Review

Therapeutic targets in systemic sclerosis

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Abstract

The precise aetiology of systemic sclerosis (SSc) remains elusive, but significant advances over the past few years have improved our understanding of the underlying pathogenic processes and identified key pathways and mediators that are potential therapeutic targets. The situation is complicated by the clinical heterogeneity of SSc and the differential pathogenesis that underlies the two commonest subsets, namely diffuse and limited cutaneous disease. However, there are common mediators that could be targeted to provide clinical benefit in both types of disease. To date, clinical success with therapies directed against logical profibrotic mediators, such as connective tissue growth factor and transforming growth factor-β, is yet to be reported, although studies are ongoing. More promising clinical results have been obtained with the dual endothelin receptor antagonist bosentan, which has been shown to manage two vascular complications of SSc effectively: pulmonary arterial hypertension and digital ulceration. It remains to be determined whether the identification of additional mediators merely furthers our knowledge of the natural history of SSc or presents targets that can be manipulated to manage SSc patients effectively.

Introduction

Ongoing studies of the pathogenesis of systemic sclerosis (SSc) have yielded improved understanding of the complex cellular interactions that occur in this heterogeneous connective tissue disease. It is clear from studies of lesional skin in SSc that there is a common sequence of pathogenic events. This sequence starts with microvascular change, including endothelial cell activation, which is followed by inflammation and immune cell activation, with a perivascular infiltration of inflammatory cells initially from the innate immune system and including cells of the monocyte/macrophage lineage. Later, there is evidence of involvement of the adaptive immune system. Lymphocytic infiltrates are noted, including T cells bearing markers of activation. Recent studies have also identified B-cell genetic signature as a hallmark feature of SSc skin. As the disease progresses there is evidence of a profibrotic fibroblastic cell population becoming established within the skin. This leads to increased extracellular matrix deposition. Early studies showed that these profibrotic cells are initially located around blood vessels but that later they are more generally distributed. As the disease becomes well established lesional skin becomes relatively avascular, and after 12 to 18 months there is often little evidence of ongoing inflammation. This suggests that there are probably distinct phases, at least in the skin, when the component processes of SSc might be amenable to therapeutic modulation [1]. Thus, targeted therapy is likely to depend critically on disease stage and subset.

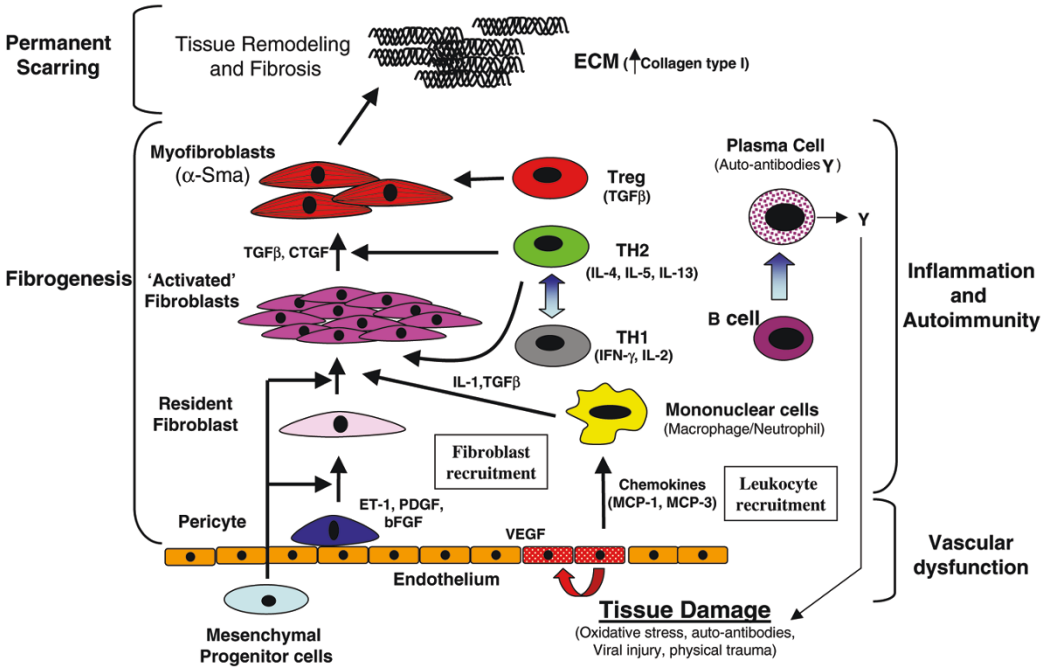
Differential pathogenesis in subsets of systemic sclerosis

