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Coagulation and Autoimmunity in Scleroderma Interstitial Lung Disease

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Abstract

Objectives—Interstitial lung disease in systemic sclerosis (SSc-ILD) is often an irreversible and progressive fibrosing process that now is the leading cause of scleroderma-related deaths. In this review we present our current understanding of the role played by coagulation and particularly by thrombin in autoimmune-mediated tissue injury and fibrosis, mainly as it relates to SSc-ILD.

Methods—We used PubMed to search for articles published up to October 2010 for keywords referring to autoimmunity, coagulation, pulmonary fibrosis, and scleroderma.

Results—SSc-ILD is an autoimmune disease associated with lymphocyte activation and release of various cytokines and growth factors. The production of autoantibodies is a central feature in SSc. Activation of the coagulation cascade with release of thrombin is 1 of the earliest events following tissue injury. Thrombin contributes to autoimmune responses by activating of pathogenic Th2 lymphocyte profile in SSc. Thrombin also modulates tissue repair responses, stimulates transformation of epithelial cells, endothelial cells, and fibroblasts into myofibroblast phenotype, and induces secretion of several pro-immune and profibrotic factors, which serve as antigens for pathogenic autoantibodies production in SSc-ILD.

Conclusions—The identification of links between autoimmunity and coagulation would provide new insights into the pathogenesis of pulmonary fibrosis associated with autoimmune diseases and further acknowledge the importance of thrombin in the development of SSc-ILD.

Keywords

thrombin; scleroderma; pulmonary fibrosis; coagulation; autoimmunity

Systemic sclerosis (SSc, scleroderma) is a complex autoimmune disease of unknown etiology that results in progressive fibrosis of skin and internal organs. Pulmonary fibrosis associated with SSc is a chronic diffuse lung disease characterized by progressive deterioration of lung function that ultimately may lead to death. It is well established that several molecular abnormalities occur after injury that may result in fibrosis (1–3). The presence of autoantibodies is a central feature of SSc, with antinuclear antibodies detected in more than 90% of SSc patients (4). The autoantibodies react to various cells, their intracellular components, and extracellular products. Furthermore, abnormal activation of immune cells including T lymphocytes, B lymphocytes, natural killers, and macrophages

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has been identified in SSc patients (5,6). The coagulation cascade, apoptosis, angiogenesis, vascular remodeling, cytokine and chemokine imbalance, and immune abnormalities have been implicated, suggesting multiple pathways involved in the pathogenesis of pulmonary fibrosis (7).

The vascular damage that exists in all patients with SSc and endothelial cell injury is believed to be an early initiating event that occurs even before the onset of fibrosis (8). Pulmonary involvement, now accounting for the majority of scleroderma-related deaths, is most often characterized by either an interstitial fibrosing process (SSc-ILD) or pulmonary arterial hypertension (SSc-PAH), or by both (1,2). The pathogenesis of SSc-ILD involves tissue injury with evidence for both epithelial and endothelial cell damage followed by activation of the immune system, and accumulation of myofibroblasts (9,10). Myofibroblasts are key mediators of pulmonary fibrosis responsible for the deposition of excessive amounts of extracellular matrix proteins in the alveolar-capillary units (10). They likely arise from multiple sources including resident fibroblasts, via the process of epithelial-mesenchymal transformation, and from bone marrow-derived fibrocytes (11,12).

Pulmonary fibrosis is also often characterized by endothelial and smooth muscle hypertrophy of the pulmonary vasculature and abnormal vascular proliferation (13). We have observed the proliferation of microvascular endothelial cells and the generation of functional capillaries in lung tissues from SSc-ILD patients (14). Early vascular dysfunction in SSc lung may result from the hyperproliferation of endothelial cells and possibly from the increased numbers of alveolar capillaries (3,14).

Pulmonary fibrosis results from recurrent alveolar epithelial injury and aberrant alveolar wound repair (15). Alveolar epithelial injury is followed by extravasation of plasma proteins and activation of the coagulation cascade, resulting in intra-alveolar fibrin deposition. Fibrin acts as provisional matrix for type II alveolar epithelial cells that restore integrity of alveolar epithelium, as well as for fibroblasts, which proliferate and differentiate into collagen-producing myofibroblasts (16).

Increasing evidence has accumulated to implicate an impaired coagulation system in respiratory conditions associated with chronic or repetitive lung injury, including SSc-ILD (17–20). This coagulation imbalance is the net result of activation of coagulation, impaired activity of natural coagulation inhibitors, and suppressed fibrinolysis. Activation of coagulation proteases, eg, thrombin, is 1 of the earliest events following tissue injury (20,21). Thrombin can modulate tissue repair responses by altering vascular permeability, stimulating fibroblast and neutrophil migration, and promoting adhesion and spreading of endothelial cells and fibroblasts (3). It activates various cell types and induces secretion of several pro-immune, pro-fibrotic, and angiogenic factors (22–26). Thrombin also promotes lung fibroblast differentiation into a myofibroblast phenotype resistant to apoptosis and induces angiogenesis in endothelial cells by promoting migration and proliferation (27,28). Thus, thrombin is involved in the development and progression of pulmonary fibrosis. The purpose of this review is to analyze the literature pertaining to autoimmune-mediated tissue injury with resultant thrombin signaling and fibrosis especially as demonstrated in SSc-ILD.

METHODS

The PubMed database was searched, using the following terms both alone or in combination: autoimmunity, coagulation, thrombin, pulmonary fibrosis, and sclero-derma. The abstracts were screened for the relevance and to interstitial lung diseases and appropriate articles were evaluated. In the interpreting these data we conclude that autoimmunity and coagulation systems are integrated in pulmonary fibrosis, particularly in

SSc-ILD. Available articles published in English between years 1980 and 2010 were reviewed. References noted in relevant articles were also accessed and critically discussed.

RESULTS

Imbalance of Coagulation System in Pulmonary Fibrosis and Other Lung Diseases

There is now increasing evidence that procoagulant signaling contributes to inflammation and fibrosis and that an activated coagulation cascade is present in the lung of patients with fibroproliferative pulmonary disease (20,29,30). The elevated procoagulant activity in alveolar compartments is mainly attributable to tissue factor (TF) induced activation of coagulation (20). TF is generally localized outside the vasculature, preventing initiation of coagulation under normal flow circumstances (31). Once tissue injury occurs, factor VII leaves the circulation and rapidly binds to the exposed TF, followed by the conversion of factor X to active Xa. Factor Xa catalyzes the activation of prothrombin to thrombin, which in turn converts fibrinogen to fibrin resulting in clot formation (Fig. 1). The small amount of thrombin produced by factor Xa feeds back to activate factor VIII, resulting in generation of additional factor Xa and activation of factor V. The combination of factors Va and Xa, in association with phospholipids and calcium, forms the prothrombinase complex that subsequently catalyzes the production of larger quantities of thrombin. This positive feedback mechanism leads to further thrombin generation and results in amplification of the coagulation cascade (32) (Fig. 1).

There is compelling evidence that thrombin is an important mediator of pulmonary fibrosis including the ILD-SSc, as well as idiopathic pulmonary fibrosis (IPF), chronic asthma, and in several animal models, employing bleomycin to induce lung fibrosis (3,20,29). Increased thrombin activity is found in bronchoalveolar lavage fluid (BALF) from SSc patients with lung fibrosis, as well as in other fibrosing lung diseases (17,18). Thrombin concentration is rapidly elevated up to 100-fold exceeding its physiological concentrations in fibrotic lung with tissue injury (Fig. 2). As noted above, thrombin is the primary bioactive factor in the pathway to fibrin formation (32). Fibrin is required for reparative processes and normal wound healing. However, persistent and excessive deposition of extravascular fibrin may contribute to the pathogenesis of chronic ILD. Fibrin has been shown to serve as a reservoir of profibrotic growth factors and to provide a provisional matrix on which fibroblasts proliferate and produce collagen (29). Furthermore, fibrin and its degradation products exhibit chemotactic activity that enhances neutrophil recruitment in the lung, increases vascular permeability, and influences migration and proliferation of inflammatory cells.

