Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology REVIEW ARTICLE doi: 10.1111/cei.13238

The immunopathogenesis of fibrosis in systemic sclerosis

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Accepted for publication 7 November 2018
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Summary

Systemic sclerosis (SSc) is an idiopathic systemic autoimmune disease. It is characterized by a triad of hallmarks: immune dysfunction, fibrosis and vasculopathy. Immune dysfunction in SSc is characterized by the activation and recruitment of immune cells and the production of autoantibodies and cytokines. How immune abnormalities link the fibrosis and vasculopathy in SSc is poorly understood. A plethora of immune cell types are implicated in the immunopathogenesis of SSc, including T cells, B cells, dendritic cells, mast cells and macrophages. How these different cell types interact to contribute to SSc is complicated, and can involve cell-to-cell interactions and communication via cytokines, including transforming growth factor (TGF)-β, interleukin (IL)-6 and IL-4. We will attempt to review significant and recent research demonstrating the importance of immune cell regulation in the immunopathogenesis of SSc with a particular focus on fibrosis.

Keywords: arthritis (including rheumatoid arthritis), B cells, T cells, Toll-like receptors (TLRs)

Introduction

Systemic sclerosis (SSc) is an idiopathic systemic autoimmune disease. The pathogenesis of SSc is mostly unknown. Immunological abnormality, which includes autoimmunity and infiltration and activation of immune cells, is one of three key features of the disease. The other two hallmarks of SSc are fibrosis and vasculopathy. How autoimmunity is linked to fibrosis and vascular damage remains poorly understood, with no single hypothesis or mechanism to explain the links. It is therefore likely that a combination of mechanisms is involved in the pathogenesis of SSc, and picking apart these mechanisms to establish how the triad of fibrosis, vasculopathy and immune cell activation progress in SSc is a mammoth task. Many studies have linked immune abnormality as a cause of or at least a contributor to fibrosis in SSc, while fibrosis may also contribute to immune cell activation. Fibrosis in SSc occurs mainly in the skin but may progress to visceral organs, including heart and lungs. Fibrosis is the result of activation of fibroblasts and excessive extracellular matrix (ECM) deposition, both of which are hallmarks of SSc. As well as fibrosis, immune abnormalities are also linked to vasculopathy in SSc, with most research implicating vascular damage as an activator of immune cells. However, this review will focus mainly on the role of immunological abnormalities in the development of fibrosis in SSc; we recommend the paper by Asano and Sato for a comprehensive review of vasculopathy in SSc [1].

Multiple different cell types of the immune system have been implicated in SSc, including T cells, B cells, dendritic cells, mast cells and macrophages. It appears that both innate and adaptive immunity is critical in the disease. We will attempt to review the old and emerging literature to examine the potential roles of these individual cell types in the immunopathogenesis of SSc. First, we will summarize major cytokines implicated in the immunopathogenesis of SSc, because these major players reappear in many studies, linking immune cell activation with other phenotypes in SSc, especially fibrosis.

Cytokines in SSc

Many cytokines are elevated in SSc, but interleukin (IL)-4, transforming growth factor (TGF)-β and IL-6 are considered to be the major fibrogenic cytokines, or are at least the most studied. Genetic deletion of both IL-4 and TGF-β prevents skin fibrosis in a mouse model of SSc [2]. Many other *in-vitro* studies support the idea that IL-4 promotes fibrosis through its ability to enhance the production of collagen [3,4] and other ECM proteins [5,6] while antibodies against IL-4 prevent dermal fibrosis in the tight skin (Tsk) mouse model [7] targeted deletion of IL-4 receptor in the Tsk mouse also reduces fibrosis [2]. Skin and lung in SSc have high levels of IL-4 [8] and increased levels of IL-4 in the blood are a common feature in patients with SSc [9–11] suggesting systemic release.

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TGF-β is a well-known potent inducer of fibrosis, with TGF-β-stimulated fibroblasts resembling those from SSc patients [12]. Activation of the TGF-β receptor following the binding of TGF-β results in the phosphorylation and activation of SMAD proteins in the cytoplasm [13]. TGF-β also activates the three mitogen-activated protein kinase (MAPK) signalling branches, c-Jun N-terminal kinase (JNK), p38 and extracellular signal-regulated kinases 1 and 2 (ERK1 and 2) [12] all of which can promote inflammatory signalling. TGF-β-induced collagen production from both healthy and SSc dermal fibroblasts was found to be dependent on p38 [14]. JNK activation has also been implicated in fibrosis [15]. However, in one study ERK activation inhibited skin fibroblast collagens I and III production while, p38 activation up-regulated collagen I [16].

IL-6 is a classic proinflammatory cytokine and is also considered to be an important protein in the immunopathogenesis of SSc. For example, IL-6 levels are increased in SSc patient sera [9] and skin [17]. IL-6 levels also correlate with SSc disease severity [18]. A mouse model with development of autoimmune disease with SSc-like skin thickening and lung fibrosis was found to be mediated by IL-6 signalling [19]. Bleomycin-induced lung inflammation with collagen deposition was significantly attenuated in IL-6-deficient mice [20]. IL-6 signalling through trans-signalling appears to be important, and we found that IL-6 and the soluble form of the IL-6 receptor are necessary for collagen production [21]. We further showed in the same study that this was critical, dependent on the downstream signalling molecule signal transducer and activator of transcription (STAT)-3.

A crucial early step hypothesized to trigger the immune abnormalities and fibrosis in SSc is vasculopathy, including the damage and apoptosis of endothelial cells, resulting in the release of internal damage-associated molecular patterns (DAMPs), which go on to activate and recruit immune cells [22]. IL-6 was found to mediate endothelial activation and apoptosis caused by the serum of patients with SSc [23], suggesting that it may play a major role in the very early stages of SSc. However, IL-6 was found to be up-regulated at the late stage of the disease using immunohistological analysis of skin biopsies from SSc patients [17]. In both IL-6 knock-out (KO) mice and mice exposed to an IL-6 blocking antibody, bleomycin-induced dermal fibrosis was greatly induced by supressing fibroblast activation [24]. The anti-IL-6 receptor antibody tocilizumab has had promising results with softening of the skin in two patients with SSc in one study [25] while a Phase II trial provided SSc patients with improvement in fibrosis of the skin [26] although statistically this was not significant. Thus, IL-6 antibody therapy could be the first biological licensed for SSc.

T cells

T cells have been identified early in SSc progression before any evidence of fibrosis [27]. SSc skin has a greater propensity to recruit/adhere T cells compared to healthy controls because of a greater expression of intercellular adhesion molecule (ICAM-1), which is a ligand for the lymphocyte function-associated antigen 1 (LFA1) receptor found on the surface of lymphocytes such as T cells [28]. T cells from SSc skin biopsies have increased expression of the early T cell activation marker CD69 [29]. TGF-β, which is elevated in SSc, was also found to be important for the recruitment of T cells to the skin in an SSc mouse model [30]. A recent paper demonstrated that abatacept, which is an antibody that interferes with T cell activation, reduced fibrosis in not one, but two animal models of fibrosis [31]. This was associated with reduced T cell activation and reduced levels of IL-6, which may be mediated by blockade of cross-talk between T cells and antigen-presenting cells such as monocytes. Abatacept works by blocking the interaction of CD80/86 with cytotoxic T lymphocyte antigen (CTLA)-4 on T cells, which is required for co-stimulatory activation of T cells alongside major histocompatibility complex (MHC) and antigen presentation from antigen-presenting cells such as dendritic cells, macrophages and B cells [32] Abatacept was initiated in three patients with morphea, and all three showed regression of the modified Rodnan skin score [33].

