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Future targets in the management of systemic sclerosis

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CTDs—such as SSc and SLE and related rheumatic diseases such as RA—have complex, underlying pathogeneses that include fibrosis, vascular dysfunction, activation of the immune system and inflammation. Although some current therapies for SSc offer benefits to patients, there is a clear need to investigate potential therapeutic targets. However, the breadth and diversity of cellular pathways and mediators implicated in these diseases, coupled with inherent redundancies in these systems, has made pre-clinical investigation difficult. Despite this, recent advances have been made in elucidating the immunological aspects of CTD, including the roles of B cells, T cells, matrix-remodelling cells and autoantibodies, enabling novel therapeutic approaches including immunoablation to be investigated. The mechanisms underlying the fibrosis that characterizes SSc are also becoming clearer; and as the putative events that trigger excessive collagen deposition are identified, so too are potential junctures at which these aberrant processes may be deactivated. Progress is also being made in understanding the vasculopathy in SSc, and the potential benefits of antioxidants and endothelin receptor antagonists. There have been some significant advances in the treatments available to SSc patients; however, this spectrum of diseases remains challenging, and continues in some cases to be associated with high morbidity, increased mortality and poor prognosis.

Key words: Connective tissue disease, Systemic sclerosis, Autoimmunity, Anti-fibrotic, vascular dysfunction.

Introduction

The term 'connective tissue disease' (CTD) can be applied to numerous conditions including SSc and SLE. Each of these diseases has a complex pathogenesis, with manifestations that include fibrosis, inflammation and vascular dysfunction (Fig. 1) [1]. The inflammatory component of these diseases is characterized by an aggressive autoimmune activation that may lead to scarring of tissue and permanent organ damage. The fibrotic aspect of these diseases can manifest in the skin, internal organs and the vasculature, and is a hallmark of CTD. The resultant damage to the vasculature from these aberrant processes leads to vascular remodelling and tissue ischaemia.

One CTD with a high case-specific mortality is SSc, which features all of the characteristic immunological, vascular and fibrotic dysfunctions and is associated in some cases with poor prognosis and high morbidity. The majority of SSc patients have a disease form that can be categorized into one of the two major subsets, dcSSc or lcSSc, depending on the degree of skin involvement and autoantibody profile [2].

Patients with dcSSc typically experience skin thickening proximal and distal to the elbows and knees, which can include the face. These patients frequently experience internal organ involvement including pulmonary fibrosis (PF), renal crisis and cardiac involvement. Whilst lcSSc patients can also experience facial manifestations, they have skin thickening distal to the elbows and knees, present less frequently with internal organ involvement, and be more prone to calcinosis, oesophageal dysfunction, sclerodactyly, telangiectases, pulmonary arterial hypertension (PAH) and digital ulcers (DUs) [2].

A third, less common subset of SSc patients can be described as having scleroderma overlap syndromes, also known as MCTD. These patients may present with features of both SSc subtypes, sometimes together with features of other autoimmune CTDs. A fourth subgroup of SSc also exists, in which patients present with the internal organ manifestations of SSc, but without detectable skin involvement. This rare and unique disease type is called SSc *sine* scleroderma.

In general, SSc treatments are determined by the extent and severity of disease manifestations, and if there is any internal organ involvement. At present, there are few efficacious treatment options for SSc, with most current therapies being targeted towards symptom relief. Understandably, interest exists in identifying new targets to address this unmet medical need.

Laboratory and animal model research to identify the mechanisms underlying CTD has proven difficult; the links between immunological activation, fibrosis and vascular dysfunction are incompletely understood. Furthermore, whilst disturbances in cell–cell communication and intracellular signalling are apparent, the complexities and redundancies inherent within these networks make their investigation difficult. Future prototypical targets include many ligands and receptors, including cytokines, vasoactive molecules, growth factors and components of metabolic pathways. This article will discuss the current state of understanding of the immunological, vascular and fibrotic mechanisms underlying CTD, specifically focusing on SSc, and evaluate potential therapeutic targets.