The clinical heterogeneity of SSc makes it likely that different pathogenic processes operate in different disease subsets. Diffuse cutaneous SSc (dcSSc) has a much more inflammatory onset than does limited cutaneous disease (lcSSc). There is often widespread inflammation in the skin and musculoskeletal system, and oedema suggestive of altered endothelial permeability. Later in the course of disease, extensive fibrotic changes are apparent. Atrophy in the late stages may be more consequential than fibrosis. The dynamic nature of the process is highlighted by the frequent regression of skin disease at later stages, which can account for the observed apparent spontaneous fall in skin sclerosis score in dcSSc over time. Raynaud's symptoms generally occur contemporaneously with skin changes in dcSSc, or they may even occur afterward. This can delay diagnosis and broaden the differential diagnosis in early stage dcSSc.

In contrast, lcSSc has a much slower onset. Raynaud's phenomenon may pre-exist for several years. In this subset of

CCL = CC chemokine ligand; CTGF = connective tissue growth factor; dcSSc = diffuse cutaneous systemic sclerosis; EC = endothelial cell; ET = endothelin; $ET_{A/B}$ = endothelin receptor subtype A/B; lcSSc = limited cutaneous systemic sclerosis; PAH = pulmonary arterial hypertension; SSc = systemic sclerosis; TGF = transforming growth factor.

Figure 1



Cellular interactions in pathogenesis of SSc. The multiple cell types implicated in pathogenesis of SSc are illustrated. Soluble mediators involved in this intercellular cross-talk are candidate targets for therapeutic intervention. bFGF, basic fibroblast growth factor; CTGF, connective tissue growth factor; EC, endothelial cell; ECM, extracellular matrix; ET, endothelin; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; PDGF, platelet-derived growth factor; SMA, smooth muscle actin; SSc, systemic sclerosis; TGF, transforming growth factor; TH, T-helper (cell); Treg, regulatory T (cell); VEGF, vascular endothelial growth factor. Reproduced with permission from [1].

disease, the vascular component of SSc is much more prominent and is responsible for many of the clinical manifestations of the disease, including pulmonary arterial hypertension (PAH), digital ulceration and scleroderma renal crises. However, some degree of fibrosis is always present, including sclerosis of skin in the face and extremities and within the gastrointestinal tract, leading to the universal presence of gastro-oesophageal reflux.

Intercellular interactions in systemic sclerosis

It seems likely that there is a complex interaction between multiple cell types during the evolution of SSc. These cell types may interact through direct contact, but soluble mediators are also likely to be critical. These regulate cell migration, differentiation and proliferation. Cellular origins may be diverse within lesional tissue, and there has been a recent focus on the role of progenitor cells locally or migrating from other sites, such as bone marrow or from peripheral blood. The myofibroblast is the effector cell for fibrosis and many other organ-based changes. Myofibroblasts are likely to be derived from at least three sources: local fibroblasts, transdifferentiation and other cell types. Circulating and locally produced soluble mediators regulate this process. The key patterns of intercellular interaction that operate in SSc pathogenesis are summarized in Figure 1.

Cellular mediators in systemic sclerosis as potential therapeutic targets

Transforming growth factor-β

Descriptive studies of SSc, including examination of explanted skin fibroblasts and gene and protein profiling studies, implicate a number of growth factors or cytokines in SSc. A consistent candidate mediator is transforming growth factor (TGF)-β. This has potent profibrotic activity *in vitro* and stimulates myofibroblast differentiation. A number of studies have demonstrated increased TGF-β ligand expression in lesional SSc skin, and altered downstream signalling has been reported in SSc fibroblasts, although the data are somewhat conflicting.

Surprisingly, circulating levels of active TGF-β_1 are reduced in patients with dcSSc compared with healthy control individuals or patients with lcSSc [2]. In addition, there is an inverse relationship between TGF-β_1 in the circulation and skin score, and a correlation with disease duration. This may be explained by sequestration of active TGF-β in lesional tissues such as the skin caused by upregulation of various TGF-β binding proteins, many of which are TGF-β inducible. There is evidence to support this from gene profiling studies [3]. Total levels of ligand are not suppressed in SSc. When skin is examined directly there is evidence of increased TGF-β_1 and

TGF-β_2 mRNA in skin biopsies in both involved and uninvolved skin compared with the skin of healthy control individuals.