T cells can be further classified into diverse subsets characterized by their distinct function, activators and the cytokines they subsequently release. Some of these T cell types have been found to play a role in SSc, which has been reviewed in more detail [34]. However, many studies have identified the T helper type 2 (Th2) subtype as playing the largest role in SSc. Th2 cells are a major IL-4-producing cell type, but also secrete IL-5, IL-6, IL-10 and IL-13. Depletion of $CD3^+$ T cells in a bleomycin mouse model reduced both fibrosis and IL-4 secretion [35]. As described above, IL-4 can directly induce fibroblasts to promote fibrosis, but in addition IL-4 can induce TGF-β production in fibroblasts [36] and macrophages [37], which then leads to further signalling to fibroblasts to produce more collagen. Therefore, it is very likely that T cells have a major role to play in the onset of fibrosis in SSc through activation of macrophages and fibroblasts. An unusual population of $CD4^+CD8^+$ double-positive T cells has been described in the skin that has very high levels of IL-4 [38]. Gross *et al.* showed almost 30 years ago that IL-4 is required to induce naive T cells to produce further IL-4 [39]. If IL-4 is required for T cells to produce IL-4, this leads to the question: 'what is the initial source of IL-4 in SSc?'. This question remains unanswered, but potentially implicates other immune cells in the onset or progression of SSc.

IL-13 is produced predominantly by activated Th2 cells [34]. IL-13 was established as a major profibrotic agent in a model of hepatic fibrosis [40]. IL-13 inhibited IL-1β-induced matrix metalloproteinase (MMP)-1 and MMP-3 production and enhanced tissue inhibitor of metalloproteinase (TIMP)-1 and collagen generation in fibroblasts [41]. In the bleomycin model of SSc pulmonary fibrosis IL-13 levels increased with pathogenesis, while neutralization of IL-13 attenuated bleomycin-induced pulmonary fibrosis [42]. The potent fibrosis-inducer, TGF-β, may also contribute to an increase in Th2-originating IL-13 because TGF-β up-regulates GATA binding protein 3 (GATA-3) expression in the T cells of patients with SSc, resulting in an increase in IL-13 synthesis [43]. As well as $CD4^+CD8^+$ double-positive T cells, $CD8^+$ single-positive cells have been described that produce exuberant levels of IL-13 [44]. These cells appear to be memory $CD8^+$ T cells [45]. We have shown that IL-13 is directly profibrotic and is STAT-6-dependent [46]. Thus, IL-13 produced from T cells probably contributes to fibrosis in SSc and a monoclonal IL-13 clinical-grade antibody exists. Very recently, IL-13 was shown to decrease MMP-1 expression in both healthy and SSc fibroblasts and therefore may have an anti-fibrotic role [47]. However, this was only demonstrated in tumour necrosis factor (TNF)-α-induced fibroblasts, thus the effect of IL-13 may be specific to the mechanism with which TNF-α induces MMP-1 expression.

Another cytokine, IL-17, prevalent in the serum of SSc patients in some studies, also implicates T cells [48]. Th17 cells, which are characterized by their production of IL-17A, IL-17F, IL-21 and IL-22, are elevated in SSc skin compared to healthy controls [49,50]. Thus, Th17 cells and the IL-17 they produce may play an important role in SSc. However, the roles of Th17 and IL-17 in SSc are controversial, and have been recently reviewed in detail [51]. Importantly, some studies have not detected differences in IL-17 levels between SSc patients and healthy controls [52,53] while IL-17 has been shown to both increase [50] and decrease [54,55] collagen production. The reasons for the differences in these studies between pro- or anti-fibrotic effects of IL-17 are not clear, and may reflect the source of the recombinant protein.

A reduction in regulatory T cells has been recorded in the skin lesions of patients with SSc [56] suggesting that there is compromised capacity to regulate immune responses in SSc. A recent study identified an imbalance of regulatory T cells and Th17 cells, with a decrease in the former and an increase in the latter in the peripheral blood of SSc patients compared to healthy controls [57]. In addition, a more recent study has confirmed that Th2 and Th17 cells are found in higher frequencies in SSc patients, and this was found to positively correlate with IL-35 levels, although a causal link has not been established [58]. Further evidence of disruption to T cell homeostasis comes from a recent study showing that SSc patients displaying severe peripheral vascular complications have an expansion of the recently discovered angiogenic T cells [59]. This expansion in angiogenic T cells may also demonstrate a link between immune cell activation and vasculopathy in SSc.

Macrophages

More than 30 years ago circulating monocytes were found to be strongly activated in patients with SSc [60]. Since then, many studies have found evidence of monocyte/macrophage activation in SSc. Higher numbers of macrophages have been observed in skin from SSc patients [27,61] while cells positive for CD163, a putative marker for M2 macrophages [62], have been found to be increased in the serum of SSc patients [61,63–65]. M2 macrophages are therefore prominent in SSc, with a very recent study involving more than 200 SSc patients, not only confirming this but also demonstrating that soluble CD163 (sCD163) is significantly elevated in the serum of SSc patients and therefore may be of use as a biomarker [66].

Microarray gene expression data have also supported a role for monocytes/macrophages in the immunopathogenesis of SSc, with studies demonstrating that peripheral blood mononuclear cells (PBMCs) from SSc patients have an increased expression of genes associated with monocyte/macrophages [67–69]. Such large-scale genomic studies have identified multiple innate immune regulators in SSc. Macrophages are also a major source of TGF-β [70], which is a potent inducer of fibrosis [71]. Many studies have implicated macrophages in the initiation or progression of fibrosis in SSc. Microarray data from SSc lung tissue confirm what has been observed in the blood with markers of macrophage activation [72] and emigration, which correlate with progressive lung fibrosis [73]. In another recent study, a novel multi-network approach to compare gene expression profiles has identified a gene expression signature indicative of profibrotic M2 macrophages in SSc tissues [74]. Interestingly, the profibrotic macrophage gene expression profile differed between skin and lung, suggesting that although a role for macrophages in the immunopathogenesis of fibrosis in SSc seems likely in both skin and lung, there may still be subtle differences in what those roles are. Recently nintedanib, an inhibitor of the receptor tyrosine kinase platelet-derived growth factor receptor, fibroblast growth factor receptor and vascular endothelial growth factor receptor, blocked myofibroblast differentiation and subsequent fibrosis in a mouse model of SSc. Interestingly, these nintedanib-mediated anti-fibrotic effects were associated with reduced numbers of M2 macrophages [75].

Many chemokines which both recruit and can be primarily secreted by macrophages are up-regulated in SSc skin [76–79] which is not surprising, given that infiltration of macrophages in SSc skin has been known since the early 1990s [80]. However, the chemokine (C-C motif) ligand 19 (CCL19) was up-regulated in SSc skin and co-localized with CD163-positive macrophages, suggesting that it has a role in the recruitment of macrophages [76]. The authors of the study also suggest that macrophages are the source of the CCL19, and *in-vivo* experiments demonstrate that Toll-like receptor (TLR)-3, -4 or -9 activation is required for CCL19 expression in monocytes. TLR activation is considered a major event in the immunopathogenesis of SSc [81,82]. Cell types which highly express TLRs such as macrophages are thought to play a major role in SSc. However, many different cell types express TLRs, and thus TLR-mediated signalling in the immunopathogenesis of SSc may not be limited to macrophages. It is possible that TLR activation in any cell type expressing TLRs is also a source of CCL19, and therefore a potential cause of macrophage recruitment in SSc. Indeed, Mathes *et al.* also observed that CCL19 expression was up-regulated from TLR activation in T cells and other cell types from PBMCs, but monocytes gave the most robust increase [76]. We have found that macrophages that respond to the TLR-8 stimulus single-stranded RNA in SSc result in up-regulation of TIMP-1 that is functional and leads to increased collagen deposition [83]. Interestingly, these monocytes seemed to be perivascular.