The underlying pathological mechanisms in SSc

Immunological disturbances

Autoaggressive immunological activation is a key component of CTD, yet the mechanisms underlying this activity are incompletely understood. In SSc, disturbances in B-cell homeostasis and the inappropriate activation of T cells are postulated to contribute to the fibrosis, microvascular dysfunction and autoimmunity that characterize this disease. Elevated serum concentrations of growth factors, ILs, chemokines and other cytokines have also been measured in these patients [3]. Populations of expanded clonal T cells have been detected in the skin and blood of SSc patients, suggesting that proliferation and clonal expansion has occurred *in situ*, in response to an as yet unidentified antigen. Circulating γ/δ T cells have been demonstrated to be involved in the early stages of SSc, with significant infiltration of the skin and expression of a cytotoxic, type 1 Th cell (Th1) orientation [4, 5].

It has also been proposed that the pattern of cytokine production exhibited by skin-infiltrating T lymphocytes in SSc is oriented towards a Th2 polarization [6]. Activated T cells produce Th2 cytokines including IL-6, -10 and MCP-1—all speculated to be inducers of fibrosis. However, this paradigm may not be so simple, since it has been shown that Th2 cells from the skin of

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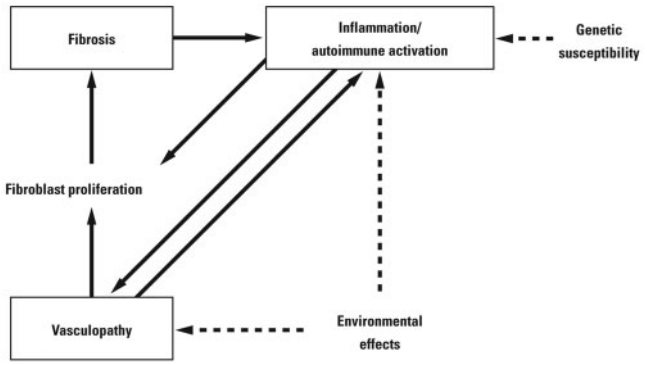

FIG. 1. Hypothesis for the pathogenesis of SSc. Adapted from the *Lancet* [1] with permission from Elsevier.

patients with early-stage SSc may inhibit fibrosis via production of TNF-α [7]. Production of TNF-α overrides the profibrotic IL-4, -6 and -10, and impedes collagen production [7]. Correspondingly, SSc patients who experience a shift in serum cytokine profile from a Th2 to a Th1 orientation, also experience improvements in skin fibrosis [8].

Additionally, disturbances in B-cell homeostasis are understood to contribute towards the SSc phenotype. Sato *et al*. [9] documented a 20% increase in B-cell CD19 expression in SSc patients; an increase comparable with one that leads to autoaggressive antibody (Ab) production in mice. Also, the up-regulated expression of CD80 and CD86 on the memory B cells of SSc patients suggests that these cells are chronically activated [10].

The effects of B-cell depletion has been investigated in a murine SSc model, and was demonstrated to suppress development of skin fibrosis, autoantibody production and hypergammaglobulinaemia in newborn mice [11]. Messenger RNA expression profiles of Th1 and Th2 cytokines were also less imbalanced in treated mice. However, this intervention was inefficacious in mice with existing fibrosis, suggesting that imbalances in B-cell homeostasis may only be important during disease onset [11].

The development of autoaggressive Abs is a central feature of CTD, with different autoantibody expression profiles being associated with different diseases. In SSc, a high (90%) prevalence of ANAs has been detected, although autoantibody expression differs within SSc subtypes. Additionally, a putative pathogenic autoantibody to PDGF receptor (PDGFR) has been recognized in SSc patients, and implicated in collagen gene overexpression by fibroblasts [12]. Together, these observations suggest future interventions that restore immunological homeostasis to CTD patients, which might yield quantifiable therapeutic benefits. However, continued investigation and characterization of the mechanisms underlying autoimmunity are clearly necessary.