Evidence for direct involvement of TGF-β activation in fibroblasts comes from mouse models, in which selective activation of TGF-β signalling was observed in fibroblasts but not in other cell types. This has been demonstrated in two mouse models. In the first there is expression of a non-signalling human type II TGF-β receptor in fibroblasts using an expression cassette subcloned from the type I collagen gene *Col1a2* [4,5]. This mouse exhibits constitutive ligand-dependent activation of TGF-β genes in skin and other organs, and the animals develop skin fibrosis reminiscent of scleroderma. Interestingly, they also appear to be susceptible to organ-based fibrosis such as lung disease, although this may require additional triggers such as epithelial cell injury. Fibroblasts from the skin of TβRIIΔk-fib mice closely resemble those from SSc skin, exhibiting myofibroblast differentiation, TGF-β activation, and over-expression of connective tissue growth factor (CTGF) and plasminogen activator inhibitor-1 on gene profiling. These animals provide a valuable platform for testing targeted therapy, and blocking TGF-β signalling attenuated the scleroderma-like phenotype, as expected.

In the second, related mouse model, a Cre-Lox strategy has been used to induce genetic recombination postnatally. In this way, a constitutively active TGF-β type I receptor can be activated, and this model develops even more severe skin changes and a generalized vasculopathy reminiscent of scleroderma. Together, these mouse strains make it highly plausible that sustained activation of TGF-β signalling in fibroblasts can induce the hallmark features of SSc.

Scleroderma fibroblasts can be explanted, and recent studies have confirmed that blocking activin-like kinase receptor 5, the TGF-β type I receptor that is active in the inducible mouse strain, attenuates some of the hallmark biochemical features of SSc fibroblasts. Interestingly, some features cannot be reversed, including CTGF over-expression, which is in contrast to the mouse models and suggests that other mediators must also be important in human SSc [6].

To test fully the role of TGF-β signalling, a mouse strain has been generated in which the TGF-β type II receptor is deleted postnatally. These animals are resistant to lung fibrosis and do not form fibrous scars in healing skin wounds or contract skin wounds appropriately. However, they develop digital contractures that are reminiscent of scleroderma. This raises the hypothesis that although TGF-β overactivity may be a key to early SSc, reduced responsiveness may operate later.

Connective tissue growth factor

Although a logical target, TGF-β blockade is potentially problematic. There are real concerns about safety, including potential hazards of neoplasia and immune cell activation. Other molecules may be more appropriate targets. One such molecule is CTGF [7]. A member of the CCN family, CTGF (also known as CCN2) is a protein that has consistently been shown to be over-expressed in SSc. It may have direct profibrotic activity, but it also plays a key role in promoting cell adhesion and other aspects of the SSc phenotype. Other CCN family members are also implicated in fibrosis. Studies of CTGF serum and blister fluid levels suggest a correlation with skin sclerosis score. It is likely that CTGF represents a valuable marker of the fibrotic process in SSc, and soluble levels of the amino-terminal portion may be a useful surrogate for fibrotic burden [8].

Endothelium-derived mediators

Mediators that are produced by endothelial cells are especially interesting, considering the early events in SSc pathogenesis. Experimental systems have identified candidate mediators, and these include chemokines such as CC chemokine ligand (CCL)2 and CCL7 [9,10]. Both were recently shown to be elevated in SSc. CCL2 elevation appears to be associated with disease severity, and high levels of CCL2 are found in patients with major internal organ involvement in early dcSSc. CCL2 may have potential as a mediator that promotes migration, transdifferentiation and matrix production by myofibroblasts in SSc. Recent studies have confirmed that a subgroup of early dcSSc fibroblasts expresses the CCL2 receptor, and so the potential for autocrine or paracrine stimulation of fibroblasts in SSc is clear. *In vitro* experiments support this.

Endothelin (ET)-1 is a prototypic endothelial cell (EC)-derived product that possesses many of the hallmark properties that could be relevant to pathogenesis in SSc. This supposition comes from studies examining the production of these cytokines. ET-1 is a peptide that was first identified as an EC product. Levels of ET-1 released by ECs in monolayer culture are impressive, particularly considering the potential for this factor to link vascular inflammatory and fibrotic processes. Experimental data confirm over-expression of ET-1 peptide by three EC lines in tissue culture and that ET-1 promotes contraction and remodelling of fibroblast-populated collagen lattices, an established model for fibrosis [11]. ET-1 can induce myofibroblast differentiation in SSc, and blocking ET-1 using the dual endothelin receptor antagonist (endothelin receptor subtype A [ET_A]/ET_B) bosentan has been shown to reduce α-smooth muscle actin expression by lung fibroblasts cultured from SSc biopsies [12]. Gene expression profiles of lung fibroblasts treated with ET-1 are similar to those seen in SSc and can be normalized by bosentan. This provides some of the strongest data *ex vivo* for a role for autocrine ET-1 production as a driver of fibrosis. The validity of targeting ET-1 in SSc is confirmed by studies demonstrating that bosentan is an effective treatment for SSc-associated PAH [13] as well as for ischaemic digital ulceration [14]. Other endothelin receptor blocking drugs are being developed. Sitaxentan is an ET_A-specific antagonist that is now licensed in Europe for treatment of PAH. Broader beneficial effects of

this agent have not yet been explored. Another single ET_A-receptor antagonist, ambrisentan, has recently been approved in the US. The significance of dual versus ET_A-specific antagonism in the clinical arena remains to be elucidated.