IL-6 appears to play an important part in the role of macrophages in SSc because inhibition of phosphodiesterase-4, which blocks M2 differentiation, also inhibits IL-6 production, fibroblast activation and fibrosis in an SSc mouse model [84]. IL-6 may also be an important activator of M2 macrophages, as indicated by IL-6 receptor blockage by tocilizumab [85] causing down-regulation of genes associated with M2 macrophages in SSc skin. Oncostatin M is another IL-6-like cytokine that appears to be involved in fibrosis, as cells treated with OSM induced ECM accumulation in fibroblasts [86].

SSc patients with higher expression of the M2 macrophage marker, MRC1, were also found to have elevated levels of IL-13 in their plasma [69] suggesting that macrophage activation in SSc results in production of cytokines from macrophages themselves or indirectly through interaction with other immune cells, because macrophages can both secrete IL-13 [87] and, through being antigen-presenting cells, can activate T cells which could lead to IL-13 production.

There have been more than 30 years of research implicating macrophages in the immunopathogenesis of SSc, with various cytokines produced by macrophages and other immune cells, internal molecules from damaged endothelial cells and chemokines all contributing to the recruitment and activation of macrophages, resulting in a profibrotic environment (Fig. 1). However, how macrophages are recruited to and activated in the tissues of SSc patients is not fully understood. What role is played by macrophages in the progression of fibrosis is also not fully known, but activation of TLRs and the subsequent profibrotic signalling remains an obvious choice. Targeting of TLRs may be a therapeutic option.

B cells

B cells are heavily implicated in the immunopathogenesis of SSc. B cells are known inducers of fibrosis generally and in SSc [88] and mounting evidence suggests that they are modified and activated in patients with SSc. SSc patients have abnormalities of B cell homeostasis in the blood, which includes an expansion of naive B cells and activated but diminished memory B cells [89]. The cytokine B cell activating factor (BAFF), which belongs to the TNF ligand family, is a potent activator of B cells [90]. Not only are BAFF levels elevated in SSc; levels also correlate with disease severity, while B cells isolated from SSc patients produce more IL-6 when exposed to BAFF [91]. A BAFF antagonist inhibited IL-6 and IL-10 expression in the skin of the Tsk mouse model of SSc, while stimulation of B cells with BAFF greatly increased IL-6 [92]. IL-6 can direct the differentiation of T cells into IL-4-producing Th2 cells [93], therefore B cell-secreted IL-6 could contribute to the Th2 phenotype detected in SSc. As well as being involved in IL-6 cytokine production, B cells are also suggested to play an important role in fibrosis. Co-cultures of B cells and fibroblasts from SSc patients induced a fibrotic response, including collagen, TGF-β1 and IL-6 secretion. Exposure of co-cultures to BAFF (and anti-IgM) further increased secretion of the profibrotic compounds [88].

The majority of B cells are IL-6-producing, termed B effector cells (B_{effs}), and have a proinflammatory and autoimmunity role. However, a small subset of B cells termed regulatory B cells (B_{regs}) are potent negative regulators of inflammation and autoimmunity, in part through their ability to express IL-10 [94]. These B_{regs} therefore have the potential to inhibit diseases with immune abnormalities such as SSc. Indeed, B_{regs} were able to inhibit the initiation of a mouse model of multiple sclerosis and depletion of B_{regs} increased symptom severity [95]. Interestingly, the frequency of blood B_{regs} was found to be significantly lower in SSc patients compared to healthy controls [96] while in a very recent study by Matsushita *et al.*, B_{regs} were found to have a

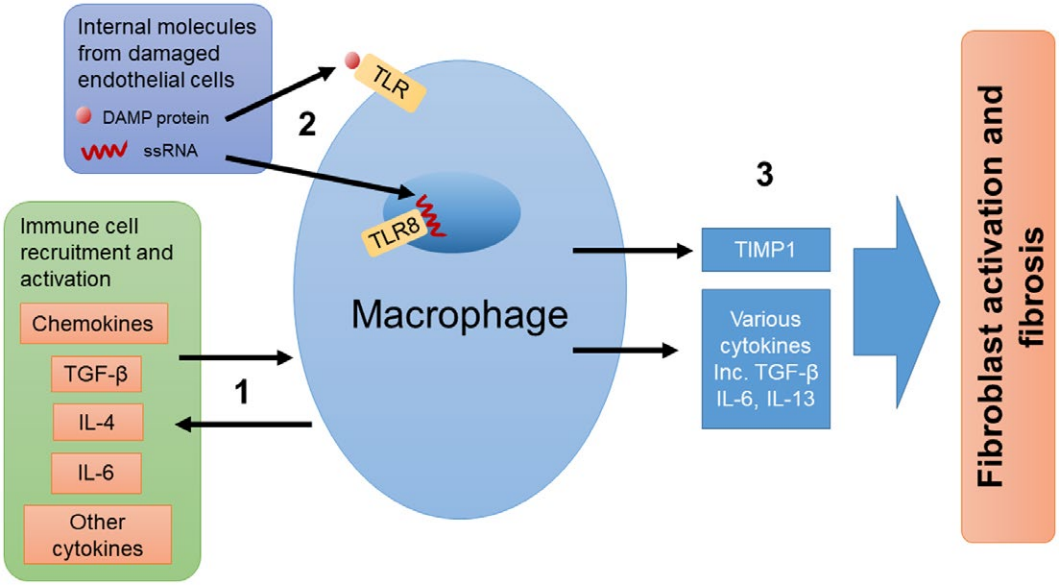

Fig. 1. Potential roles for macrophages in the immunopathogenesis of SSc. (1) Macrophages can both be activated by and release cytokines. Cytokines and chemokines released by macrophages may recruit and activate other immune cells to further promote an inflammatory environment. (2) Internal molecules released from damaged endothelial cells can activate macrophages by Toll-like receptors (TLRs). (3) Activation of macrophages leads to the secretion of profibrotic molecules and the activation of fibroblasts resulting in fibrosis.

similar role in a mouse model of SSc. Depletion of IL-6-producing B_{effs} reduced skin and lung fibrosis, while depletion of IL-10-producing B_{regs} caused more severe fibrosis [97] backing up findings from a previous study that B_{regs} can suppress skin fibrosis in a graft-*versus*-host disease mouse model of SSc [98]. A tipping of the balance of B_{regs} and B_{effs} in favour of fewer B_{regs} is therefore a potential important event in the initiation of fibrosis in SSc. However, the cause of this shift in B cell homeostasis remains unknown, and future studies to identify the trigger/s may provide useful early therapeutic targets.

Various response regulators on the surface of B cells exist, with some increasing and others decreasing B cell receptor (BCR) signals. Of these, the positive B cell regulator, CD19, is the most established in SSc disease research. CD19 is an important regulatory molecule expressed by B cells. $CD19^+$ B cells increase in SSc, with expression of CD19 on the surface of B cells significantly higher in SSc patients compared to healthy controls [99]. CD19-deficient mice have decreased sera levels of several autoantibodies [100] suggesting that B cells could be responsible for the autoantibodies detected in SSc patients. In agreement, a transgenic mouse model, with an elevation in CD19 expression similar to SSc human patients, shows an increase in the levels of autoantibodies [101,102]. It is also worth noting that CD19 over-expression has not been observed in the Tsk mouse model, suggesting that a role for CD19 in B cell activation in SSc is not straightforward or that the animal models can only replicate the human disease phenotypes to a degree. However, the apparent lack of CD19 over-expression may be explained partly through constitutive activation of CD19 and the downstream signalling pathway via up-regulated CD19 tyrosine phosphorylation, which could negate the need for over-expression [102].