Vascular dysfunction

The mechanisms underlying SSc vasculopathy include activation of vascular endothelial cells and disordered angiogenesis. Endothelial activation can be measured indirectly by quantifying the plasma concentration of von Willebrand factor (vWf), produced by the vascular endothelium following activation or damage [13]. Plasma vWf concentrations are increased in SSc patients, particularly when endothelial damage begins to impair control of vascular tone [14]. Indeed, plasma vWf propeptide concentrations are understood to correlate with early pulmonary involvement and other biochemical markers of disease activity [13]. Morphological evidence of endothelial cell activation and damage in CTD can be obtained by measuring the frequency and nature of microvascular lesions [15]. Apoptotic endothelial cells characteristic of SSc have also been detected in the early inflammatory stages of dcSSc and lcSSc [16].

TABLE 1. Clinical outcomes up to 60 months for 57 patients with SSc treated using immunoablation plus HSCT [32]

Patient status	Proportion, %
Improved, then progressed	25
Sustained improvement	34
TRM	9
Not transplanted	10
Progressed, no response	7
Stable	2
Too early	10
Unknown	3

Angiogenic homeostasis is understood to become destabilized in SSc patients, leading to ischaemia and vascular remodelling [17, 18]. Increased serum concentrations of the angiogenic VEGF are detectable, and are understood to correlate with disease progression [18–21]. Elevated expression of the angiogenic inhibitor endostatin has also been measured in SSc patients, and is associated with the presence of giant capillaries in nail-fold capillaroscopy [22–24].

The expression of urokinase-type plasminogen activator (uPA) and the uPA receptor (uPAR) by microvascular endothelial cells (MVECs) is understood to be important to angiogenic control. However, the MVECs of patients with SSc exhibit increased expression of a functionally impaired, truncated uPAR [25]. Additionally, disordered regulation of angiogenic remodelling by the angiopoietins, and their associated receptor tyrosine kinases, is also understood to occur in some vascular diseases [26, 27]. Furthermore, the expression of activation markers and hyperplasia of microvascular pericytes have been postulated to be important links between chronic microvascular damage and fibrosis.

Fibrotic mechanisms

The fibrosis that affects the skin and internal organs of SSc patients is underpinned by the transition of fibroblasts from a quiescent state to one of sustained activation. These activated myofibroblasts characteristically overproduce type I collagen, collagen-modifying enzymes, and other extracellular matrix (ECM) components. However, the events that trigger this activation are unknown. Interestingly, the SSc fibroblast phenotype is comparable with fibroblasts that have been exposed to excessive signalling by TGF-β, hinting at a potential underlying mechanism [28, 29]. Additionally, interactions between activated SSc fibroblasts and the ECM may also contribute to excessive collagen deposition, since the ECM is understood to regulate the activity of mediators such as TGF-β and fibrillin [30, 31].

Future immunological therapies

Conventional treatments for CTD offer only limited efficacy, leaving patients with few therapeutic options. The need for improved therapies is particularly acute for patients with severe autoimmune diseases, and therefore the combination of immunoablation plus autologous haematopoietic stem cell transplantation (HSCT) has been piloted in these individuals. Local protocols differ, but this process essentially combines the mobilization and harvest of peripheral blood stem cells, with their purification, selection and transplantation into patients following chemotherapy [32]. To date, over 1000 patients have undergone this technique, with sustained remission achieved in several recipients, including over 150 patients with SSc (Table 1) [32, 33]. The success of this procedure varies between diseases, as does the risk of treatment-related mortality (TRM). However, with growing experience and technical refinements, the TRM frequency is declining [32, 34].

