REVIEW ARTICLE

T cell abnormalities in systemic sclerosis with a focus on Th17 cells*

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ABSTRACT. Systemic sclerosis (SSc) is a connective tissue disorder characterized by vascular alterations and deregulated fibroblast activation leading to fibrosis of the skin and internal organs. SSc is thought to be an autoimmune disease, owning the presence of auto-antibodies. Genetic studies lend support to the critical role exerted by the immune response in the physiopathology of the disease, since several of the SSc-associated polymorphisms have been found in genes involved in the immune response. Oligoclonal T cells, preferentially producing type 2 cytokines, are present in affected tissues and peripheral blood early in the disease course, and their soluble mediators favor the production of pro-fibrotic and pro-angiogenic factors by fibroblasts, most likely participating in the establishment of fibrosis. More recently, we and others have reported an increased expression of additional T cell subsets, including Th17 cells, and their hallmark cytokines in the peripheral blood, serum and skin of SSc individuals. Here, we will review recent data on the presence of various T helper cells in SSc, and discuss the potential role of Th17 cells in promoting inflammatory responses while keeping fibrosis in check. An understanding of the immune abnormalities characteristic of SSc and their significance, represents a critical step towards the identification of novel therapies that could modify the course of the disease.

Key words: systemic sclerosis, T cells, Th17 cells, IL-17A, fibrosis

Systemic sclerosis (SSc, scleroderma) is a systemic, autoimmune disorder characterized by inflammation and vascular abnormalities resulting in progressive fibrosis of the skin and internal organs. The prevalence of SSc is estimated at 276/million in the USA with an incidence of 19.3 new cases/million adults/year, and with a mean age at diagnosis in the fifth decade. Susceptibility to the disease differs significantly according to sex, age, race and geographical factors. SSc occurs more frequently in African-Americans than Caucasians, and demonstrates a remarkable female predominance with a female-to-male incidence ranging from 3 to 14:1 [1, 2]. SSc is a clinically heterogeneous disease, and the overall rate of progression may vary from a relatively benign to a rapidly progressive form leading to extremely reduced life expectancy. Two major clinical subsets of SSc, namely limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) forms, have been described according to the extent of fibrosis and autoantibody profile [3]. The lcSSc form is dominated by vascular manifestations with milder skin and internal organ fibrosis, generally accompanied by a low progression rate. In contrast, dcSSc patients present a rapidly progressive fibrosis of the skin and internal organs, leading to a high mortality risk. The cumulative survival rate for SSc has improved during the last 30 years, with the diffuse form of the disease having a worse prognosis than the limited form [4]. An increased frequency of malignancy in SSc patients has been recently reported [5]. No curative treatment exists for SSc, and current therapies are tailored to treat clinical manifestations and SSc-related pathology.

The etiology of SSc is unknown, although environmental factors acting on a predisposing genetic background, resulting in abnormal immune-inflammatory responses are thought to play a role. SSc occurs more frequently in families (1.6%) than in the general population (0.026%), nevertheless it is not inherited in a Mendelian manner, since both monozygotic and dizygotic twins show a relatively low disease concordance rate [6]. It is currently believed that SSc is a complex, polygenic disease and the identification of the genetic determinants represents an area of active research. Viruses, including but not exclusively human cytomegalovirus (CMV), along with drugs and environmental and occupational exposure to silica, organic solvents and vinyl chloride have been identified as possible causative agents [7]. CMV-specific antibodies have been detected in the blood of SSc patients, although no viral mRNA was found in affected tissues. In addition, antibody cross-reactivity between the human CMV late protein UL94 and NAG2, an adhesion molecule expressed on endothelial cells and fibroblasts, has been identified in SSc serum. Binding of NAG2 by these antibodies induces endothelial cell apoptosis and increased collagen synthesis in fibroblasts, effects that represent important pathogenic events [8].

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Vascular damage occurs early in the course of SSc due to an abnormal vasoreactivity accompanied by structural fibroplastic angiopathy, finally leading to severe tissue hypoxia. The resulting decreased capillary blood flow, intimal proliferation and adventitial fibrosis manifest as clinical symptoms such as Raynaud's phenomenon, digital ulcers, arterial pulmonary hypertension and hypertensive renal crisis. Vascular remodeling is preceded by activation of endothelial cells (EC), which express adhesion molecules promoting the formation of a perivascular inflammatory infiltrate, ultimately triggering EC injury and apoptosis [9]. Activated EC also release endothelin-1 (ET-1), a potent vasoconstrictor promoting leukocyte adhesion, vascular smooth muscle cells proliferation and fibroblast activation. Levels of ET-1 were shown to be elevated in the blood and bronchoalveolar lavage (BAL) fluid from SSc patients. Despite severe tissue hypoxia, angiogenesis in SSc patients is impaired while, high levels of angiogenic factors, such as vascular endothelial growth factor (VEGF), have been identified in both serum and tissues. VEGF induction in SSc appears to be mediated by cytokines present in the microenvironment, such interleukin (IL)-1, platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β, rather than by hypoxia [10]. Recent studies indicate that the defects in vasculogenesis may also be associated with a reduction in bone marrow-derived CD34+ circulating endothelial progenitor cells, as well as their impaired differentiation into mature EC [11].