Autoantibody production is prevalent, and can be used diagnostically in SSc. Furthermore, autoantibodies against platelet-derived growth factor receptor (PDGFR) were found in SSc patients, and caused collagen I-increased expression and transition of normal human primary fibroblasts to a myofibroblast phenotype through activation of an intracellular loop that involves Ha-Ras, ERK1/2 and reactive oxygen species (ROS) [103]. However, it is still possible that B cell activation is the cause of both autoantibody production and fibrosis, as the two may be linked. Interestingly, B cell activation via CD19 specifically may also be a cause of other immune cell type infiltration in SSc. CD19-deficient mice showed less infiltration of mast cells, macrophages, T cells and B cells after bleomycin treatment compared to wild-type cells exposed to bleomycin [104]. In the same study, it was discovered that B cells, via hyaluronan-induced TLR-4 activation, produced various cytokines which were dramatically reduced in CD19-deficient cells. Therefore, B cells have the potential to contribute to fibrosis directly as well as indirectly through recruitment and activation of other immune cells

to promote a cycle of cytokine and fibrogenic compound release.

Mast cells

Mast cell infiltration was detected from lip biopsy in very early stages of SSc and preceded the onset of skin changes [105] and more recently cutaneous mastocytosis was found to precede the onset of SSc in a 36-year-old woman [106] suggesting that mast cells may play an early role in the onset of SSc. Mast cell infiltration has also been detected late in the development of SSc [107] and in a subset of SSc patients with localized scleroderma-like lesions [108]. An increase in mast cell numbers has also been detected in SSc skin [109].

Mast cells are able to produce TGF-β and are found localized to fibroblasts in the skin of SSc patients [110]. Interestingly, mast cells are not only a source of TGF-β; they can also directly transfer TGF-β to fibroblasts mediated by transgranulation via cell–cell contacts [111], which has also been observed in the dermis of SSc patients [112]. The triggers of mast infiltration in SSc or other fibrotic diseases are not known, but a very recent study has identified Snail-dependent production of plasminogen activator inhibitor-1 (PAI1) in keratinocytes as a chemoattractant of mast cells, a mediator of mast cell–fibroblast adhesion and a promoter of fibrogenesis [113]. Interestingly, PAI1 up-regulated tenascin-C (TENC) expression, which is the main activator of TLR-4 to promote fibrosis and found to be elevated in SSc [114]. Therefore, the role of PAI1 in SSc could involve mast cell recruitment and activation of fibroblasts to promote an inflammatory environment.

Dendritic cells

Two major types of dendritic cell are known: conventional (cDC) and plasmacytoid (pDC). pDC are a specialized cell type that when activated produce large amounts of interferon (IFN). A very recent study observed that pDCs infiltrate the skin of SSc patients and are chronically activated, producing IFN-α and chemokine (C-X-C motif) ligand 4 (CXCL4). Fibrosis was reverted in a mouse model of SSc after pDCs depletion [115]. In the same study, TLR-8 signalling was found to be important for CXCL4 production and recruitment of pDCs to fibrotic skin. TLR involvement has also been found to be important for mediating the increased cytokine production in DCs in SSc, which has been comprehensively reviewed [116]. pDCs isolated from SSc patients show up-regulation of CXCL4 [115,117], which can also be detected at elevated levels and correlates with disease severity in SSc patient plasma [118] and has been suggested as a biomarker in SSc.

In another recent study, pDC depletion in a bleomycin mouse model improved the clinical score, lung histopathology score, skin thickness and collagen content. The expression of genes involved in chemotaxis, dendritic cell differentiation, inflammation and fibrosis in the lungs of pDC-depleted mice was also significantly reduced. Alongside this, B and T cells were also found to be reduced in the lungs, suggesting an important role for ongoing immune abnormalities induced by DCs [119]. In another bleomycin-induced pulmonary fibrosis mouse model, DC accumulation in the lung was reduced through blocking of TGF-β with the use of inhibitor SB431542 [120]. TGF-β was also found to be important for the recruitment of pDCs (and T cells) to the skin in an SSc mouse model [30] suggesting an important role for TGF-β in DC location and activation. An important role for microRNAs in the pathogenesis SSc is emerging [121]. Recently, microRNA expression profiling has identified miRNA-618 (miR-618) as being up-regulated in pDCs from SSc patients, while overexpression in healthy pDCs resulted in an SSc-like pDC [122]. Thus, microRNAs are disturbed, leading to altered functionality of immune cells. Alterations of microRNAs by restoration using microRNA therapeutics may be one way to reset dendritic cells to a 'tolerogenic' phenotype.

Anti-nuclear antibodies, which are a subset of autoantibodies, are found to be elevated in SSc patients [123]. DNA topoisomerase I (TopoI) is an intracellular target of anti-nuclear antibody anti-TopoI. The presence of anti-TopoI antibodies has been clinically associated with a more severe form of SSc [124]. Immunization of mice with TopoI peptide-loaded DCs induces anti-TopoI autoantibody response and long-term fibrosis of skin and lung, which is preceded by inflammation with increased IL-17A and CXCL4 expression [125]. TopoI peptide-loaded DCs also induce proliferation of SSc and healthy-derived T cells, but activation of T cells requires a fragmented form of TopoI and activation of full-length TopoI is IL-2-dependent [126]. Interestingly, antigen-presenting cells within PBMCs were able to activate T cells more efficiently than dendritic cells, and even processed TopoI peptides differently, suggesting that there is a complex regulation of T cells by dendritic cells and other antigen-presenting cells with regard to anti-nuclear antibodies. Overall, experimental manipulation of DCS can mimic some major events in the pathogenesis of SSc, and therefore raises the intriguing prospect that DCs may be capable of initiating SSc. A broken loop that may perpetuate inflammation with cytokine-mediated up-regulation of co-stimulatory molecules may also exist in SSc.

Other immune cells

Dysregulation and activation of immune cells is clearly a major hallmark of SSc involving many cell types. This

complex network of signalling between different immune cells is only now beginning to become unravelled, with new evidence emerging regularly to provide new players in SSc immunopathogenesis. For example, only a few studies have highlighted the potential for platelets [127–129], neutrophils [130,131], natural killer cells [132,133] and innate lymphoid cells [134,135] to be dysregulated and contribute to the pathogenesis of SSc. Type 2 innate lymphoid cells are found in higher numbers in SSc and correlate strongly with the Rodnan skin score and also the presence of lung fibrosis.

Although not considered to be innate immune cells, fibroblasts play a crucial role in the fibrotic phenotype of SSc, but they also express TLRs and are therefore capable of responding to DAMPs. Expression of TLR-2 was found to be increased in fibroblasts from SSc skin [136]. Recently, fibroblasts exposed to TGF-β and IL-17A responded with a 100-fold increase in the production of IL-6 [137], a known profibrotic mediator.