Nitric oxide is an important mediator that has proinflammatory effects at high levels and is an important mediator of endothelium-dependent vasodilatation. The effects of nitric oxide are mediated via cGMP, and levels of this second messenger are increased when the degradative enzyme phosphodiesterase V is inhibited. This mechanism underlies the beneficial effect of agents such as sildenafil in treatment of PAH complicating SSc.

Platelet-derived growth factor

Pericytes may be important in the development of fibrosis by transdifferentiation into fibroblasts or myofibroblasts. This process may be intrinsic to wound healing and is regulated by platelet-derived growth factor. Its importance in organ-based fibrosis is unclear, but in the skin recent studies have clearly shown that platelet-derived growth factor signalling is critical to pericyte activation and that this sequence occurs as SSc progresses [15].

Therapeutic intervention: targeting pathways or mediators

The many mediators that have been implicated in SSc pathogenesis, and especially in fibroblast activation, are likely to signal via convergent signalling pathways intracellularly. This may include common signalling intermediates and modulation of likely transcription factors that regulate gene expression [16]. Whether these convergent pathways could be targeted for therapy depends on their involvement in other cellular processes and on whether there can be selective attention in disease states. At present this seems unlikely, and major concern about pathway directed drugs remains.

It is more likely that ligand-directed strategies will be effective, and this is certainly the case for many other diseases, including inflammatory arthritis. Cross-talk between mediators must be considered and it is possible that multiple proteins will need to be targeted either sequentially or together. It is very encouraging that there is a growing armamentarium of potential ligand-directed antagonists, some of which are listed in Table 1. Some of these agents have already been evaluated in fibrotic disease, and although none has yet been found to be effective, early-stage proof of concept studies are being developed. In a safety study of human anti-TGF-β_1 there was no evidence of a treatment effect, but the treatment did not appear to be toxic [17]. Further evaluation of a pan-specific anti-TGF-β agent in idiopathic lung fibrosis is ongoing.

It is encouraging that one of these strategies, namely endothelin blockade, has already been shown to be effective in treating two major complications of SSc. Studies in lung fibrosis complicating SSc were unable to demonstrate efficacy, but this may reflect disease progression and trial design rather than lack of antifibrotic potential. It is very tempting to speculate that blocking a mediator such as ET-1 would be a valuable strategy in treating SSc, and the fact that - for the first time - a manifestation of the disease can be prevented (digital ulceration) is a major finding. In addition, the fact that several of the agents listed in Table 1 have acceptable safety profiles makes evaluation in SSc of their impact on advanced clinical outcomes imperative.

Table 1

Attenuation of key pathways in systemic sclerosis: agents for evaluation

Candidate therapy	Target pathway
Bosentan	ET_A/ET_B pathway
Imatinib	PDGF receptor signalling
Infliximab	TNF-α
Adalimumab	TNF-α
Etanercept	TNF-α
CAT-192	TGF-β_1
GC-1008	TGF-β_1, TGF-β_2, TGF-β_3
FG-3019	CTGF ligand
Alefacept	LFA3/CD2
Basiliximab	IL-2Rα
MLM-1202	CCR2
Efalizumab	LFA/ICAM-1

CCR, CC chemokine receptor; $ET_{A/B}$, endothelin receptor subtype A/B; CTGF, connective tissue growth factor; ICAM, intercellular adhesion molecule; IL-2Rα, interleukin-2 receptor α; LFA, lymphocyte function associated; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumour necrosis factor.

Conclusion

Substantial advances have been made in elucidating the pathogenesis of SSc, which has been facilitated in part by the development of more appropriate animal models. Targeting of several putative mediators is now feasible [16]. Convergence of signalling pathways suggests synergy between antagonists. A hierarchy of mediators may determine stage-specific efficacy. It remains to be determined whether these exciting strategies only inform about disease processes or confer real therapeutic advantage. A key question, however, is whether targeted therapies will ultimately prove to be more effective than broader approaches, such as high-dose immunosuppression. Ongoing trials of immunosuppression with cyclophosphamide and haemopoietic stem cell transplantation are important studies that will better define the benefit of these treatments in early-stage dcSSc [18].

Competing interests

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