Beside vascular damage, tissue fibrosis is another relevant characteristic of the disease. Fibrosis results from an overproduction of extracellular matrix (ECM) components, especially collagen, by deregulated fibroblasts and activated mesenchymal cells, accompanied by impaired ECM degradation and turnover. ECM also functions as a reservoir for TGF-β, connective tissue growth factor (CTGF), and other growth factors that participate in controlling mesenchymal cell differentiation, function and survival. Progressive replacement of tissue architecture by collagen-rich ECM results in the functional impairment of affected organs and ultimately, pathology. Soluble mediators produced by platelets, EC, epithelial cells, and inflammatory cells in the local microenvironment control fibroblast activation and differentiation [12]. In SSc, fibroblasts display an inappropriate, activated myofibroblast phenotype characterized by, among others, increased collagen, TGF-β, CTGF, IL-6, ET-1, monocyte chemotactic protein-1 (MCP-1) production, resistance to IFN-γ and T cell contact-mediated inhibitory signals, as well as increased proliferation and decreased apoptosis [12, 13].

EVIDENCE FOR IMMUNE ALTERATIONS IN SSC

Altered innate and acquired immune responses are frequently observed in SSc and several pieces of evidence indicate that immune cells may be among the initiators of the complex molecular and biochemical process culminating in fibrosis, although the factors driving their activation remain unknown. Direct evidence for an immune involvement in SSc pathogenesis was provided more than three decades ago when histological examination of early SSc lesions revealed that an inflammatory infiltrate, mainly composed of monocytes/macrophages and activated αβ and γδ T lymphocytes, precedes the development of vasculopathy and fibrosis [14-17]. Of interest, fibroblasts with markedly increased expression of type I and III procollagen mRNA are frequently detected near the mononuclear cell infiltrate [18, 19]. The majority of infiltrating T cells express activation markers, such as HLA-DR and CD49d [15, 20], and products of activated T cells, such as soluble IL-2R, are found in suction skin blister fluid from SSc patients [21]. The skin infiltrate is more prominent early during the edematous, inflammatory phase of the disease, and decreases with transition to the sclerotic phase. CD4+ T cells dominate over CD8+ T cells in the skin infiltrate and peripheral blood of SSc patients, while the CD4:CD8 ratio is reduced in BAL fluids [22]. Consistently, most T cell clones obtained from lesional skin of SSc individuals express the CD4 co-receptor [23]. More recently, we identified CD4+CD8+ double positive T cells in the skin of SSc individuals [24]. Analysis of TCR usage in skin infiltrating T lymphocytes revealed oligoclonality, thus suggesting that T cells had undergone proliferation *in situ* in the skin and clonal expansion in response to, as yet, unidentified, specific (auto-) antigens [25]. Of interest, T-cell clones exhibiting the Y chromosome have been generated from the peripheral blood and/or skin of SSc women who had previously delivered male infants. These male-offspring T cells were alloreactive with maternal MHC antigens, supporting the possibility that a chronic graft-versus-host reaction, attributable to long-term microchimerism, plays a pathogenic role in SSc [26].

Activated T cells may trigger activation of adjacent fibroblasts by direct contact and by paracrine action of secreted cytokines and chemokines. The effects induced by the different T cell subsets on fibroblasts are summarized in *figure* 1 and discussed in detail in the next sections. In addition, autoreactive T cells may interact with B cells and drive the production of autoantibodies, whose presence in the serum represents a common feature of the disease. Up to 95% of SSc patients present anti-nuclear antibodies (ANA), the most frequent being anti-topoisomerase-I (ATA), anti-centromere (ACA) and anti-RNA polymerase III antibodies (ARA). Of interest, a contact-mediated and HLA-DR-restricted collaboration between topoisomerase I-specific T and B cells has been shown to be essential for *in vitro* production of ATA in SSc patients [27]. The specific pattern of ANA is important for diagnosis, and provides clues for clinical patterns and prognosis while as yet there is no evidence indicating that such antibodies may directly contribute to tissue fibrosis [28]. Additional auto-antibodies, not directed against ubiquitous auto-antigens, have been identified in SSc and appear to have direct roles in ECM turnover. For instance, anti-MMP-1 and MMP-3 antibodies have been shown to inhibit the enzymatic activity of these enzymes, whereas antibodies against fibrillin-1 and PDGF-R activated fibroblasts and induced collagen synthesis [29]. Along the same line, we have identified the presence of antibodies directed against the surface of fibroblasts (AFA) in the serum of SSc individuals. AFA+ IgG promoted a pro-inflammatory and pro-adhesive fibroblast phenotype, at least partially, via toll-like receptor (TLR)4 binding [30-32], suggesting an involvement of receptors belonging to the innate immunity in the pathogenesis of SSc. Of note, TLR4 was found constitutively expressed, and