The role of fibrin in the development of pulmonary fibrosis was assessed in 2 different transgenic mouse models in which fibrin is significantly reduced. In the first, a fibrinogen-deficient mouse lacks the necessary precursor to make fibrin polymers (33); in the second, fibrin is degraded in a mouse deficient in plasminogen activator inhibitor (34). Both of these murine strains possess undetectable levels of lung fibrin in response to injury; however, neither is protected from experimental pulmonary fibrosis induced by bleomycin (33,34). Such data suggest that very early events in the coagulation system, and its downstream cellular effects, may be a more probable link to the profibrotic effects of procoagulant factors than are fibrin polymers per se. The coagulation cascade is locally activated in various lung diseases, including SSc-ILD, and the resultant generation of thrombin plays a critical role in the evolution of lung fibrosis.

Alterations of the alveolar hemostatic balance and excessive deposition of intra-alveolar fibrin have been observed in chronic fibrosing interstitial lung disease with considerable inflammation of the lung parenchyma (29). Procoagulant and antifibrinolytic activities with excessive fibrin deposition in alveolar space have been demonstrated in both SSc-ILD and

IPF (35). However, changes in the alveolar hemostatic balance and excessive deposition of intraalveolar fibrin seem to be independent of the driving mechanisms (inflammation vs alveolar epithelial injury/ altered wound repair) and appear to reflect a rather uniform response pattern to chronic lung injury (29).

Besides pulmonary fibrosis, apparent coagulation imbalance occurs in patients with acute and chronic pulmonary thromboembolism (PTE) and asthma (36,37). Thrombin is also activated in PTE and asthma; however, it does not usually exhibit profibrotic action in these conditions. In both asthma and PTE, activation of the coagulation cascade and fibrinolysis are each increased, while in pulmonary fibrosis, coagulation is increased but fibrinolysis is decreased. Increased fibrinolysis at least partially attenuates thrombin's actions in asthma and PTE and, therefore, profibrotic effects do not occur or are delayed in these diseases. Recently it was reported that venous thromboembolism is associated with idiopathic interstitial pneumonia and pulmonary fibrosis. These studies were performed on a large population (7 million individuals, in a 27-year follow-up period) and represent mainly a white Danish population, so may not apply to other ethnic groups (38). Recently it has also been shown that acute exacerbation of moderate asthma appears to be associated with a shift to a profibrogenic, possibly antifibrinolytic, condition in the airways (39). These results support the notion that thrombin may be a profibrotic factor in these diseases as well.

Protease Activated Receptors (PARs) in Coagulation, Autoimmunity, and Pulmonary Fibrosis

High-affinity thrombin signaling receptors, the PARs, play a central role in orchestrating events relating coagulation to inflammation and fibroproliferation (40,41). There are 4 known members (PAR-1, -2, -3, and -4) belonging to this family of 7-transmembrane G-protein-coupled receptors. These receptors are activated by proteolytic cleavage of their N-terminal domains, leading to the exposure of a new amino-terminus, a "tethered ligand" that in turn activates the receptor (41) (Fig. 3). PARs have recently been shown to play important roles as effectors in the pathophysiology of various lung diseases, including SSc-ILD (40,42,43). PARs are present in different combinations in a variety of cell types, including leukocytes, platelets, T cells, endothelial cells, vascular smooth muscle cells, and fibroblasts (44). PAR-1, the major profibrotic signaling receptor, appears to play a critical role in conditions characterized by lung injury. Expression of PAR-1 is increased in patients with either IPF (20) or SSc-ILD (27). In the bleomycin model of pulmonary fibrosis, PAR-1-deficient mice show a significant reduction of inflammatory cells in BALF as compared with wild-type mice, and significant protection from lung fibrosis is seen in the PAR-1-deficient mice (40). The relative protection from pulmonary fibrosis observed in this model is a reduction in expression of 2 major fibrogenic growth factors, connective tissue growth factor (CTGF) and transforming growth factor- β , as well as a reduction of the chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemoattractant protein 1 (40).

Stimulation of PARs by coagulation proteases on different cell types leads to induction of a number of pro-inflammatory factors including interleukins (IL-1, IL-6, IL-8), and monocyte chemoattractant protein 1 (25,26). Thrombin via PAR-1 also induces CTGF, transforming growth factor- β , and platelet-derived growth factor (PDGF) (17,22–24) (Fig. 1). We demonstrated that following PAR-1 activation thrombin's mitogenic effect on fibroblasts is critically dependent on induction of both PDGF-AA and the PDGF α -receptor (17). Recently discovered auto-antibodies directed against the PDGF α -receptor may play an important role in the pathogenesis of SSc lung fibrosis (45), although the relevance of such auto-antibodies remains controversial (46). Nevertheless, blocking PDGF signaling attenuates bleomycin-induced pulmonary fibrosis (47), suggesting that PDGF or its upstream effectors might be considered to be potential therapeutic targets for treating lung fibrosis.

During the development of pulmonary fibrosis, thrombin activates lung fibroblasts (Fig. 4), which in turn interact with other cell types, such as immune cells, as well as nonimmune cells, such as endothelial cells. Similarly, alveolar epithelial-mesenchymal transformation and endo-thelial-mesenchymal transformation, which have been shown to participate in the progression of lung fibrosis, occurs on stimulation by thrombin via PAR-4 and PAR-1, respectively (48,49). Beneficial effects of anticoagulant therapy have been observed in a bleomycin-induced lung fibrosis model (40) and in patients with pulmonary fibrosis (50,51). A direct thrombin inhibitor UK-156406 has been shown to attenuate lung collagen accumulation by lowering the profibrotic effects of thrombin and by suppressing CTGF synthesis in bleomycin-induced lung fibrosis (52). Other direct thrombin inhibitors, eg, lepirudin, bivalirudin, argatroban, and melagatran, which interact with thrombin by blocking its catalytic activity, are currently approved for clinical therapy in cardiovascular and thrombotic diseases. Deletion of the thrombin receptor PAR1 (knockout mice) also attenuates pulmonary fibrosis in a murine model, suggesting a protective role of thrombin blockade in bleomycin-induced lung inflammation and fibrosis (40). Recently, we have shown that the direct thrombin inhibitor, dabigatran, inhibits thrombin-induced and PAR-1-mediated profibrotic signaling. Dabigatran blocks myofibroblast differentiation and reverses SSc lung myofibroblasts to a fibroblast phenotype (53) by reducing collagen, CTGF, and α -SMA in these cells (Fig. 5). These data suggest that targeting thrombin's actions may result in successful treatment in SSc-ILD and other types of pulmonary fibrosis.