Conclusion

Various cell types of the immune system appear to be involved to some degree in the immunopathogenesis of SSc through promoting other immune cell activation, fibrosis or vascular damage. Very recent studies have highlighted the importance of this plethora of immune cell types in SSc (Table 1). However, research to date provides evidence for macrophages, T cells and B cells having the most important role in SSc. Whether this accurately represents their importance in SSc or is a result of research interests combined with the practicality of investigating specific cell types remains to be seen. Nevertheless, immune cell activation is a recurring observation in SSc research and is leading to the development and testing of therapeutic interventions. Although the over-production of cytokines is a well-accepted symptom of SSc, TGF-β, IL-6 and IL-4 specifically seem to be integral to many of the immune abnormalities recorded. Thoroughly understanding how these and other cytokines fully regulate, and are regulated by, all the different immune cells and their subcategories in SSc will be a difficult task, but every study into immune cell activation, regardless of cell type, brings us closer to achieving it and as a result developing therapeutic interventions. Current trials such as the Fassinate trial in SSc blocking IL-6 seem promising, with the Phase II trial positive, although the primary end-point was not reached. Phosphodiesterase type 4 (PDE4) inhibitors appear to regulate macrophages leading to reduced fibroblast activation. There is an ongoing trial concerning morphea.

Table 1. A selection of recent and important studies implicating each of the innate cell types in the immunopathogenesis of systemic sclerosis (SSc)

Immune cell	Recent findings	Reference
T cell	Angiogenic T cells were elevated in SSc patients displaying digital ulcers, which is a severe peripheral vascular complication	[59]
Macrophage	The soluble form of the M2 macrophage marker CD163 is elevated in the serum of patients with SSc, highlighting it as a potential biomarker	[66]
B cell	Used a mouse model of SSc to demonstrate the importance of B cell homeostasis. Depletion of IL-6-producing B effector cells reduced fibrosis while depleting of IL-10-producing B regulatory cells had the opposite effect	[97]
Mast cell	A subset of SSc patients with localized scleroderma-like lesions were found to have an inflammatory phenotype leading to the activation of mast cells in the dermis of mechanically stressed skin	[108]
Dendritic cell	After depletion of pDCs fibrosis was reverted in mice with established SSc-like disease. pDC depletion prior to induction of disease also prevented fibrosis	[115]
Platelets	Activated platelets induced the production of the profibrotic mediator thymic stromal lymphopoietin (TSLP) in human dermal endothelial cells	[128]
Neutrophils	Neutrophil activation was induced by SSc microparticles. Microparticles were derived from platelets and expressed the damage-associated molecular pattern HMGB1. An inhibitor of HMGB1 attenuated neutrophil activation	[131]
Natural killer	A peculiar natural killer cell phenotype in SSc patients was identified characterized by decreased chemokine and activation receptors expression. These SSc-derived natural killer cells were potent inducers of endothelial microparticle release suggesting that there may be a role for natural killer cells in the activation of endothelial cells in SSc	[133]
Innate lymphoid	Type 2 innate lymphoid (ILC2) cells are elevated in patients with SSc. ILC2 counts correlated with skin fibrosis. This study highlights that there may be a profibrotic role for ILC2 cells in SSc	[134]
Fibroblast	Although not an immune cell type, fibroblasts exposed to TGF-β and IL-17a responded with a 100-fold increase in the production of IL-6	[137]

DCs = dendritic cells; IL = interleukin; TGF = transforming growth factor; HMGB1 = high mobility group box 1.

Disclosures

None.

References

1 Asano Y, Sato S. Vasculopathy in scleroderma. Semin Immunopathol 2015; **37**:489–500.

2 McGaha T *et al*. Lack of skin fibrosis in tight skin (TSK) mice with targeted mutation in the interleukin-4R alpha and transforming growth factor-beta genes. J Invest Dermatol 2001; **116**:136–43.

3 Fertin C *et al*. Interleukin-4 stimulates collagen synthesis by normal and scleroderma fibroblasts in dermal equivalents. Cell Mol Biol 1991; **37**:823–9.

4 Sempowski GD, Derdak S, Phipps RP. Interleukin-4 and interferon-gamma discordantly regulate collagen biosynthesis by functionally distinct lung fibroblast subsets. J Cell Physiol 1996; **167**:290–6.

5 Postlethwaite AE *et al*. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. J Clin Invest 1992; **90**:1479–85.

6 Wegrowski Y, Paltot V, Gillery P, Kalis B, Randoux A, Maquart FX. Stimulation of sulphated glycosaminoglycan and decorin production in adult dermal fibroblasts by recombinant human interleukin-4. Biochem J 1995; **307**:673–8.

7 Ong C, Wong C, Roberts CR, Teh H-S, Jirik FR. Anti-IL-4 treatment prevents dermal collagen deposition in the tight-skin mouse model of scleroderma. Eur J Immunol 1998; **28**:2619–29.

8 Luzina IG, Atamas SP, Wise R *et al*. Occurrence of an activated, profibrotic pattern of gene expression in lung CD8+ T cells from scleroderma patients. Arthritis Rheum 2003; **48**:2262–74.

9 Needleman BW, Wigley FM, Stair RW. Interleukin-1, interleukin-2, interleukin-4, interleukin-6, tumor necrosis factor alpha, and interferon-gamma levels in sera from patients with scleroderma. Arthritis Rheum 1992; **35**:67–72.

10 Hasegawa M *et al*. Elevated serum levels of interleukin 4 (IL-4), IL-10, and IL-13 in patients with systemic sclerosis. J Rheumatol 1997; **24**:328–32.

11 Sakkas LI *et al*. Increased levels of alternatively spliced interleukin 4 (IL-4delta2) transcripts in peripheral blood mononuclear cells from patients with systemic sclerosis. Clin Diagn Lab Immunol 1999; **6**:660–4.

12 Ihn H. The role of TGF-beta signaling in the pathogenesis of fibrosis in scleroderma. Arch Immunol Ther Exp (Warsz) 2002; **50**:325–31.

13 Zi Z, Chapnick DA, Liu X. Dynamics of TGF-beta/Smad signaling. FEBS Lett 2012; **586**:1921–8.

14 Sato M *et al*. Role of p38 MAPK in transforming growth factor beta stimulation of collagen production by scleroderma and healthy dermal fibroblasts. J Invest Dermatol 2002; **118**:704–11.

15 Hocevar BA, Brown TL, Howe PH. TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. EMBO J 1999; **18**:1345–56.

16 Reunanen N, Foschi M, Han J, Kähäri V-M. Activation of extracellular signal-regulated kinase 1/2 inhibits type I collagen expression by human skin fibroblasts. J Biol Chem 2000; **275**:34634–9.

17 Koch AE, Kronfeld-Harrington LB, Szekanecz Z *et al*. In situ expression of cytokines and cellular adhesion molecules in the skin of patients with systemic sclerosis. Their role in early and late disease. Pathobiology 1993; **61**:239–46.

18 Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. J Dermatol Sci 2001; **27**:140–6.

19 Yoshizaki K. Pathogenic role of IL-6 combined with TNF-alpha or IL-1 in the induction of acute phase proteins SAA and CRP in chronic inflammatory diseases. Adv Exp Med Biol 2011; **691**:141–50.

20 Saito F, Tasaka S, Inoue K-I *et al*. Role of interleukin-6 in bleomycin-induced lung inflammatory changes in mice. Am J Respir Cell Mol Biol 2008; **38**:566–71.

21 O'Reilly S *et al*. Interleukin-6 (IL-6) trans signaling drives a STAT3-dependent pathway that leads to hyperactive transforming growth factor-beta (TGF-beta) signaling promoting SMAD3 activation and fibrosis via Gremlin protein. J Biol Chem 2014; **289**:9952–60.

22 Henderson J, Bhattacharyya S, Varga J, O'Reilly S. Targeting TLRs and the inflammasome in systemic sclerosis. Pharmacol Ther 2018; **192**:163–9.

23 Barnes TC *et al*. Endothelial activation and apoptosis mediated by neutrophil-dependent interleukin 6 trans-signalling: a novel target for systemic sclerosis? Ann Rheum Dis 2011; **70**:366–72.