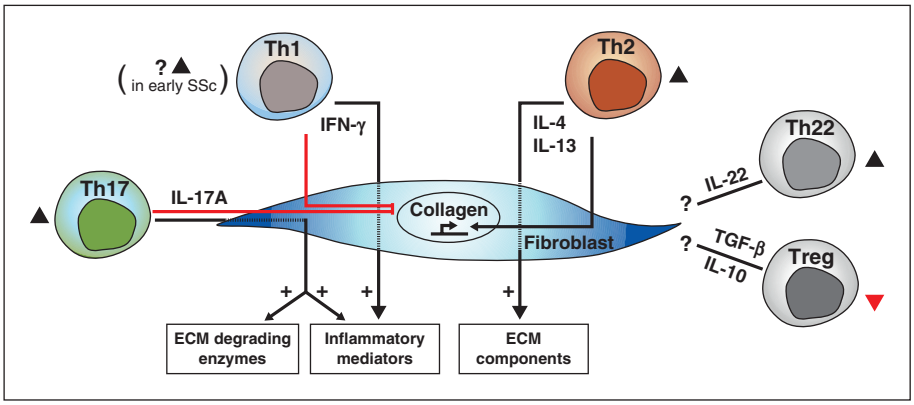

Figure 1

Effects of CD4+ T cells on fibroblasts in SSc. Increased or decreased numbers of T cell subsets in SSc are indicated by black or red arrowheads, respectively. Black lines with arrowhead indicate positive effects; red lines with blunted end indicate negative effects; Th2 refers to CD4+ as well as CD8+ T cells producing IL-4 and/or IL-13; ?:unknown effect.

accumulation of endogenous TLR4 ligands significantly elevated, in SSc lesional skin and lung [33]. Moreover, TLR4-stimulated monocyte-derived dendritic cells (moDC) have been shown to have an increased production of the profibrotic chemokine CCL18, in an IL-10-dependent manner [34]. Additional studies have found that anti-topoisomerase I-containing serum induces a significantly higher level of interferon (IFN)α as compared to controls. In this context, IFNα was produced by plasmactoid DCs, and required uptake of immune complexes through FcγRII and the presence of RNA, presumably upon interaction with TLR7. Higher IFNα induction was observed in serum from patients with dcSSc than in that from lcSSc patients, and an IFNα signature was significantly associated with lung fibrosis and digital ulcers [35, 36]. Interestingly, peripheral blood cells from SSc and systemic lupus erythematosus (SLE) patients share a similar IFN-inducible gene expression pattern. In particular, a subset of SSc patients shows a "lupus-like", high IFN-inducible phenotype that correlates with the presence of anti-topisomerase-I and anti-U1 RNA antibodies [37]. Furthermore, IFNα2 has been reported to induce TLR3 in dermal fibroblasts, and stimulation with poli I:C resulted in increased production of the profibrotic cytokine IL-6 [38]. In addition, TLR3 appears to be increased in the skin of SSc individuals. Confirming the importance of TLR signaling in SSc, transcutaneous administration to mice of several TLR ligands, especially of TLR3, induced a marked progression of fibrosis [39]. Finally, TLR-ligands have been recently shown to be crucial in the priming of inflammasome in antigen-presenting cells (APC), and might participate in IL-1-mediated fibrosis [40].

The importance of the immune response in the pathogenesis of SSc is underlined by the fact that all SSc susceptibility genes that have been replicated thus far are found among genes coding for proteins important for innate and adaptive responses. As for many autoimmune diseases, SSc has been associated with HLA alleles, mostly belonging to the HLA class II genes. Nevertheless, these associations have been reported to occur within autoantibody-positive subgroups rather than the overall disease. Apart from HLA, other susceptibility genes identified include *TBX21*, *STAT4*, *IRF5*, *IL-23R*, *C8orf12-BLK*, *BANK1*, *TNFSF4*, *IL10R2*, *CD266* and the T cell receptor zeta-chain CD247 (reviewed in [41]). *STAT4* and *IRF5* polymorphisms had a synergistic effect in increasing susceptibility to SSc [42] and, interestingly, are both involved in type I interferon responses, a cytokine that characterizes at least a subset of SSc individuals [35, 36].

Immunosuppressive agents have long been used in the clinical management of SSc patients with a marginal advantage in the control of fibrosis. However, pilot studies have demonstrated a major improvement in skin and overall function with high dose immunosuppressive therapy and autologous hematopoietic cell transplantation (HSCT) in patients with dcSSc, providing grounds for the seminal role exerted by the immune system in the pathogenesis of SSc [43]. To date, autologous HSCT appears to be the most effective therapy for reversing skin fibrosis in SSc patients. Three randomized clinical trials of autologous HSCT, namely the American scleroderma stem cell versus immune suppression trial (ASSIST, [44]), the autologous stem cell transplantation international scleroderma (ASTIS) trial, and the scleroderma cyclophosphamide or transplantation (SCOT) trial, have been undertaken to establish better the effect of HSCT on scleroderma. Results from the ASSIST confirmed the highly beneficial effects of this approach on the disease course [44].

Thus, SSc appears to be a disease of autoimmune origin where a tight interplay between innate and adaptive immune responses takes place and contributes to initiate and/or favor the pathological process leading to fibrosis. The causative events triggering the altered immune reaction in SSc remain however, unknown.