Autoimmune-Mediated Tissue Injury in Scleroderma Pulmonary Fibrosis

Scleroderma is characterized by the presence of various immunologic abnormalities, such as T lymphocyte activation, elevated serum and BALF levels of various cytokines, and chronic activation of memory B lymphocyte responsible for the occurrence of autoantibodies (5). Various fibrotic diseases of lungs including SSc-ILD are frequently associated with excessive accumulation of T lymphocytes, which is 1 of the major inflammatory cell type (54,55). T lymphocytes are present diffusely throughout the alveolar septae and interstitium, with focal perivascular aggregates and within alveolar spaces. The location of T cells accumulation is the greatest in the areas of lung tissue with interstitial fibrosis and honeycomb change, and there are few T cells present in areas of relatively normal lung tissue (56). T lymphocytes constitute a relatively small population in a normal lung; however, they expand and undergo phenotypic changes with pulmonary inflammation and fibrosis (55). Evidence from human and animal models suggests that, depending on the phenotype, T lymphocytes may act as either profibrotic or antifibrotic, or have no function in the fibrotic process (55). Infiltration by inflammatory T cells is thought to precede the development of lung fibrosis (6). There are 2 functional subsets of T cells with distinct cytokine-secretion profiles. Type 1 (Th1/Tc1) T cells produce predominantly interferon- γ and interleukin-2 (IL-2) that are involved in delayed-type hypersensitivity and defense against intracellular pathogens. Type 2 (Th2/Tc2) T cells secrete IL-4, IL-5, IL-10, and IL-13 and participate in humoral immune responses (6). An abnormal profibrotic Th2/Tc2-polarized T cell response has been postulated to mediate tissue damage and fibrosis in SSc-ILD (6). Thrombin has been reported to modulate the Th1/Th2 response by promoting a Th2 profile in human peripheral blood mononuclear cells (57), suggesting a role for thrombin in the formation of a pathogenic Th2 profile in SSc-ILD.

Natural killer (NK) cells are innate immune effectors that produce various immunoregulatory cytokines. Human NK cells appear to exist as 2 functionally distinct subsets, NK1 and NK2, analogous to Th1 and Th2 T-cell subsets (58). Increased levels of several cytokines have been shown in nonstimulated NK cells from SSc patients when compared with controls, suggesting that NK cell dysfunction may contribute to immunological abnormalities in scleroderma (59). NK and T cells can exert cyto-toxicity via

exocytosis of their cytotoxic granules (60). The major components of cytotoxic granules are the pore-forming protein perforin and the lymphocyte-specific granule serine protease granzyme B (61). Perforin and granzyme induce target cell death cooperatively. Granzyme triggers caspase activation directly by caspase processing and indirectly by upregulating proapoptotic genes, while perforin supports the appropriate delivery of granzyme by inducing transmembrane pores (62). A role for each in the pathogenesis of lung injury and fibrosis has been postulated (62). Perforin and granzyme B expression was found to be upregulated in infiltrating lymphocytes in lung tissue from patients with SSc-ILD or IPF when compared with normal controls, as well as in infiltrating mononuclear cells in lungs of bleomycin-treated mice (63,64). Furthermore, the degree of inflammation and fibrosis was decreased following bleomycin instillation in perforin knockout mice (64). Similarly, mice deficient in granzyme A and B show significantly delayed clinical response to fatal pneumovirus infection by reducing alveolar injury (64). These results implicate cell-mediated immunity in tissue injury, resulting in thrombin generation and followed by secretion of pro-inflammatory and pro-fibrotic cytokines and growth factors.

Autoantibodies in Scleroderma

Scleroderma is associated with several autoantibodies, some of which are important diagnostic and prognostic markers. SSc patients have autoantibodies against a variety of intracellular antigens, thus providing evidence that SSc is indeed an autoimmune disease. In particular, anti-topoisomerase-I (anti-Scl-70) antibodies are often found in patients with severe SSc-ILD and have been associated with a poor prognosis and higher mortality rate (9,65). Antiendothelial cell antibodies and antiphospholipid antibodies have also been found to be correlated with SSc-ILD (66,67). In contrast, patients with isolated SSc-PAH are less likely to have Scl-70 autoantibodies and more prone to have anticentromere antibodies. Patients with antibodies to RNA polymerase III are prone to develop scleroderma renal crisis but less apt to develop significant pulmonary fibrosis. Circulating autoantibodies against several cellular and intracellular antigens are characteristic features of certain autoimmune diseases including scleroderma. They are nearly universal in SSc patients, present in more than 90% of all patients (65,68–70). In recent years several studies have provided evidence that autoantibodies in SSc not only provide clinically useful prognostic information but also may play an important role in pathogenesis of disease (65).

Antiendothelial Cell Antibodies

Total and activated circulating endothelial cell counts are significantly higher in SSc patients than in controls, and this increase was positively correlated with the severity of the disease (71,72). Del Papa and coworkers also reported that bone marrow samples contained reduced numbers of endothelial precursor cells and stromal cells, both of which were functionally impaired (71). Other studies suggest that bone marrow mesenchymal cells are defective in scleroderma and are not able to repair or replace endothelial cells after injury (72). Antiendothelial cell antibodies (AECA) are heterogeneous immunoglobulins directed against a variety of endothelial cell antigens. AECA are detectable in 25 to 85% of SSc patients and have been correlated with pulmonary fibrosis (66). AECA are linked to vascular injury and may reflect endothelial damage (66,73–75). Patient sera containing AECA induce cytotoxicity and reduce the migration of endothelial cells in vitro (76). In addition to in vitro studies showing a direct effect of AECA on endothelial cell function, several other lines of investigation have established a link between AECA and severity of disease in various autoimmune disorders, including SSc-ILD (66,76,77). A potential pathogenic role of AECA has been suggested by their ability to induce endothelial cell apoptosis, perhaps 1 of the earliest pathologic events to be observed in patients and in animal models of SSc (78). Recently, Tobon and coworkers (77) suggested that AECA trigger the translocation of anionic phospholipids, most notably phosphatidylserine, from the inner to the outer leaflet of

the plasma membrane, and thereafter activation of caspase 3 and cleavage of poly (ADP-ribose) polymerase. Apoptotic cell death generates oxidative modified moieties, which can induce autoimmune and local inflammatory responses (77). Earlier studies demonstrated that sera containing AECA can induce antibody-dependent cytotoxicity in endothelial cells (75). AECA IgG increased the expression by endothelial cells of the adhesion protein E-selectin, as well as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, each of which is elevated in SSc patients (73).

AECA may exhibit procoagulant properties by inducing tissue factor production in endothelial cells. The binding of AECA from patients with SSc to endothelial cells results in apoptosis and has pro-inflammatory and procoagulant effects (74,79). Patients with ILD, including patients with SSc, in addition to having enhanced thrombin activity also exhibit elevated levels of other procoagulant factors, such as Factor VIII, Xa, and C-reactive protein, additionally supporting the presence of endothelial cell injury in the lungs (20,74,79). Indeed, an association between endothelial cell injury and pulmonary fibrosis has been suggested (14,79), and a role for thrombin in the endothelial cell/fibroblast interaction has been implied (3). It has been also observed that media conditioned by apoptotic endothelial cells derived from SSc patients inhibit apoptosis of fibroblasts, whereas media conditioned by endothelial cells in which apoptosis was blocked did not stimulate fibroblasts (80).

Antifibroblast Antibodies

Activated fibroblasts or myofibroblasts that are resistant to apoptosis are major players in fibrotic diseases including SSc-ILD (11,27). Previously, we showed that thrombin induces the myofibroblast phenotype in normal lung fibroblasts and that these cells are resistant to apoptosis (27). Similarly, myofibroblasts isolated from SSc-ILD patients are resistant to several apoptotic stimuli including FasL, whereas normal lung fibroblasts are sensitive to these stimuli (27). We have also shown that thrombin stimulates unrestricted proliferation of fibroblasts and protects these cells from apoptosis via activation PAR-1 receptor, which we found to be abundantly expressed in SSc lung within fibroproliferative foci (27). Downstream, PAR1 activates PKC ϵ , which promotes activation of p21/Cip/WAF1 pathway leading to the resistance of apoptosis by myofibroblasts (27). Autoantibodies to fibroblasts have been shown to induce an activated fibroblast phenotype responsible for ECM deposition in lungs. Such antibodies are found in SSc patients, mainly those with the diffuse form of disease who are most likely to develop SSc-ILD (81,82).

