasegawa M, Fujimoto M, Takehara K: Function blocking autoantibodies against matrix metalloproteinase-1 in patients with systemic sclerosis. *J Invest Dermatol* 2003, 120:542–547.

13. Abraham D, Muir H, Olson J: Adhesion of T and B lymphocytes to fibroblasts in tissue culture. *Immunology* 1988, 65:385–392.