THE TH1/TH2 PARADIGM IN SSC

T cells infiltrating the skin and organs of SSc patients are functionally heterogeneous. Evidence points to a predominant, type 2 cytokine profile for the disease, characterized by an increased frequency of αβ CD4 T cells producing high levels of IL-4, IL-5 and IL-13 in skin, lung and peripheral blood [26, 45-50]. Similarly, CD4+CD8+ double positive T cells and CD8+ T cells found in the skin of SSc patients, were capable of producing high levels of IL-4 [24]. In addition, male-offspring T cells present in the blood and skin of women who had previously delivered male infants exhibited a Th2-oriented profile [26]. More recently, CD8+ T cells with increased IL-13 production

have been identified in the peripheral blood of SSc individuals, especially in the dcSSc form, and were shown to correlate with the extent of skin fibrosis [51]. A significant association between IL-13 receptor IL13Rα2 gene polymorphisms and SSc has been found, underling a possible role of IL-13 in SSc pathogenesis [52]. In the CD8+IL13+ T cells from SSc individuals, IL-13 production could be specifically reduced by silencing of GATA-3. Of interest, these cells expressed high levels of both T-bet and GATA-3, suggesting that T-bet was, to some extent, unable to exert its normal regulatory, down-modulating activity on GATA-3 [53]. Intriguingly, polymorphisms of *TBX21*, the gene coding for T-bet, are associated with increased risk of SSc [54]. Increased levels of type 2 cytokines, including IL-4 and IL-13, have been found in the serum, skin and lung of SSc patients [55-61]. Despite these findings, some results support Th1 activation [23, 62, 63], increased CD8+IFN-γ+ cells [64], or a mixed type 1 and type 2 cytokine phenotype in SSc [65]. Although controversy persists, Th1 responses appear to be greater in the early inflammatory stages of the disease, while being decreased in late stages.

In vitro, IL-4 strongly stimulates collagen synthesis by both normal and SSc fibroblasts, whereas IFN-γ has inverse effects [66-68]. This notwithstanding, IFN-γ completely suppresses the stimulation induced by IL-4 when fibroblasts are simultaneously incubated with the two cytokines [68]. In addition, transcriptome analysis in animal models has shown that genes involved in wound healing and fibrosis are associated with Th2-polarized responses [69, 70], IL-13 being crucial in inducing fibrosis in the bleomycin model [71]. Cell membranes of activated Th1 and Th2 cell clones inhibited collagen I production by normal fibroblasts via a cell-cell, contact-dependent mechanism mediated by IFN-γ and membrane-bound TNFα, respectively [72]. Of note, SSc fibroblasts appeared to be resistant to Th2-mediated, cell-contact inhibition, and membranes from Th1 cells were more potent inhibitors of collagen synthesis than those of Th2 cells [72, 73]. In addition, Th1 cells preferentially stimulated fibroblasts to produce interferon-induced protein-10 (IP-10), which possesses anti-angiogenic and anti-fibrotic properties, whereas Th2 induced IL-8, which rather plays a role in promoting angiogenesis and fibrosis [74]. On the other hand, both T cell subsets promote the production of monocyte chemotactic protein-1 (MCP-1), a CC chemokine greatly increased in SSc [75]. Of interest, MCP-1 may polarize T cells towards Th2 subsets in the mouse, and both MCP-1-/- and CCR2 (the receptor for MCP-1) -/- mice were shown to be resistant to bleomycin-induced dermal fibrosis [76].

Both *in vivo* and *in vitro* studies suggest that Th1 responses may be crucial in mediating early inflammatory processes of the disease, while Th2 responses actively promote fibrotic responses. These latter may indeed activate fibroblasts, promote their differentiation into myofibroblasts and directly stimulate ECM production and deposition.

TH17 AND IL-17A IN SSC PATHOGENESIS

The presence and role of Th17 cells in SSc have attracted great interest in the last few years and they remain an area of intense investigation. Th17 cells, firstly described in 2005, produce IL-17A and IL-17F in conjunction, or not, with IL-22, and are implicated in mounting protective responses against extracellular bacteria and fungi [77]. In addition, Th17 cells and IL-17 cytokines are implicated in the pathogenesis of several inflammatory and autoimmune diseases, including collagen-induced arthritis and experimental autoimmune encephalomyelitis [78]. Studies in mice showed that Th17 cells require retinoid-related orphan receptor-γ to differentiate in response to a combination of TGF-β, IL-1, IL-6 and IL-21. The role of TGF-β in Th17 cell polarization in humans is somehow more controversial, with some data arguing for its essential function in full polarization [79-81] and others indicating that TGF-β may be dispensable [82, 83]. IL-23 is essential for pathogenic Th17 cell expansion in both humans and mice [77]. Th17 cells are enriched in a subset of cells expressing CC chemokine receptor (CCR)6 in the absence of CXC chemokine receptor (CXCR)3 [84, 85], and have been shown to be enriched in CD161+ cells in humans, a molecule which is present on virtually all Th17 cells [86]. IL-17A and IL-17F are secreted as disulfide-linked homo- or hetero-dimers, and both signal through a heterodimeric receptor complex consisting of IL-17RA and IL-17RC chains. In the context of SSc, it is worth noting that both fibroblast and endothelial cells express functional IL-17 receptors [87].