Antiphospholipid Antibodies

Recent evidence suggests that antiphospholipid antibodies, which alter the regulation of hemostasis and induce the activation of complement (83), are detected in several autoimmune disorders (84). They are recognized as a group of antibodies directed not only toward phospholipids such as cardiolipin, β 2 glycoprotein I (β 2GPI), but also toward lupus anticoagulant antibodies, and antiphosphatidylserine-prothrombin complex antibodies that are detectable in SSc patients (67,69). It has been suggested that the level of anti-PS-PT antibodies may reflect severity of endothelial injury and prognosis in the development and progression of pulmonary fibrosis (69). The clinical manifestations of antiphospholipid syndrome (APS) are also observed in SSc and a series of systemic sclerosis cases associated with APS have been reported (67,85). Lopez-Pedraza and coworkers recently demonstrated that monocytes from APS patients have increased expression of PAR receptors (85). Moreover, monocytes produce PAR-1 and PAR-2 in vitro when treated with aCL, suggesting that these receptors may play a role as regulatory factors in aCL-mediated monocyte activation and TF expression, thereby contributing to inflammation (85). SSc patients with aCL/ β 2GPI antibodies are more likely to develop microvascular dysfunction

(pulmonary arterial hypertension, digital infarcts) and macrovascular impairment (69). Next, there is a strong correlation between the presence of aCL and pulmonary hypertension and the possibility that aCL/ $\alpha\beta 2$ GPI and lupus anticoagulant antibodies may increase local production of endothelin and cause pulmonary hypertension (84). Also, patients with anti-PS-PT antibodies may represent a unique group of SSc patients with peripheral ischemia and lung disease, but these antibodies have not been found to be associated with severity of skin disease (86).

Autoantibodies to Matrix Metalloproteinases (MMPs)

In general, tissue fibrosis reflects an imbalance between collagen production and degradation by MMPs. A marked decrease in collagenase activity and production by SSc fibroblasts has been observed (87), and such imbalance may play a potential role in the pathogenesis of SSc. MMPs are powerful degradative enzymes that target ECM molecules (interstitial collagens, lamins, fibronectin, and heparin sulfate proteoglycans), cell-cell adhesion molecules, cell surface receptors, growth factors, cytokines, chemoattractant proteins, as well as other proteinases (88). Thrombin is known to induce MMP, particularly MMP-1, in lung fibroblasts (89). Anti-MMP-1 autoantibodies are present in SSc and are capable of inhibiting enzymatic activity of MMP-1 (90). There is no evidence that these autoantibodies penetrate into viable cells, thereby directly inducing tissue damage; however, they may be involved in the accumulation of ECM proteins in SSc by reducing ECM degradation (91). It has been reported that 50 to 70% of SSc patients have elevated levels of anti-MMP-3 autoantibodies, and there is a correlation with the extent of fibrosis in skin, lung, and renal blood vessels, which reflects the severity of this disease (90). Along with anti-MMP-1 antibodies, anti-MMP-3 antibodies may contribute to fibrosis by inhibiting MMP activity and reducing the turnover of ECM. CTGF, which is considered to be a major profibrotic factor in pulmonary fibrosis and in SSc, is induced in lung fibroblasts by thrombin and has been shown to be a substrate for both MMP-1 and MMP-3 (24,91). Autoantibodies directed against MMPs might inhibit the cleavage of CTGF, thereby leading to overexpression of CTGF and fibrosis (70,90).

Autoantibodies to PDGF Receptors

The PDGF-AA and PDGF-BB isoforms play key roles in the pathogenesis of SSc and other fibrotic disorders (92–94). Increased expression of PDGF has been observed in BALF from patients with SSc-ILD (93,95). Elevated expression of PDGF and PDGF α and β receptors was found in endothelial cells and macrophages in skin fibrosis (96) and in lung fibroblasts from SSc patients (17). Thrombin has been shown to increase production of both PDGF and PDGF receptors (PDGFRs) in SSc-ILD (17). Recently, Olson and Soriano (97) showed that increased activation of PDGFR α is sufficient to disrupt connective tissue development and drive systemic fibrosis in mouse tissues, and involvement of PDGFRs autoantibodies in SSc has been postulated (98).

These autoantibodies have been shown to trigger an intracellular loop involving Ha-Ras-extracellular-signal regulated kinases and reactive oxygen species that leads to increased collagen type I and α -SMA gene expression in lung fibroblasts (70,99). This strongly suggests a role for anti-PDGFR antibodies in the pathogenesis of SSc. Antibodies directed to the PDGFR α were reported initially to be specific for SSc, since all patients with SSc who were studied had autoantibodies to PDGFR, whereas patients with lupus, rheumatoid arthritis, primary Raynaud's disease, or ILD not associated with SSc did not (99). However, recent studies have shown no differences in PDGFR autoantibodies between SSc patients and healthy controls (46). These conflicting results might be due to selective conformation of PDGFR. In the absence of ligands PDGFRs are either monomeric or dimeric, but are inactive (46,96). SSc autoantibodies recognize a different epitope on the PDGFR- α molecule

that triggers its stimulatory effect when analyzed by functional assays. Alternatively, naturally occurring autoantibodies may become pathogenic after affinity maturation and class switching in genetically susceptible subjects (46). PDGFR autoantibodies found in scleroderma patients can bind to $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$ PDGF receptors. The epitope bound by the stimulatory autoantibodies is a conformational motif recognized only in its native configuration and awaits molecular identification. A recent report by Baroni and coworkers (98) suggested that fibroblast activation in scleroderma may be caused by stimulatory autoantibodies to the PDGFR- α and - β subunits, which are members of the type III receptor tyrosine kinase family. These autoantibodies have also been found in chronic graft-versus-host disease, which is characterized by fibrotic lesions similar to those observed in scleroderma (45). In contrast to most serum biomarkers of autoimmune disorders, these autoantibodies were described as being fully specific, since they were detected in all patients with scleroderma or extensive chronic graft-versus-host disease, but in none of the study patients with other autoimmune disorders and none of the healthy controls. PDGFR antibodies are thought to induce PDGFR α chain and/or β chain dimerization, mimicking the effect of the natural dimeric ligands (98). Selective inhibition of PDGF signaling attenuates pulmonary fibrosis induced by bleomycin (45,99), and therapies designed to block PDGF are currently under investigation for the treatment of pulmonary fibrosis (100–102).

DISCUSSION

The pathophysiology of scleroderma is diverse and includes abnormal immunologic processes such as cytokine and chemokine dysregulation, abnormal T cell signaling, B cell dysfunction, autoantibody production, persistent wound healing, and abnormalities in the coagulation and fibrinolytic system. Evidence exists that the immune and coagulation systems are simultaneously activated in some systemic auto-immune disorders, both inflammatory and fibrotic. Thrombin, acting via PARs, promotes inflammation via immune cells and induces several profibrotic factors and participates in dysregulation of matrix homeostasis (103).