24 Kitaba S, Murota H, Terao M *et al*. Blockade of interleukin-6 receptor alleviates disease in mouse model of scleroderma. Am J Pathol 2012; **180**:165–76.

25 Shima Y, Kuwahara Y, Murota H *et al*. The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. Rheumatology (Oxf) 2010; **49**:2408–12.

26 Khanna D, Denton CP, Jahreis A *et al*. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. Lancet 2016; **387**:2630–40.

27 Prescott RJ, Freemont AJ, Jones CJP, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. J Pathol 1992; **166**:255–63.

28 Abraham D, Lupoli S, McWhirter A *et al*. Expression and function of surface antigens on scleroderma fibroblasts. Arthritis Rheum 1991; **34**:1164–72.

29 Kalogerou A, Gelou E, Mountantonakis S, Settas L, Zafiriou E, Sakkas L. Early T cell activation in the skin from patients with systemic sclerosis. Ann Rheum Dis 2005; **64**:1233–5.

30 Gerber EE, Gallo EM, Fontana SC *et al*. Integrin-modulating therapy prevents fibrosis and autoimmunity in mouse models of scleroderma. Nature 2013; **503**:126–30.

31 Ponsoye M, Frantz C, Ruzehaji N *et al*. Treatment with abatacept prevents experimental dermal fibrosis and induces regression of established inflammation-driven fibrosis. Ann Rheum Dis 2016; **75**:2142–9.

32 Gaud G, Lesourne R, Love PE. Regulatory mechanisms in T cell receptor signalling. Nat Rev Immunol 2018; **18**:485–97.

33 Adeeb F, Anjum S, Hodnett P *et al*. Early- and late-stage morphea subtypes with deep tissue involvement is treatable with Abatacept (Orencia). Semin Arthritis Rheum 2017; **46**:775–81.

34 O'Reilly S, Hugle T, van Laar JM. T cells in systemic sclerosis: a reappraisal. Rheumatology (Oxf) 2012; **51**:1540–9.

35 Sharma SK, MacLean JA, Pinto C, Kradin RL. The effect of an anti-CD3 monoclonal antibody on bleomycin-induced lymphokine production and lung injury. Am J Respir Crit Care Med 1996; **154**:193–200.

36 Kodera T *et al*. Disrupting the IL-4 gene rescues mice homozygous for the tight-skin mutation from embryonic death and diminishes TGF-beta production by fibroblasts. Proc Natl Acad Sci USA 2002; **99**:3800–5.

37 Seder RA *et al*. Factors involved in the differentiation of TGF-beta-producing cells from naive CD4+ T cells: IL-4 and IFN-gamma have opposing effects, while TGF-beta positively regulates its own production. J Immunol 1998; **160**:5719–28.

38 Parel Y, Aurrand-Lions M, Scheja A, Dayer J-M, Roosnek E, Chizzolini C. Presence of CD4+CD8+ double-positive T cells with very high interleukin-4 production potential in lesional skin of patients with systemic sclerosis. Arthritis Rheum 2007; **56**:3459–67.

39 Gross A, Ben-Sasson SZ, Paul WE. Anti-IL-4 diminishes in vivo priming for antigen-specific IL-4 production by T cells. J Immunol 1993; **150**:2112–20.

40 Fallon PG, Richardson EJ, McKenzie GJ, McKenzie ANJ. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. J Immunol 2000; **164**:2585–91.

41 Oriente A *et al*. Interleukin-13 modulates collagen homeostasis in human skin and keloid fibroblasts. J Pharmacol Exp Ther 2000; **292**:988–94.

42 Belperio JA, Dy M, Burdick MD *et al*. Interaction of IL-13 and C10 in the pathogenesis of bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2002; **27**:419–27.

43 Baraut J *et al*. Transforming growth factor-beta increases interleukin-13 synthesis via GATA-3 transcription factor in T-lymphocytes from patients with systemic sclerosis. Arthritis Res Ther 2015; **17**:196.

44 Fuschiotti P, Medsger TA Jr, Morel PA. Effector CD8+ T cells in systemic sclerosis patients produce abnormally high levels of interleukin-13 associated with increased skin fibrosis. Arthritis Rheum 2009; **60**:1119–28.

45 Li G, Larregina AT, Domsic RT *et al*. Skin-resident effector memory CD8(+)CD28(–) T cells exhibit a profibrotic phenotype in patients with systemic sclerosis. J Invest Dermatol 2017; **137**:1042–50.

46 OReilly S *et al*. IL-13 mediates collagen deposition via STAT6 and microRNA-135b: a role for epigenetics. Sci Rep 2016; **6**:25066.

47 Brown Lobbins ML *et al*. Chronic exposure of interleukin-13 suppress the induction of matrix metalloproteinase-1 by tumour necrosis factor alpha in normal and scleroderma dermal fibroblasts through protein kinase B/Akt. Clin Exp Immunol 2018; **191**:84–95.

48 Kurasawa K, Hirose K, Sano H *et al*. Increased interleukin-17 production in patients with systemic sclerosis. Arthritis Rheum 2000; **43**:2455–63.

49 Truchetet M-E, Brembilla N-C, Montanari E *et al*. Interleukin-17A+ cell counts are increased in systemic sclerosis skin and their number is inversely correlated with the extent of skin involvement. Arthritis Rheum 2013; **65**:1347–56.

50 Yang X, Yang J, Xing X, Wan L, Li M. Increased frequency of Th17 cells in systemic sclerosis is related to disease activity and collagen overproduction. Arthritis Res Ther 2014; **16**:R4.

51 Chizzolini C, Dufour AM, Brembilla NC. Is there a role for IL-17 in the pathogenesis of systemic sclerosis? Immunol Lett 2018; **195**:61–7.

52 Gourh P, Arnett FC, Assassi S *et al*. Plasma cytokine profiles in systemic sclerosis: associations with autoantibody subsets and clinical manifestations. Arthritis Res Ther 2009; **11**:R147.

53 Olewicz-Gawlik A, Danczak-Pazdrowska A, Kuznar-Kaminska B *et al*. Interleukin-17 and interleukin-23: importance in the pathogenesis of lung impairment in patients with systemic sclerosis. Int J Rheum Dis 2014; **17**:664–70.

54 Brembilla N, Montanari E, Truchetet M-E, Raschi E, Meroni P, Chizzolini C. Th17 cells favor inflammatory responses while inhibiting type I collagen deposition by dermal fibroblasts: differential effects in healthy and systemic sclerosis fibroblasts. Arthritis Res Ther 2013; **15**:R151.

55 Nakashima T, Jinnin M, Yamane K *et al*. Impaired IL-17 signaling pathway contributes to the increased collagen expression in scleroderma fibroblasts. J Immunol 2012;**188**:3573–83.

56 Klein S, Kretz CC, Ruland V *et al*. Reduction of regulatory T cells in skin lesions but not in peripheral blood of patients with systemic scleroderma. Ann Rheum Dis 2011; **70**:1475–81.

57 Krasimirova E, Velikova T, Ivanova-Todorova E *et al*. Treg/Th17 cell balance and phytohaemagglutinin activation of T lymphocytes in peripheral blood of systemic sclerosis patients. World J Exp Med 2017; **7**:84–96.

58 Tang J, Lei L, Pan J, Zhao C, Wen J. Higher levels of serum interleukin-35 are associated with the severity of pulmonary fibrosis and Th2 responses in patients with systemic sclerosis. Rheumatol Int 2018; **38**:1511–9.

59 Manetti M *et al*. Angiogenic T cell expansion correlates with severity of peripheral vascular damage in systemic sclerosis. PLOS ONE 2017; **12**:e0183102.