We and others have reported that SSc individuals have a higher frequency of Th17 cells in their peripheral blood than healthy controls [50, 57, 63, 88, 89]. The percentage of CD161+CD4+ T cells was consistently increased in SSc, and correlated with the percentage of IL-17A-producing cells [50]. The Th17 profile was mostly attributable to CCR6-expressing CD4+ T cells [88], with IL-17 levels correlating with CCR6 expression in SSc, but not healthy controls [50], suggesting enhanced skin- and lung-homing properties [90]. In addition, increased levels of IL-17A have been detected in the serum of SSc patients [91, 92], although some inconsistencies exist in the literature [93, 94], and increased IL-17A mRNA levels were identified in involved skin and lung [91, 95]. Similarly, we and others observed an increased IL-17A positivity in skin biopsies from SSc patients on immunohistochemical analysis [96, 150]. IL-17F, which shares an amino acid sequence identity of more than 50% with IL-17A, was not found to be increased in the involved skin and serum of SSc individuals [96]. Of interest, we also observed IL-17A-positive cells in normal skin, which raises the question of its role in physiological conditions, possibly in relation to host defense and skin homeostasis. Beside Th17 cells, we found that tryptase-positive mast cells stain positive for IL-17A in the dermis of SSc. IL-17A+ mast cells have been described in psoriatic skin lesion [97], and their numbers are increased in the inflamed joint of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and osteoarthritis patients [98, 99]. Remarkably, in contrast to SSc, these conditions are characterized by tissue destruction rather than fibrosis.

As mentioned above, evidence suggests that TGF-β, IL-1 and IL-6 participate in Th17 cell priming. These cytokines, which promote pro-fibrotic responses in fibroblasts [59, 100], are increased in tissues and serum of SSc patients [55-58, 60]. It is important to note that commitment to Th17 cells, which are crucial in mounting inflammatory responses, is reciprocally linked with that of T regulatory cells (Treg), thought to be involved in dampening

immune reactions and inducing peripheral tolerance. Indeed, TGF-β appears to be involved in the generation of both subsets, whereas the presence or absence of IL-1 and IL-6 skews the differentiation process towards inflammatory Th17 or suppressive Treg cells, respectively. While Th17 cells have been mostly found increased in the disease, Treg cells have been reported to be reduced in number or functionally defective in several studies [88, 89, 101-104]. The fact that TGF-β overexpression is accompanied by increased IL-1 and IL-6 levels in SSc individuals, could be one of the reasons explaining the altered Th17/Treg cell ratio in the disease. Of interest, Il-1β, in combination with IL-2, was reported to convert memory Treg cells into IL-17-producing cells [105], and TLR-stimulated monocytes producing IL-1β can trigger Th17 differentiation [82]. In line with the hypothesis that the cytokine environment may favor Th17 differentiation, increased levels of IL-23, required for Th17 cell expansion, have been reported in SSc patients [106]. Interestingly, polymorphisms of the *IL23R* gene are associated with the diffuse form of SSc and ATA positivity and exert a protective effect for the development of pulmonary hypertension [107]. How the polymorphisms in the *IL23R* gene alter IL23R signaling and Th17 development has not been address so far.

Efforts have been made to understand the functional significance of the Th17 cell imbalance in SSc. Studies in rodents indicate that IL-17 acts in a pro-fibrotic manner, while data for humans suggest that Th17 cells and IL-17A may play a role in controlling rather than promoting fibrosis. The distinct roles exerted by these cells and their hallmark cytokines in the development of fibrosis in humans and mice are summarized in *table I*. In animal models, IL-17A was shown to be involved in bleomycin-induced lung and skin fibrosis [108-111], and IL-17A deficiency attenuated skin thickness in tight skin-1 (TSK-1/$^{+}$) mice [110]. In addition, neutralization of IL-17A inhibited silica-induced chronic inflammation and pulmonary fibrosis [108], and Th17-polarized responses mediated lung fibrosis in a model of chronic hypersensitivity [112]. Consistently, IL-17 increased TGF-β, CTGF, and collagen production by mouse skin fibroblasts [110], and promoted collagen production and epithelial-mesenchymal transition in mouse alveolar epithelial cells in a TGF-β-dependent manner [108]. In humans, IL-17 has been shown to enhance proliferation, IL-6, IL-8 production and ICAM-1 expression by fibroblasts [113, 114], and to promote IL-1 and IL-6 production by EC [91]. Thus, it is likely that IL-17 may establish a positive loop that promotes a cytokine environment favorable to Th17 commitment via fibroblast and endothelial cell activation. In a recent report, Nakashima and coworkers reported that IL-17A possesses direct anti-fibrogenic effects in normal

Table 1
IL-17 induces distinctly different fibrotic responses in mice and humans.