The main function of the coagulation cascade is the formation of fibrin clots to plug injured vessels and prevent further blood loss. Thrombin, as well as upstream coagulation factors, may play an important role in auto-immune processes that regulate tissue repair. For example, formation of antiphosphatidylserine-prothrombin complex autoantibodies against the antiphosphatidyl-serine-prothrombin complex contributes to endothelial injury in patients with autoimmune diseases (Fig. 1). Thrombin, generated at the site of tissue injury, acting via PAR-1, promotes the formation of a pathogenic Th2 subset of T lymphocytes involved in lung tissue injury. Several autoantibodies may be involved in the pathogenesis of SSc either by amplifying the immune response or by targeting those cell types that are relevant in the patho-physiology of the disease (65,104,105). Thrombin also activates endothelial cells and fibroblasts that may contribute to the generation of pathogenic antibodies against these cells. Additionally, thrombin stimulates production of PDGF, PDGF receptors α and β , and several MMPs. Autoantibodies directed against such antigens are pathogenic, leading to ECM deposition and vascular abnormalities characteristic of SSc-ILD (45,71,106). Thrombin's role in affecting intracellular targets of these autoantibodies provides the link between coagulation and autoimmunity. Additionally, thrombin indirectly contributes to autoimmune responses in the lung by stimulating major profibrotic factors such as TGF β 1 and CTGF.

The findings presented suggest a close association between the presence of specific autoantibodies, procoagulant activity, and clinical features in pulmonary fibrosis, particularly in SSc-ILD. The review provides many evidences suggesting the integration of these systems in the development and progression of pulmonary fibrosis. Thrombin, through

PAR-1 stimulation, mediates a variety of effects independent of hemostasis and, thereby, can amplify inflammation initiated by several diverse stimuli in various diseases including lung fibrosis. During the development of pulmonary fibrosis, thrombin promotes differentiation of myofibroblasts which, in turn, interact with other cell types including immune cells. Thrombin has specific effects on cells involved in adaptive immunity affecting production of pathogenic auto-antibodies in SSc, which also provides a link between coagulation and autoimmunity.

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References

1. Steen VD. The many faces of scleroderma. *Rheum Dis Clin North Am*. 2008; 34:1–15. [PubMed: 18329529]
2. Fischer A, Meehan RT, Feghali-Bostwick CA, West SG, Brown KK. Unique characteristics of systemic sclerosis sine scleroderma-associated interstitial lung disease. *Chest*. 2006; 130:976–81. [PubMed: 17035427]
3. Ludwicka-Bradley A, Bogatkevich G, Silver RM. Thrombin-mediated cellular events in pulmonary fibrosis associated with systemic sclerosis (scleroderma). *Clin Exp Rheumatol*. 2004; 22(Suppl 33):S38–46. [PubMed: 15344597]
4. Okano Y. Antinuclear antibody in systemic sclerosis (sclero-derma). *Rheum Dis Clin North Am*. 1996; 22(4):709–35. [PubMed: 8923592]
5. Sato S, Fujimoto M, Hasegawa M, Takehara K, Tedder TF. Altered B lymphocyte function induces systemic autoimmunity in systemic sclerosis. *Mol Immunol*. 2004; 41:1123–33. [PubMed: 15482848]
6. Boin F, De Fanis U, Bartlett SJ, Wigley FM, Rosen A, Casolaro V. T cell polarization identifies distinct clinical phenotypes in scleroderma lung disease. *Arthritis Rheum*. 2008; 58:1165–74. [PubMed: 18383361]
7. Maher TM, Wells AU, Laurent GJ. Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? *Eur Respir J*. 2007; 30(5):835–9. [PubMed: 17978154]
8. Kahaleh MB, LeRoy EC. Autoimmunity and vascular involvement in systemic sclerosis (SSc). *Autoimmunity*. 1999; 31(3):195–214. [PubMed: 10739336]
9. Ostojic P, Cerinic MM, Silver R, Highland K, Damjanov N. Interstitial lung disease in systemic sclerosis. *Lung*. 2007; 185(4):211–20. [PubMed: 17717851]
10. Scotton CJ, Chambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. *Chest*. 2007; 132:1311–21. [PubMed: 17934117]
11. Bogatkevich GS, Tourkina E, Silver RM, Ludwicka-Bradley A. Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via the proteolytically activated receptor-1 and a protein kinase C-dependent pathway. *J Biol Chem*. 2001; 276:45184–92. [PubMed: 11579091]
12. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol*. 2007; 170(6):1807–16. [PubMed: 17525249]
13. Zisman DA, Kawut SM. Idiopathic pulmonary fibrosis: a shot through the heart? *Am J Respir Crit Care Med*. 2008; 178(12):1192–3. [PubMed: 19050202]
14. Beon M, Harley RA, Wessels A, Silver RM, Ludwicka-Bradley A. Myofibroblast induction and microvascular alteration in scleroderma lung fibrosis. *Clin Exp Rheumatol*. 2004; 22(6):733–42. [PubMed: 15638048]
15. Geiser T. Mechanisms of alveolar epithelial repair in acute lung injury—a translational approach. *Swiss Med Wkly*. 2003; 133(43–44):586–90. [PubMed: 14745653]
16. Van Leer C, Stutz M, Haerberli A, Geiser T. Urokinase plasminogen activator released by alveolar epithelial cells modulates alveolar epithelial repair in vitro. *Thromb Haemost*. 2005; 94(6):1257–64. [PubMed: 16411403]