60 Andrews BS *et al*. Changes in circulating monocytes in patients with progressive systemic sclerosis. J Rheumatol 1987; **14**:930–5.

61 Higashi-Kuwata N *et al*. Characterization of monocyte/macrophage subsets in the skin and peripheral blood derived from patients with systemic sclerosis. Arthritis Res Ther 2010; **12**:R128.

62 Stifano G, Christmann RB. Macrophage involvement in systemic sclerosis: do we need more evidence? Curr Rheumatol Rep 2016; **18**:2.

63 Bielecki M, Kowal K, Lapinska A, Chyczewski L, Kowal-Bielecka O. Increased release of soluble CD163 by the peripheral blood mononuclear cells is associated with worse prognosis in patients with systemic sclerosis. Adv Med Sci 2013; **58**:126–33.

64 Nakayama W *et al*. CD163 expression is increased in the involved skin and sera of patients with systemic lupus erythematosus. Eur J Dermatol 2012; **22**:512–7.

65 Shimizu K, Ogawa F, Yoshizaki A *et al*. Increased serum levels of soluble CD163 in patients with scleroderma. Clin Rheumatol 2012; **31**:1059–64.

66 Frantz C *et al*. Soluble CD163 as a potential biomarker in systemic sclerosis. Dis Markers 2018; **2018**:8509583.

67 Grigoryev DN, Mathai SC, Fisher MR *et al*. Identification of candidate genes in scleroderma-related pulmonary arterial hypertension. Transl Res 2008; **151**:197–207.

68 Pendergrass SA, Hayes E, Farina G *et al*. Limited systemic sclerosis patients with pulmonary arterial hypertension show biomarkers of inflammation and vascular injury. PLOS ONE 2010; **5**:e12106.

69 Christmann RB, Hayes E, Pendergrass S *et al*. Interferon and alternative activation of monocyte/macrophages in systemic sclerosis-associated pulmonary arterial hypertension. Arthritis Rheum 2011; **63**:1718–28.

70 Shapouri-Moghaddam A, Mohammadian S, Vazini H *et al*. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 2018; **233**:6425–40.

71 Hu HH *et al*. New insights into TGF-beta/Smad signaling in tissue fibrosis. Chem Biol Interact 2018; **292**:76–83.

72 Hsu E, Shi H, Jordan RM, Lyons-Weiler J, Pilewski JM, Feghali-Bostwick CA. Lung tissues in patients with systemic sclerosis have gene expression patterns unique to pulmonary fibrosis and pulmonary hypertension. Arthritis Rheum 2011; **63**:783–94.

73 Christmann RB *et al*. Association of Interferon- and transforming growth factor beta-regulated genes and macrophage activation with systemic sclerosis-related progressive lung fibrosis. Arthritis Rheumatol 2014; **66**:714–25.

74 Taroni JN, Greene CS, Martyanov V *et al*. A novel multi-network approach reveals tissue-specific cellular modulators of fibrosis in systemic sclerosis. Genome Med 2017; **9**:27.

75 Huang J, Maier C, Zhang Y *et al*. Nintedanib inhibits macrophage activation and ameliorates vascular and fibrotic manifestations in the Fra2 mouse model of systemic sclerosis. Ann Rheum Dis 2017; **76**:1941–8.

76 Mathes AL, Christmann RB, Stifano G *et al*. Global chemokine expression in systemic sclerosis (SSc): CCL19 expression correlates with vascular inflammation in SSc skin. Ann Rheum Dis 2014; **73**:1864–72.

77 Distler O, Pap T, Kowal-Bielecka O *et al*. Overexpression of monocyte chemoattractant protein 1 in systemic sclerosis: role of platelet-derived growth factor and effects on monocyte chemotaxis and collagen synthesis. Arthritis Rheum 2001; **44**:2665–78.

78 Yamamoto T, Eckes B, Krieg T. High expression and autoinduction of monocyte chemoattractant protein-1 in scleroderma fibroblasts. Eur J Immunol 2001; **31**:2936–41.

79 Hasegawa M, Sato S, Takehara K. Augmented production of chemokines (monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1alpha (MIP-1alpha) and MIP-1beta) in patients with systemic sclerosis: MCP-1 and MIP-1alpha may be involved in the development of pulmonary fibrosis. Clin Exp Immunol 1999; **117**:159–65.

80 Ishikawa O, Ishikawa H. Macrophage infiltration in the skin of patients with systemic sclerosis. J Rheumatol 1992; **19**:1202–6.

81 O'Reilly S. Toll like receptors in systemic sclerosis: an emerging target. Immunol Lett 2018; **195**:2–8.

82 Fullard N, O'Reilly S. Role of innate immune system in systemic sclerosis. Semin Immunopathol 2015; **37**:511–7.

83 Ciechomska M, Huigens CA, Hügle T *et al*. Toll-like receptor-mediated, enhanced production of profibrotic TIMP-1 in monocytes from patients with systemic sclerosis: role of serum factors. Ann Rheum Dis 2013; **72**:1382–9.

84 Maier C, Ramming A, Bergmann C *et al*. Inhibition of phosphodiesterase 4 (PDE4) reduces dermal fibrosis by interfering with the release of interleukin-6 from M2 macrophages. Ann Rheum Dis 2017; **76**:1133–41.

85 Sebba A. Tocilizumab: the first interleukin-6-receptor inhibitor. Am J Health Syst Pharm 2008; **65**:1413–8.

86 Mozaffarian A, Brewer AW, Trueblood ES *et al*. Mechanisms of oncostatin M-induced pulmonary inflammation and fibrosis. J Immunol 2008; **181**:7243–53.

87 Hancock A, Armstrong L, Gama R, Millar A. Production of interleukin 13 by alveolar macrophages from normal and fibrotic lung. Am J Respir Cell Mol Biol 1998; **18**:60–5.

88 Francois A *et al*. B lymphocytes and B-cell activating factor promote collagen and profibrotic markers expression by dermal

fibroblasts in systemic sclerosis. Arthritis Res Ther 2013; **15**:R168.

89 Sato S, Fujimoto M, Hasegawa M, Takehara K. Altered blood B lymphocyte homeostasis in systemic sclerosis: expanded naive B cells and diminished but activated memory B cells. Arthritis Rheum 2004; **50**:1918–27.

90 Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol 2009; **9**:491–502.

91 Matsushita T, Hasegawa M, Yanaba K, Kodera M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. Arthritis Rheum 2006; **54**:192–201.

92 Matsushita T, Fujimoto M, Hasegawa M *et al*. BAFF antagonist attenuates the development of skin fibrosis in tight-skin mice. J Invest Dermatol 2007; **127**:2772–80.

93 Rincon M *et al*. Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4+ T cells. J Exp Med 1997; **185**:461–9.

94 Iwata Y, Matsushita T, Horikawa M *et al*. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood 2011;**117**:530–41.

95 Matsushita T, Yanaba K, Bouaziz J-D, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest 2008; **118**:3420–30.

96 Matsushita T, Hamaguchi Y, Hasegawa M, Takehara K, Fujimoto M. Decreased levels of regulatory B cells in patients with systemic sclerosis: association with autoantibody production and disease activity. Rheumatology (Oxford) 2016; **55**:263–7.

97 Matsushita T *et al*. BAFF inhibition attenuates fibrosis in scleroderma by modulating the regulatory and effector B cell balance. Sci Adv 2018; **4**:eaas9944.

98 Le Huu D, Matsushita T, Jin G *et al*. Donor-derived regulatory B cells are important for suppression of murine sclerodermatous chronic graft-versus-host disease. Blood 2013; **121**: 3274–83.