Species	Effects on fibrosis	Ref
Mice	***In vivo***	
	- reduced bleomycin-induced skin fibrosis in IL-17A$^{-/-}$ mice	[110]
	- reduced skin thickness in IL-17A$^{-/-}$ tight skin-1 mice	[110]
	- reduced lung inflammation and fibrosis in a model of hypersensitivity pneumonitis in IL-17A$^{-/-}$ mice	[112]
	- reduced bleomycin- and IL-1β-induced inflammation and pulmonary fibrosis in IL-17A$^{-/-}$ mice	[108, 109, 111]
	- IL-17A neutralization inhibited silica-induced chronic inflammation and pulmonary fibrosis	[108]
	- Intratracheal delivery of IL-17A induced collagen release in the BAL and deposition in the lung	[111]
	In vitro	
	- IL-17A increased collagen, TGF-β and CTGF production by skin fibroblasts	[110]
	- IL-17A increased collagen production by mouse type II alveolar epithelial cells (MLE-12) in a TGF-β-dependent manner	[108]
Humans	***In vivo***	
	- the number of IL-17+ cells in the skin of SSc patients correlates inversely with skin fibrosis	[150]
	In vitro	
	- IL-17 enhances the production of pro-inflammatory cytokines (MCP-1, IL-6, IL-8) by synoviocytes and fibroblasts from the lung and the skin	[113, 114, 151]
	- IL-17 enhances GM-CSF and PGE_2 production by synovial fibroblasts	[113]
	- IL-17 increases the expression of ICAM-1 by endothelial cells and foreskin fibroblasts	[91, 114]
	- IL-17 induces IL-1 and IL-6 production, and VCAM-1 expression by endothelial cells	[91, 113]
	- IL-17 enhances fibroblast proliferation	[91]
	- IL-17A enhances the production of ECM degrading enzyme (MMP-1, MMP-3) by synoviocytes, cardiac and skin fibroblasts	[120, 121]
	- IL-17A does not stimulate type I and type III collagen production	[91, 151]
	- IL-17A reduces type I collagen and CTGF production via upregulation of miR-129-5p in dermal fibroblasts	[96]
	- IL-17A inhibits αSMA induction by TGF-β	[150]
	- Th17 cell clone supernatants induce pro-inflammatory cytokines and ECM degrading enzymes by dermal fibroblasts	[151]
	- Th17 cell clone supernatants strongly inhibit type I collagen production by dermal fibroblasts	[151]

BAL bronchoalveolar; IL: interleukin; TGF: transforming growth factor; CTGF: connective tissue growth factor; MCP: monocyte chemoattractant protein; GM-CFS: granulocyte macrophage colony stimulating factor; ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; ECM: extra-cellular matrix; MMP: matrix metalloproteinase; αSMA: α-smooth muscle actin

human fibroblasts via upregulation of miR-129-5p and downregulation of connective tissue growth factor and type I collagen. According to these authors, SSc fibroblasts may escape the negative control of IL-17A because of a reduced expression of the IL-17RA subunit [91]. Similarly, IL-17A was shown to stimulate proliferation, but failed to increase type I and type III procollagen mRNA expression in fibroblasts from healthy controls and SSc patients [91]. In our experimental settings, IL-17A did not favor type I collagen production at either mRNA or protein levels in control and SSc fibroblasts and, importantly, did not modified type I collagen production induced by TGF-β. In addition, IL-17A significantly reduced the increase in α-smooth muscle actin (αSMA) induced by TGF-β, thus controlling and limiting the differentiation of fibroblasts into pro-fibrotic myofibroblasts [150, 151]. Of note, immunofluorescence analysis of SSc skin sections revealed the proximity of IL-17A-, but not IL-4-, producing cells to αSMA+ myofibroblasts, suggesting an interplay between these two cell types *in vivo*. Of interest, Th17 cell clone supernatants strongly inhibited type I collagen synthesis by dermal fibroblast from SSc and control individuals in an IL-17A- and TNF- dependent manner. Consistent with the work by Nakashima, SSc fibroblasts were more resistant to inhibition when cultured in the presence of Th17 cell supernatants, where IL-17A and TNF were simultaneously neutralized. While participating in limiting collagen synthesis and myofibroblast differentiation, Th17 cells and IL-17A actively promoted an inflammatory microenvironment, favoring MCP-1 and IL-8 production by fibroblasts. These cytokines have been reported to be increased in the skin and serum of SSc patients [115, 116] and appeared to be critical in mediating lung and dermal fibrosis in bleomycin-treated mice [76, 117]. However, whether these mediators have direct pro-fibrotic actions on fibroblasts in humans remains controversial [115, 118, 119]. The role of these mediators in tissue fibrosis observed in mice may indeed be related more to chemoattractant and angiogenetic properties than to a direct pro-fibrotic activity on fibroblasts. On the other hand, Th17 and IL-17A have been shown to promote MMP-1 production in dermal fibroblasts [151], fibroblast-like synoviocytes [120] and cardiac fibroblasts [121]. Despite its role as matrix-degrading enzyme, MMP-1 levels have been paradoxically shown to be increased in human lung fibrosis [122], and variably reported to be increased, unchanged or decreased in SSc [123-126]. Of interest, our data indicate that IL-17A induces the production of pro-inflammatory chemokines preferentially via NF-κB and p38 signaling, while inducing MMP-1 via the JNK pathway. Thus, in contrast to that which is observed in mice, in the human setting Th17 cells appear to limit and counterbalance direct fibrotic stimuli on fibroblasts while participating in the establishment of a pro-inflammatory microenvironment. In support of this model, we have found that the frequency of IL-17+ cells observed in SSc skin correlated inversely with the extent of skin fibrosis as assessed by the modified Rodnan Skin Score (MRSS). A further important observation we recently made indicates that the *in vivo* administration of iloprost, a PGI2 analog used in the clinical management of digital ulcers in SSc with beneficial effects on the disease course, results in increased Th17 cell numbers in the peripheral blood [127]. Thus, the bulk of our observations tip the balance in favor of a role for Th17 in controlling fibrosis in SSc (*figure 2*). The discrepancy between studies in humans and mice remain however, unexplained and may stress species-specific differences in the responses induced by IL-17. Whether Th17 cells and IL-17 might have indirect pro-fibrotic effects via interaction with endothelial/epithelial cells or via the enhanced production of pro-angiogenic factors (IL-8, MCP-1) remains to be investigated. Similarly, the role of Th17 cells in autoantibody generation in SSc has not, as yet, been investigated. This notwithstanding, IL-17 has been shown to promote autoantibody generation in BXD2 mice by orchestrating the spontaneous formation of autoreactive germinal centers [128].