17. Ohba T, McDonald JK, Silver RM, Strange C, LeRoy EC, Ludwicka A. Scleroderma bronchoalveolar lavage fluid contains thrombin, a mediator of human lung fibroblast proliferation via induction of platelet-derived growth factor alpha-receptor. *Am J Respir Cell Mol Biol.* 1994; 10:405–12. [PubMed: 7510986]
18. Hernández-Rodríguez NA, Cambrey AD, Harrison NK, Chambers RC, Gray AJ, Southcott AM, et al. Role of thrombin in pulmonary fibrosis. *Lancet.* 1995; 346:1071–3. [PubMed: 7564789]
19. Howell DC, Laurent GJ, Chambers RC. Role of thrombin and its major cellular receptor, protease-activated receptor-1, in pulmonary fibrosis. *Biochem Soc Trans.* 2002; 30:211–6. [PubMed: 12023853]
20. Chambers RC. Procoagulant signalling mechanisms in lung inflammation and fibrosis: novel opportunities for pharmacological intervention? *Br J Pharmacol.* 2008; 153(Suppl 1):S367–78. [PubMed: 18223674]
21. Krupiczkoj MA, Scotton CJ, Chambers RC. Coagulation signalling following tissue injury: focus on the role of factor Xa. *Int J Biochem Cell Biol.* 2008; 40:1228–37. [PubMed: 18420447]
22. Bachhuber BG, Sarembok IJ, Gimple LW, Owens GK. alpha-Thrombin induces transforming growth factor-beta1 mRNA and protein in cultured vascular smooth muscle cells via a proteolytically activated receptor. *J Vasc Res.* 1997; 34:41–8. [PubMed: 9075824]
23. Chambers RC, Leoni P, Blanc-Brude OP, Wembridge DE, Laurent GJ. Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1. *J Biol Chem.* 2000; 275:35584–91. [PubMed: 10952976]
24. Bogatkevich, GS.; Ludwicka-Bradley, A.; Nietert, PJ.; Silver, RM. Scleroderma lung fibroblasts: contractility and connective tissue growth factor. In: Gabbiani, G.; Chaponnier, C.; Desmouliere, A., editors. *Myofibroblasts*. Georgetown (TX): Landes Bioscience; 2006. p. 25-31.
25. Mercer PF, Deng X, Chambers RC. Signaling pathways involved in proteinase-activated receptor1-induced proinflammatory and profibrotic mediator release following lung injury. *Ann NY Acad Sci.* 2007; 1096:86–8. [PubMed: 17405919]
26. Ludwicka-Bradley A, Tourkina E, Suzuki S, Tyson E, Bonner M, Fenton JW 2nd, et al. Thrombin upregulates interleukin-8 in lung fibroblasts via cleavage of proteolytically activated receptor-I and protein kinase C- γ activation. *Am J Respir Cell Mol Biol.* 2000; 22:235–43. [PubMed: 10657945]
27. Bogatkevich GS, Gustilo E, Oates JC, Feghali-Bostwick C, Harley RA, Silver RM, et al. Distinct PKC isoforms mediate cell survival and DNA synthesis in thrombin-induced myofibroblasts. *Am J Physiol Lung Cell Mol Physiol.* 2005; 288:L190–201. [PubMed: 15447940]
28. Blackburn JS, Brinckerhoff CE. Matrix metalloproteinase-1 and thrombin differentially activate gene expression in endothelial cells via PAR-1 and promote angiogenesis. *Am J Pathol.* 2008; 173(6):1736–46. [PubMed: 18988801]
29. Wygrecka M, Jablonska E, Guenther A, Preissner KT, Markart P. Current view on alveolar coagulation and fibrinolysis in acute inflammatory and chronic interstitial lung diseases. *Thromb Haemost.* 2008; 99(3):494–501. [PubMed: 18327397]
30. Ruppert C, Markart P, Wygrecka M, Preissner KT, Günther A. Role of coagulation and fibrinolysis in lung and renal fibrosis. *Hamostaseologie.* 2008; 28(1–2):30–2. 34–6. [PubMed: 18278159]
31. Smith SA. The cell-based model of coagulation. *J Vet Emerg Crit Care (San Antonio).* 2009; 19(1):3–10. [PubMed: 19691581]
32. Licari LG, Kovacic JP. Thrombin physiology and pathophysiology. *J Vet Emerg Crit Care (San Antonio).* 2009; 19(1):11–22. [PubMed: 19691582]
33. Ploplis VA, Wilberding J, McLennan L, Liang Z, Cornelissen I, DeFord ME, et al. A total fibrinogen deficiency is compatible with the development of pulmonary fibrosis in mice. *Am J Pathol.* 2000; 157:703–8. [PubMed: 10980108]
34. Allen GB, Cloutier ME, Larrabee YC, Tetenev K, Smiley ST, Bates JH. Neither fibrin nor plasminogen activator inhibitor-1 deficiency protects lung function in a mouse model of acute lung injury. *Am J Physiol Lung Cell Mol Physiol.* 2009; 296:L277–85. [PubMed: 19060228]
35. Imokawa S, Sato A, Hayakawa H, Kotani M, Urano T, Takada A. Tissue factor expression and fibrin deposition in the lungs of patients with idiopathic pulmonary fibrosis and systemic sclerosis. *Am J Respir Crit Care Med.* 1997; 156(2 Pt 1):631–6. [PubMed: 9279250]

36. Wagers SS, Norton RJ, Rinaldi LM, Bates JH, Sobel BE, Irvin CG. Extravascular fibrin, plasminogen activator, plasminogen activator inhibitors, and airway hyperresponsiveness. *J Clin Invest.* 2004; 114(1):104–11. [PubMed: 15232617]
37. Matthay MA, Clements JA. Coagulation-dependent mechanisms and asthma. *J Clin Invest.* 2004; 114(1):20–3. [PubMed: 15232607]
38. Sode BF, Dahl M, Nielsen SF, Nordestgaard BG. Venous thromboembolism and risk of idiopathic interstitial pneumonia: a nationwide study. *Am J Respir Crit Care Med.* 2010; 181(10):1085–92. [PubMed: 20167844]
39. Brims FJ, Chauhan AJ, Higgins B, Shute JK. Up-regulation of the extrinsic coagulation pathway in acute asthma—a case study. *J Asthma.* 2010; 47(6):695–8. [PubMed: 20615166]
40. Howell DC, Johns RH, Lasky JA, Shan B, Scotton CJ, Laurent GJ, et al. Absence of proteinase-activated receptor-1 signaling affords protection from bleomycin-induced lung inflammation and fibrosis. *Am J Pathol.* 2005; 166:1353–65. [PubMed: 15855637]
41. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature.* 2000; 407:258–64. [PubMed: 11001069]
42. Reed CE, Kita H. The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol.* 2004; 114:997–1008. [PubMed: 15536399]
43. Esmon CT. The interactions between inflammation and coagulation. *Br J Haematol.* 2005; 131:417–30. [PubMed: 16281932]
44. Shrivastava S, McVey JH, Dorling A. The interface between coagulation and immunity. *Am J Transplant.* 2007; 7:499–506. [PubMed: 17229071]
45. Svegliati S, Olivieri A, Campelli N, Luchetti M, Poloni A, Trap-polini S, et al. Stimulatory autoantibodies to PDGF receptor in patients with extensive chronic graft-versus-host disease. *Blood.* 2007; 110:237–41. [PubMed: 17363728]
46. Balada E, Simeón-Aznar CP, Ordi-Ros J, Rosa-Leyva M, Selva-O'Callaghan A, Pardos-Gea J, et al. Anti-PDGFR-alpha antibodies measured by non-bioactivity assays are not specific for systemic sclerosis. *Ann Rheum Dis.* 2008; 67:1027–9. [PubMed: 18272670]
47. Yoshida M, Sakuma-Mochizuki J, Abe K, Arai T, Mori M, Goya S, et al. In vivo gene transfer of an extracellular domain of platelet-derived growth factor beta receptor by the HVJ-liposome method ameliorates bleomycin-induced pulmonary fibrosis. *Biochem Biophys Res Commun.* 1999; 265:503–8. [PubMed: 10558898]
48. Ando S, Otani H, Yagi Y, Kawai K, Araki H, Fukuhara S, et al. Proteinase-activated receptor 4 stimulation-induced epithelial-mesenchymal transition in alveolar epithelial cells. *Respir Res.* 2007; 8:31. [PubMed: 17433115]
49. Archiniegas E, Neves CY, Candelle D, Cardier JE. Thrombin and its protease-activated receptor-1 (PAR1) participate in the endothelial-mesenchymal transdifferentiation process. *DNA Cell Biol.* 2004; 23(12):815–25. [PubMed: 15684708]
50. Ask K, Martin GE, Kolb M, Gauldie J. Targeting genes for treatment in idiopathic pulmonary fibrosis: challenges and opportunities, promises and pitfalls. *Proc Am Thorac Soc.* 2006; 3:389–93. [PubMed: 16738206]
51. Kubo H. Anticoagulant therapy for idiopathic pulmonary fibrosis. *Rev Port Pneumol.* 2005; 11:591–2. [PubMed: 16521238]
52. Howell DC, Goldsack NR, Marshall RP, McAnulty RJ, Starke R, Purdy G, et al. Direct thrombin inhibition reduces lung collagen, accumulation, and connective tissue growth factor mRNA levels in bleomycin-induced pulmonary fibrosis. *Am J Pathol.* 2001; 159:1383–95. [PubMed: 11583966]
53. Bogatkevich GS, Ludwicka-Bradley A, Silver RM. Dabigatran, a direct thrombin inhibitor, demonstrates antifibrotic effects on lung fibroblasts. *Arthritis Rheum.* 2009; 60(11):3455–64. [PubMed: 19877031]
54. Luzina IG, Todd NW, Nacu N, Lockett V, Choi J, Hummers LK, et al. Regulation of pulmonary inflammation and fibrosis through expression of integrins alphaVbeta3 and alphaVbeta5 on pulmonary T lymphocytes. *Arthritis Rheum.* 2009; 60:1530–9. [PubMed: 19404954]
55. Luzina IG, Todd NW, Iacono AT, Atamas SP. Roles of T lymphocytes in pulmonary fibrosis. *J Leukoc Biol.* 2008; 83(2):237–44. [PubMed: 17962367]