99 Sato S, Hasegawa M, Fujimoto M, Tedder TF, Takehara K. Quantitative genetic variation in CD19 expression correlates with autoimmunity. J Immunol 2000; **165**:6635–43.

100 Sato S *et al*. CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. J Immunol 1996; **157**:4371–8.

101 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.

102 Asano N, Fujimoto M, Yazawa N *et al*. B Lymphocyte signaling established by the CD19/CD22 loop regulates autoimmunity in the tight-skin mouse. Am J Pathol 2004; **165**:641–50.

103 Baroni SS, Santillo M, Bevilacqua F *et al*. Stimulatory autoantibodies to the PDGF receptor in systemic sclerosis. N Engl J Med 2006; **354**:2667–76.

104 Yoshizaki A, Iwata Y, Komura K *et al*. CD19 regulates skin and lung fibrosis via Toll-like receptor signaling in a model of bleomycin-induced scleroderma. Am J Pathol 2008; **172**:1650–63.

105 Hebbar M *et al*. Early expression of E-selectin, tumor necrosis factor alpha, and mast cell infiltration in the salivary glands of patients with systemic sclerosis. Arthritis Rheum 1996; **39**:1161–5.

106 Bagnato G *et al*. Mastocytosis and systemic sclerosis: a clinical association. Clin Mol Allergy 2016; **14**:13.

107 Frigui M, Dupin N, Carlotti A *et al*. Telangiectasic mastocytosis with systemic sclerosis. Presse Med 2013; **42**:902–4.

108 Saigusa R *et al*. Systemic sclerosis complicated with localized scleroderma-like lesions induced by Kobner phenomenon. J Dermatol Sci 2018; **89**:282–9.

109 Seibold JR, Giorno RC, Claman HN. Dermal mast cell degranulation in systemic sclerosis. Arthritis Rheum 1990; **33**:1702–9.

110 Hugle T *et al*. Mast cells are a source of transforming growth factor beta in systemic sclerosis. Arthritis Rheum 2011; **63**:795–9.

111 Trautmann A *et al*. Human mast cells augment fibroblast proliferation by heterotypic cell-cell adhesion and action of IL-4. J Immunol 1998; **160**:5053–7.

112 Hugle T, White K, van Laar JM. Cell-to-cell contact of activated mast cells with fibroblasts and lymphocytes in systemic sclerosis. Ann Rheum Dis 2012; **71**:1582.

113 Pincha N, Hajam EY, Badarinath K *et al*. PAI1 mediates fibroblast-mast cell interactions in skin fibrosis. J Clin Invest 2018; **128**:1807–19.

114 Bhattacharyya S *et al*. Tenascin-C drives persistence of organ fibrosis. Nat Commun 2016; **7**:11703.

115 Ah Kioon MD, Tripodo C, Fernandez D *et al*. Plasmacytoid dendritic cells promote systemic sclerosis with a key role for TLR8. Sci Transl Med 2018; **10**:8458.

116 Affandi AJ, Carvalheiro T, Radstake TRDJ, Marut W. Dendritic cells in systemic sclerosis: Advances from human and mice studies. Immunol Lett 2018; **195**:18–29.

117 van Bon L, Affandi AJ, Broen J *et al*. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. N Engl J Med 2014; **370**:433–43.

118 Volkmann ER, Tashkin DP, Roth MD *et al*. Changes in plasma CXCL4 levels are associated with improvements in lung function in patients receiving immunosuppressive therapy for systemic sclerosis-related interstitial lung disease. Arthritis Res Ther 2016; **18**:305.

119 Kafaja S, Valera I, Divekar AA *et al*. pDCs in lung and skin fibrosis in a bleomycin-induced model and patients with systemic sclerosis. JCI Insight 2018; **3**.

120 Chakraborty K, Chatterjee S, Bhattacharyya A. Modulation of CD11c+ lung dendritic cells in respect to TGF-beta in experimental pulmonary fibrosis. Cell Biol Int 2017; **41**:991–1000.

121 Horsburgh S, Fullard N, Roger M *et al*. MicroRNAs in the skin: role in development, homoeostasis and regeneration. Clin Sci (Lond) 2017; **131**:1923–40.

122 Rossato M, Affandi AJ, Thordardottir S *et al*. Association of MicroRNA-618 Expression With Altered Frequency and Activation of Plasmacytoid Dendritic Cells in Patients With Systemic Sclerosis. Arthritis Rheumatol 2017; **69**:1891–902.

123 Okano Y. Antinuclear antibody in systemic sclerosis (scleroderma). Rheum Dis Clin North Am 1996; **22**:709–35.

124 Sato S, Hamaguchi Y, Hasegawa M, Takehara K. Clinical significance of anti-topoisomerase I antibody levels determined by ELISA in systemic sclerosis. Rheumatology (Oxford) 2001; **40**:1135–40.

125 Mehta H, Goulet P-O, Nguyen V *et al*. Topoisomerase I peptide-loaded dendritic cells induce autoantibody response as well as skin and lung fibrosis. Autoimmunity 2016; **49**:503–13.

126 Oriss TB, Hu PQ, Wright TM. Distinct autoreactive T cell responses to native and fragmented DNA topoisomerase I: influence of APC type and IL-2. J Immunol 2001; **166**:5456–63.

127 Dees C, Akhmetshina A, Zerr P *et al*. Platelet-derived serotonin links vascular disease and tissue fibrosis. J Exp Med 2011; **208**:961–72.

128 Truchetet M-E, Demoures B, Eduardo Guimaraes J *et al*. Platelets induce thymic stromal lymphopoietin production by endothelial cells: contribution to fibrosis in human systemic sclerosis. Arthritis Rheumatol 2016; **68**:2784–94.

129 Ntelis K, Solomou EE, Sakkas L, Liossis S-N, Daoussis D. The role of platelets in autoimmunity, vasculopathy, and fibrosis: Implications for systemic sclerosis. Semin Arthritis Rheum 2017; **47**:409–17.

130 Barnes TC, Anderson ME, Edwards SW, Moots RJ. Neutrophil-derived reactive oxygen species in SSc. Rheumatology (Oxf) 2012; **51**:1166–9.

131 Maugeri N *et al*. Platelet microparticles sustain autophagy-associated activation of neutrophils in systemic sclerosis. Sci Transl Med 2018; **10**.

132 Almeida I, Silva SV, Fonseca AR, Silva I, Vasconcelos C, Lima M. T and NK cell phenotypic abnormalities in systemic sclerosis: a cohort study and a comprehensive literature review. Clin Rev Allergy Immunol 2015; **49**:347–69.

133 Benyamine A *et al*. Natural killer cells exhibit a peculiar phenotypic profile in systemic sclerosis and are potent inducers of endothelial microparticles release. Front Immunol 2018; **9**:1665.

134 Roan F *et al*. CD4+ Group 1 innate lymphoid cells (ILC) form a functionally distinct ILC subset that is increased in systemic sclerosis. J Immunol 2016; **196**:2051–62.

135 Wohlfahrt T, Usherenko S, Englbrecht M *et al*. Type 2 innate lymphoid cell counts are increased in patients with systemic sclerosis and correlate with the extent of fibrosis. Ann Rheum Dis 2016; **75**:623–6.

136 O'Reilly S *et al*. Serum amyloid A induces interleukin-6 in dermal fibroblasts via Toll-like receptor 2, interleukin-1 receptor-associated kinase 4 and nuclear factor-kappaB. Immunology 2014; **143**:331–40.

137 Dufour AM *et al*. Interleukin-6 and type-I collagen production by systemic sclerosis fibroblasts are differentially regulated by interleukin-17A in the presence of transforming growth factor-beta 1. Front Immunol 2018; **9**:1865.