TH22 AND IL-22 IN THE PATHOGENESIS OF SSC

Th22 cells typically produce IL-22 in the absence of IL-17 and IFN-γ, and are enriched in a subset of skin-homing cells expressing CCR4, CCR6, CCR10 in the absence of CXCR3 [129, 130]. The transcription factor(s) governing the commitment towards Th22 cells remain unknown, although roles for the aryl-hydrocarbon receptor (AhR) and STAT3 have been proposed [130-133]. While IL-6 and TNF may be involved in Th22 cell differentiation [129], they did not increase the production of IL-22 by human naïve T cells in an independent study, while being efficiently produced in response to IL-12 and

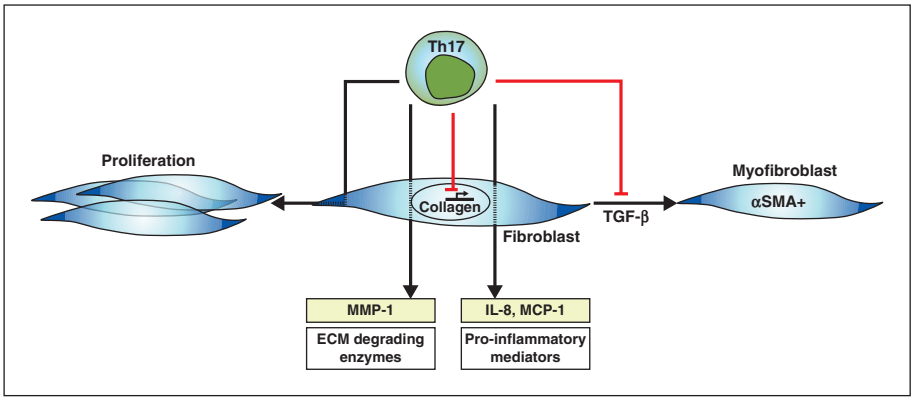

Figure 2
Th17 cell effects on fibroblasts in humans. Black lines with arrowhead indicate positive effects; red lines with blunted end indicate negative effects. αSMA: α-smooth muscle actin.

IL-23 [134]. IL-22 does not participate in the communication between immune cells since cells of hematopoietic origin do not express IL-22R1. It acts mainly on epithelial cells of mucosal origin, and the skin, where it promotes antimicrobial defense, protection against damage, epithelial homeostasis and regeneration [135]. Of interest, IL-22R1 is also expressed on keratinocytes and, to a lesser extent, on some fibroblasts. IL-22 may be involved in the pathogenesis of several immune disorders, including rheumatoid arthritis, SLE and allergic asthma, and Th22 cells have been shown to be enriched in inflammatory skin diseases, including psoriasis and atopic dermatitis [136].