56. Parra ER, Kairalla RA, Ribeiro de Carvalho CR, Eher E, Cape-lozzi VL. Inflammatory cell phenotyping of the pulmonary interstitium in idiopathic interstitial pneumonia. *Respiration*. 2007; 74(2):159–69. [PubMed: 17108669]
57. Naldini A, Morena E, Filippi I, Pucci A, Bucci M, Cirino G, et al. Thrombin inhibits IFN-gamma production in human peripheral blood mononuclear cells by promoting a Th2 profile. *J Interferon Cytokine Res*. 2006; 26(11):793–9. [PubMed: 17115897]
58. Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Differentiation of human NK cells into NK1 and NK2 subsets. *J Immunol*. 1998; 161(11):5821–4. [PubMed: 9834059]
59. 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(4):731–7. [PubMed: 16185273]
60. Topham NJ, Hewitt EW. Natural killer cell cytotoxicity: how do they pull the trigger? *Immunology*. 2009; 128(1):7–15. [PubMed: 19689731]
61. Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. *Nat Rev Immunol*. 2002; 2(10):735–47. [PubMed: 12360212]
62. Schachna L, Wigley FM, Morris S, Gelber AC, Rosen A, Cas-ciola-Rosen L. Recognition of Granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. *Arthritis Rheum*. 2002; 46:1873–84. [PubMed: 12124872]
63. Miyazaki H, Kuwano K, Yoshida K, Maeyama T, Yoshimi M, Fujita M, et al. The perforin mediated apoptotic pathway in lung injury and fibrosis. *J Clin Pathol*. 2004; 57(12):1292–8. [PubMed: 15563670]
64. Bem RA, van Woensel JB, Lutter R, Domachowske JB, Medema JP, Rosenberg HF, et al. Granzyme A- and B-cluster deficiency delays acute lung injury in pneumovirus-infected mice. *J Immunol*. 2010; 184(2):931–8. [PubMed: 20018616]
65. Boin F, Rosen A. Autoimmunity in systemic sclerosis: current concepts. *Curr Rheumatol Rep*. 2007; 9:165–72. [PubMed: 17502048]
66. Pignone A, Scaletti C, Matucci-Cerinic M, Vázquez-Abad D, Meroni PL, Del Papa N, et al. Anti-endothelial cell antibodies in systemic sclerosis: significant association with vascular involvement and alveolo-capillary impairment. *Clin Exp Rheumatol*. 1998; 16:527–32. [PubMed: 9779298]
67. Sanna G, Bertolaccini ML, Mameli A, Hughes GR, Khamashta MA, Mathieu A. Antiphospholipid antibodies in patients with scleroderma: prevalence and clinical significance. *Ann Rheum Dis*. 2005; 64:1795–6. [PubMed: 16284349]
68. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G, 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. [PubMed: 19035499]
69. Marie I, Jouen F, Hellot MF, Levesque H. Anticardiolipin and anti-beta2 glycoprotein I antibodies and lupus-like anticoagulant: prevalence and significance in systemic sclerosis. *Br J Dermatol*. 2008; 158:141–4. [PubMed: 18028496]
70. Grassegger A, Pohla-Gubo G, Frauscher M, Hintner H. Auto-antibodies in systemic sclerosis (scleroderma): clues for clinical evaluation, prognosis and pathogenesis. *Wien Med Wochenschr*. 2008; 158:19–28. [PubMed: 18286246]
71. Del Papa N, Quirici N, Soligo D, Scavullo C, Cortiana M, Borsotti C, et al. Bone marrow endothelial progenitors are defective in systemic sclerosis. *Arthritis Rheum*. 2006; 54(8):2605–15. [PubMed: 16868984]
72. Cipriani P, Guiducci S, Miniati I, Cinelli M, Urbani S, Marrelli A, et al. Impairment of endothelial cell differentiation from bone marrow-derived mesenchymal stem cells: new insight into the pathogenesis of systemic sclerosis. *Arthritis Rheum*. 2007; 56(6):1994–2004. [PubMed: 17530639]
73. Ihn H, Sato S, Fujimoto M, Igarashi A, Yazawa N, Kubo M, et al. Characterization of autoantibodies to endothelial cells in systemic sclerosis (SSc): association with pulmonary fibrosis. *Clin Exp Immunol*. 2000; 119:203–9. [PubMed: 10606984]

74. Magro CM, Ross P, Marsh CB, Allen JN, Liff D, Knight DA, et al. The role of anti-endothelial cell antibody-mediated microvascular injury in the evolution of pulmonary fibrosis in the setting of collagen vascular disease. *Am J Clin Pathol.* 2007; 127:237–47. [PubMed: 17210529]
75. Guilpain P, Mouthon L. Antiendothelial cells autoantibodies in vasculitis-associated systemic diseases. *Clin Rev Allergy Immunol.* 2008; 35:59–65. [PubMed: 18228162]
76. Chan TM, Cheng IK. A prospective study on anti-endothelial cell antibodies in patients with systemic lupus erythematosus. *Clin Immunol Immunopathol.* 1996; 78:41–6. [PubMed: 8599882]
77. Tobón GJ, Alard JE, Youinou P, Jamin C. Are autoantibodies triggering endothelial cell apoptosis really pathogenic? *Autoimmun Rev.* 2009; 8(7):605–10. [PubMed: 19393202]
78. Farkas L, Farkas D, Ask K, Möller A, Gaudie J, Margetts P, et al. VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats. *J Clin Invest.* 2009; 119:1298–311. [PubMed: 19381013]
79. Magro CM, Waldman WJ, Knight DA, Allen JN, Nadasdy T, Frambach GE, et al. Idiopathic pulmonary fibrosis related to endothelial injury and antiendothelial cell antibodies. *Hum Immunol.* 2006; 67(4–5):284–97. [PubMed: 16720208]
80. Laplante P, Raymond MA, Gagnon G, Vigneault N, Sasseville AM, Langelier Y, et al. Novel fibrogenic pathways are activated in response to endothelial apoptosis: implications in the pathophysiology of systemic sclerosis. *J Immunol.* 2005; 174:5740–9. [PubMed: 15843576]
81. Hénault J, Robitaille G, Sénécal JL, Raymond Y. DNA topo-isomerase I binding to fibroblasts induces monocyte adhesion and activation in the presence of anti-topoisomerase I autoantibodies from systemic sclerosis patients. *Arthritis Rheum.* 2006; 54:963–73. [PubMed: 16508979]
82. Fineschi S, Goffin L, Rezzonico R, Cozzi F, Dayer JM, Meroni PL, et al. Antifibroblast antibodies in systemic sclerosis induce fibroblasts to produce profibrotic chemokines, with partial exploitation of toll-like receptor 4. *Arthritis Rheum.* 2008; 58:3913–23. [PubMed: 19035500]
83. Ostrowski RA, Robinson JA. Antiphospholipid antibody syndrome and autoimmune diseases. *Hematol Oncol Clin North Am.* 2008; 22:53–65. [PubMed: 18207065]
84. Zandman-Goddard G, Tweezer-Zaks N, Shalev T, Levy Y, Ehrenfeld M, Langevitz P. A novel overlap syndrome: systemic sclerosis associated with antiphospholipid syndrome—a case series. *Ann NY Acad Sci.* 2007; 1108:497–504. [PubMed: 17894015]
85. López-Pedraza C, Aguirre MA, Buendía P, Barbarroja N, Ruiz-Limón P, Collantes-Estevez E, et al. Differential expression of protease-activated receptors in monocytes from patients with primary antiphospholipid syndrome. *Arthritis Rheum.* 2010; 62(3):869–77. [PubMed: 20131237]
86. Hasegawa M, Sato S, Yanaba K, Komura K, Yamazaki M, Takehara K. Autoantibodies against phosphatidylserine-prothrombin complex in patients with systemic sclerosis. *Ann Rheum Dis.* 2004; 63:1514–7. [PubMed: 15479907]
87. Takeda K, Hatamochi A, Ueki H, Nakata M, Oishi Y. Decreased collagenase expression in cultured systemic sclerosis fibroblasts. *J Invest Dermatol.* 1994; 103:359–63. [PubMed: 8077701]
88. Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol.* 2004; 16:558–64. [PubMed: 15363807]
89. Fang Q, Liu X, Al-Mugotir M, Kobayashi T, Abe S, Kohyama T, et al. Thrombin and TNF-alpha/IL-1beta synergistically induce fibroblast-mediated collagen gel degradation. *Am J Respir Cell Mol Biol.* 2006; 35(6):714–21. [PubMed: 16858010]
90. Nishijima C, Hayakawa I, Matsushita T, Komura K, Hasegawa M, Takehara K, et al. Autoantibody against matrix metalloproteinase-3 in patients with systemic sclerosis. *Clin Exp Immunol.* 2004; 138:357–63. [PubMed: 15498049]
91. Hashimoto G, Inoki I, Fujii Y, Aoki T, Ikeda E, Okada Y. Matrix metalloproteinases cleave connective tissue growth factor and reactivate angiogenic activity of vascular endothelial growth factor. *J Biol Chem.* 2002; 277:36288–95. [PubMed: 12114504]
92. Zhang J, Cao R, Zhang Y, Jia T, Cao Y, Wahlberg E. Differential roles of PDGFR-alpha and PDGFR-beta in angiogenesis and vessel stability. *FASEB J.* 2009; 23:153–63. [PubMed: 18827023]
93. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev.* 2004; 15:255–73. [PubMed: 15207816]