The European multicentre, prospective, randomized Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial is currently under way. This study will compare

immunoablation and autologous HSCT *vs* monthly, intravenous pulse cyclophosphamide therapy in patients with early dcSSc at high risk of mortality [35]. The primary end point in the ASTIS trial is event-free survival, defined as the duration between randomization and death, or development of end-stage organ failure [35]. Secondary end points include progression-free survival, TRM and toxicity. To date, 104 out of a planned 108 patients have been enrolled, with 54 having received HSCT. There have been no graft failures or unexpected toxicities, and only two probable TRMs.

Whilst completion of the ASTIS data set is pending, confidence in this technique is growing, and a sustained 'reset' of an autoaggressive immune system appears possible [36]. However, the associated risks mean that strict inclusion and exclusion criteria must be implemented to optimize outcomes when evaluating this technique.

Future cellular and molecular targets

The complex underlying pathologies of CTD provide numerous prototypical targets for therapeutic intervention, including ET-1, a key mediator of vascular hypertrophy, proliferation, inflammation, fibrosis and vasoconstriction. ET-1 exerts its effects via the ET_A and ET_B receptor subtypes, and can induce collagen synthesis and secretion [37, 38]. Elevated ET-1 expression has been detected in several organs, during both early and late disease stages in SSc patients, and can induce expression of an SSc phenotype in normal fibroblasts [39, 40]. Clinical data have shown that the oral, dual ET-1 receptor antagonist, bosentan, has beneficial effects in SSc patients particularly with respect to PAH secondary to their disease [41] and DUs, a debilitating manifestation in many SSc patients [42] and results in an overall improvement in quality of life.

As discussed earlier, TGF-β has been implicated in SSc pathogenesis, and a randomized, placebo-controlled trial to evaluate a recombinant human anti-TGF-β Ab (CAT-192) in patients with early dcSSc has been undertaken [43]. In this pilot study, CAT-192 showed no evidence of efficacy. In addition, more adverse events and more serious adverse events were recorded in patients receiving CAT-192 *vs* placebo, although these events were not more frequent in the high-dose treatment group [43]. Therefore, based on current data, therapies targeting TGF-β may offer limited value.

The tyrosine kinase inhibitor imatinib has been demonstrated to inhibit fibrosis in several models, and to alter the gene and protein expression profiles of signalling kinases and immunological mediators in mast cells [44, 45]. In a murine model of SLE, imatinib prolonged survival, delivered significant renal protection and reduced concentrations of biochemical disease markers [46]. Patients with autoimmune CTD may therefore benefit from the evaluation of imatinib in a controlled trial [44].

The cytokine B-cell activating factor (BAFF) is also understood to activate B cells, facilitating their maturation and survival [47–49]. In comparison with normal controls, increased serum BAFF concentrations are detectable in CTD patients, and recent data suggest that serum BAFF concentrations may correlate with the extent of disease in SSc and primary SS [50, 51]. Observations *in vivo* suggest that by reversing autoantibody production in a setting of established autoimmunity and ameliorating B-cell-dependent disease manifestations, BAFF blockade may offer a novel therapeutic approach, [52].

Additionally, the Toll-like receptors (TLR) are key signalling components in innate immunity, binding lipopolysaccharide and oligonucleotides, and exerting disease-specific immunomodulatory functions. The TLR-9 signalling pathway has been implicated in autoimmune disease, and postulated as a prototypical target. In a murine model of SLE, TLR-9 signalling appeared to confer a protective effect, with TLR-9-deficient lupus mice having a more severe disease form. Human studies also document a higher proportion of TLR-9^+ B cells and monocytes in patients with active SLE [53, 54]. Similar results were seen in peripheral blood mononuclear cells from SSc patients cultured *in vitro* with TLR agonists. SSc patients showed increased expression of a cluster of IFN-regulated genes, including Siglec-1 (CD169, sialoadhesin) [55].

Future targets in the treatment of pulmonary fibrosis

Several organ-specific targets, such as renal, gastrointestinal and cardiac targets, are important in SSc. Lung involvement is, however, the leading cause of death in SSc, and PF is estimated to account for 25% of SSc-related mortality [56]. For SSc patients with PF, the extent of connective tissue deposition in the lungs is a determinant of prognosis, making early detection an important goal. However, a scarcity of symptoms or the presence of nonspecific symptoms makes early detection challenging [57].