94. Gay S, Jones RE Jr, Huang GQ, Gay RE. Immunohistologic demonstration of platelet-derived growth factor (PDGF) and sis-oncogene expression in scleroderma. *J Invest Dermatol.* 1989; 92:301–3. [PubMed: 2918236]
95. Ludwicka A, Ohba T, Trojanowska M, Yamakage A, Strange C, Smith EA, et al. Elevated levels of platelet derived growth factor and transforming growth factor-beta 1 in bronchoalveolar lavage fluid from patients with scleroderma. *J Rheumatol.* 1995; 22:1876–83. [PubMed: 8991985]
96. Dragun D, Distler JH, Riemekasten G, Distler O. Stimulatory autoantibodies to platelet-derived growth factor receptors in systemic sclerosis: What functional autoimmunity could learn from receptor biology. *Arthritis Rheum.* 2009; 60:907–11. [PubMed: 19333918]
97. Olson LE, Soriano P. Increased PDGFRalpha activation disrupts connective tissue development and drives systemic fibrosis. *Dev Cell.* 2009; 16(2):303–13. [PubMed: 19217431]
98. Baroni SS, Santillo M, Bevilacqua F, Luchetti M, Spadoni T, Mancini M, et al. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. *N Engl J Med.* 2006; 354(25):2667–76. [PubMed: 16790699]
99. Tan FK. Autoantibodies against PDGF receptor in scleroderma. *N Engl J Med.* 2006; 354:2709–11. [PubMed: 16790706]
100. Houssiau FA, Demoulin JB. Lack of evidence of stimulatory auto-antibodies to platelet-derived growth factor receptor in patients with systemic sclerosis. *Arthritis Rheum.* 2009; 60:1137–44. [PubMed: 19333949]
101. Abdollahi A, Li M, Ping G, Plathow C, Domhan S, Kiessling F, et al. Inhibition of platelet-derived growth factor signaling attenuates pulmonary fibrosis. *J Exp Med.* 2005; 201:925–35. [PubMed: 15781583]
102. Asano Y. Future treatments in systemic sclerosis. *J Dermatol.* 2010; 37(1):54–70. [PubMed: 20175840]
103. Cugno M, Tedeschi A, Asero R, Meroni PL, Marzano AV. Skin autoimmunity and blood coagulation. *Autoimmunity.* 2010; 43(2):189–94. [PubMed: 19883336]
104. Gabrielli A, Svegliati S, Moroncini G, Avvedimento EV. Pathogenic autoantibodies in systemic sclerosis. *Curr Opin Immunol.* 2007; 19:640–5. [PubMed: 18083509]
105. Cepeda EJ, Reveille JD. Autoantibodies in systemic sclerosis and fibrosing syndromes: clinical indications and relevance. *Curr Opin Rheumatol.* 2004; 16:723–32. [PubMed: 15577611]
106. Arnett FC. Is scleroderma an autoantibody mediated disease? *Curr Opin Rheumatol.* 2006; 18:579–81. [PubMed: 17053501]

Figure 1.

Crossing points between coagulation and autoimmunity in lung tissue injury. After tissue injury factor VIIa binds to the exposed TF followed by the conversion of factor X to active Xa. Factor Xa catalyzed the activation of prothrombin to thrombin, which in turn converts fibrinogen to fibrin follow-on clot formation. The small amount of thrombin produced by factor Xa feeds back to activate factor VIII, resulting in more generation of factor Xa and in activation of factor V. The combination of factors Va and Xa, in association with phospholipids and calcium, catalyzes the production of larger quantities of thrombin, resulting in amplification of the coagulation cascade and stimulation of PAR-1. Thrombin via PAR-1 activates endothelial cells and fibroblasts that lead to production of PDGF and MMP followed by the formation of pathogenic autoantibodies. Antiphosphatidylserine-prothrombin complex (aPS-PT) autoantibodies represent a complex phospholipid bound to prothrombin expressed on the surface of endothelial cells, which together with other pathogenic antibodies participate in lung tissue injury. Thrombin also contributes to tissue injury, inducing inflammatory T cells and shifting Th1/Th2 cells toward pathogenic Th2 subset. Additionally, thrombin induces TGF β 1 and CTGF, indirectly stimulating production of autoantibodies and Th2 profile.

Figure 3.

Mechanism of PAR-1 activation. Proteolytic cleavage of the N-terminus results in the unmasking of a tethered ligand SFLLRN, which in turn interacts with extracellular loop-2 of the receptor and initiates cell signaling via activation of heterotrimeric G-proteins.

Figure 4.

Thrombin differentiates lung fibroblasts to an activated myofibroblast phenotype. Smooth muscle- α actin expression and organization in normal lung fibroblasts in serum-free medium (left panel), after incubation with thrombin (0.5 unit/mL) for 24 hours (middle panel), and in scleroderma lung fibroblasts in serum-free medium (right panel).

Figure 5. Effect of direct thrombin inhibitor, dabigatran, on SSc lung fibroblasts. A, Dabigatran inhibits collagen type I, CTGF, and α -SMA expression in scleroderma lung fibroblasts. B, Dabigatran inhibits α -SMA expression and organization in scleroderma lung fibroblasts.