Traditional immunosuppression can offer modest efficacy in the treatment of PF, although this approach plays a relatively minor role in current management. Long-term cyclophosphamide treatment can deliver moderate improvements in forced vital capacity [58, 59], although questions remain about optimal treatment regimens. Therapies that are efficacious in vasculitis, as well as the immunosuppressants mycophenolate mofetil, prednisone and AZA, might also be beneficial in SSc patients with PF. In addition, the future use of selective, targeted therapies for PF may offer improved efficacy with lower toxicity. For example, imatinib has been shown to exhibit potent anti-fibrotic effects *in vitro* and *in vivo*, and may therefore reduce collagen gene overexpression in SSc [12, 45].

The Bosentan Use in Interstitial Lung Disease (BUILD 1) trial evaluated bosentan for the treatment of idiopathic pulmonary fibrosis (IPF). In these patients, a trend favouring bosentan in the combined outcome of time-to-disease-progression or death was observed, an effect that was pronounced in a pre-identified subpopulation of patients with surgical lung biopsy-proven IPF [60]. The BUILD 2 trial evaluated bosentan in patients with interstitial lung disease secondary to SSc, but observed no significant treatment effects [61]. However, patients in this study were observed to exhibit relatively stable disease. In addition, the suitability of the 6-min walk test for the assessment of treatment effects in parenchymal lung disease has subsequently been questioned [62]. The effects of bosentan in PF related to SSc therefore require further investigation.

Future PF treatments may otherwise involve simultaneous targeting of multiple pathways; a pleiotropic approach. For example, the Ifigenia study assessed high doses of the antioxidant *N*-acetylcysteine (NAC) added to standard therapy of prednisone plus AZA in patients with IPF [63]. Patients treated with NAC experienced reduced declines in pulmonary function at months 6 and 12 *vs* placebo [63]. A future evaluation of this treatment regimen in patients with PF related to SSc may therefore be worthy of consideration. Similarly, pirfenidone has demonstrated activity in multiple fibrotic conditions, with combined anti-inflammatory, anti-oxidant and anti-fibrotic effects [64].

Regardless of approach, however, future therapeutic advances in PF may only offer incremental improvements in efficacy, and therefore trials for novel agents need to be meticulously planned. Specifically, an appropriate consideration of inclusion and exclusion criteria, choice of sample sizes, data quality, statistical analysis and the selection of appropriate and robust end points are essential.

Conclusion

Whilst the underlying pathogeneses of CTD are complex and incompletely characterized, dysfunction in signalling networks and cellular processes, including immunity, vascular function

and connective tissue deposition, are apparent. Accordingly, research into future CTD therapies is uncovering a diverse multitude of prototypical targets that includes cytokines, growth factors, vasoactive mediators and cellular targets. Whilst differing volumes of evidence support each prototypical target, some approaches, such as the use of anti-TGF-β Abs, have been demonstrated to be of limited value in one small study designed to test feasibility and safety. Other compounds and treatments, such as bosentan, NAC and immunoablation plus HSCT, may offer value as therapeutic tools in future.

Experience suggests that early identification and aggressive management of CTD may improve long-term outcomes, and this will no doubt continue to apply in an era of novel therapeutic approaches. Questions addressing patient selection, optimal administration routes and therapeutic combinations will, however, need to be addressed by meticulously planned, controlled trials, sufficiently powered to detect incremental advances in safety and efficacy. In conclusion, the continued evaluation of prototypical targets combined with more effective management of CTD patients will place us on course to deliver improved outcomes in the future.

Rheumatology key messages

- The CTDs have complex, underlying pathogeneses that include fibrosis, vascular dysfunction, activation of the immune system and inflammation.
- The continued evaluation of prototypical targets combined with more effective management of patients will place us on course to deliver improved outcomes in the future.

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