Relatively little is known about Th22 and IL-22 in the field of fibrosis. We found that Th22 cells, defined as CD4+IL-22+IL-17-IFN-γ-IL4- cells, were increased in the peripheral blood of SSc individuals, in addition to Th2 and Th17 cells [50]. Others have confirmed that IL-22 transcripts were significantly more abundant in SSc than control skin, and their levels were similarly increased in clinically affected, transitional and unaffected areas [103]. Of interest, a genetic association between *IL-10R2*, a gene coding for the common receptor for IL-10, IL-22 and IL-26, dcSSc and presence of ATA has been reported [137]. Similar to that which has been observed for IL-17-producing cells in SSc, we found a positive correlation between IL-22-producing cell numbers and CCR6, underlying the enhanced skin- and lung-homing properties of these cells in the disease. Importantly, SSc interstitial lung disease (ILD) was strongly associated with higher numbers of IL-22-producing cells [50]. Lung inflammation was ameliorated in IL-22-deficient mice receiving high doses of bleomycin compared to IL-22-sufficient mice [138], and IL-22 levels were decreased in the BAL fluid from mice protected by pretreatment with collagen type V when challenged with bleomycin [139]. In the other hand, protection mediated by IL-22 produced by γδ T cells has been reported in a mouse model of lung fibrosis induced by hypersensitivity to *Bacillus subtilis* [140]. In humans, subcutaneous intralesional injection of polymerized type I collagen in localized scleroderma patients resulted in restored normal skin architecture and decreased Th22 as well as Th17 and Treg numbers [141]. It is therefore possible that Th22 cells could contribute to tissue fibrosis, especially in the lung, via IL-22 production. This notwithstanding, fibroblasts from control and SSc patients did not up-regulate type I collagen, nor the levels of pro-inflammatory cytokines IL-8 and MCP-1 in response to IL-22 (unpublished observation). Whether IL-22 might have indirect pro-fibrotic roles in scleroderma via epithelial cell activation remains to be established. In this context, human keratinocytes submitted to the influence of Th22 cell clone supernatants showed enhanced transcription of genes involved in tissue repair and fibrosis. Of interest, these supernatants enhanced wound-healing in an *in vitro* injury model, in an IL-22-dependent manner [142].

T REGULATORY CELLS IN SSC

The analysis of Treg cells in SSc has produced conflicting data. Some studies have reported an increase in circulating CD4+CD25+FoxP3+ Treg cells [94, 143], especially in active and severe disease [144]. Despite this increase, Treg cells from SSc patients harbored a defective suppressive capacity, most likely related to skewed X chromosome inactivation [145], which correlated with decreased CD69 expression and TGF-β levels [94]. One report did not find any differences between control and SSc groups [63]. Finally, several studies showed a reduced frequency/impaired function of CD4+ Treg cells in SSc [88, 89, 101, 102]. Similarly, decreased FoxP3+ Treg cells were identified by an immunohistochemical approach in skin from patients with SSc and morphea [101] and decreased FoxP3 transcripts were isolated from SSc skin lesions [103]. Discrepancies between these results most likely reflect technical difficulties in the discrimination between Treg cells, and possible contamination of the population studied by activated CD25+ T cells, which may also include non-Treg cells expressing FoxP3 [104]. In this context, Mathian and coworkers further characterized the Treg cell compartment in SSc by discriminating resting Treg (rTreg), activated Terg (aTreg) and non-regulatory Foxp3+ cells, based on the expression of CD4, CD45RA and FoxP3. They found that the proportion and absolute counts for both aTreg and rTreg were reduced in SSc compared to controls, but not those for non-regulatory FoxP3+ CD4 T cells [103]. Reduced numbers of Treg cells and an increased Th17 cell frequency appear thus to coexist in SSc individuals. An attractive hypothesis, yet to be demonstrated, is that Treg cells may specifically convert into Th17 cells in the context of the disease. Along this line, several studies have reported that conversion of Treg to Th17 cells is possible [146-148]. Of interest, IL-1β and IL-6, which are increased in SSc, have been shown to participate to this conversion [149]. It is also possible that Treg cell-priming in SSc is skewed towards Th17 cells by the cytokine microenvironment typical of the disease, where high levels of TGF-β, IL1β and IL-6 coexist. While TGF-β is a well-documented, pro-fibrotic cytokine, the role of Treg cells in the pathogenesis of SSc remains to be clarified.

CONCLUDING REMARKS

SSc is a devastating systemic, autoimmune disorder associated with a shorten life expectancy and no curative treatment. Evidence gathered during recent years has revealed a crucial role for immune cells in the establishment and maintenance of fibrosis, with Th1 and Th2 cells contributing to the induction of pro-inflammatory and pro-fibrotic responses, respectively. The role of newly identified T helper subsets, including Th17 and Th22 cells is currently being addressed, and undoubtedly will represent an area of active research in future years. In humans, but not in mice, Th17 cells may counterbalance fibrosis via induction of ECM-degrading enzymes, inhibition of collagen synthesis and myofibroblast differentiation, while favoring a pro-inflammatory environment (*figure 2*). Evidence indicating that Th17 cells and IL-17A may exert opposing roles in humans and mice with respect to fibrosis underlines important species-specific differences that have to be taken into account when designing further research and clinical trials. Indeed, there is an urgent need for new approaches to counterbalance and reduce fibrosis, ideally with a high degree of specificity. Understanding the role exerted by T cells and their soluble mediators in the establishment and maintenance of fibrosis will provide novel

molecular and cellular targets for therapeutic intervention. Future areas of investigation should focus on the role of IL-17 family members beyond IL-17A, as well as the role of Th22 and Treg cells. Additional efforts should be devoted to understanding the functional consequences of gene polymorphisms associated with the disease. Investigations on the interplay between T cells and neighboring cells, taking into account the three-dimensional tissue architecture, will better characterize the functional activity of mediators involved in ECM deposition.

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REFERENCES

1. Chifflot H, Fautrel B, Sordet C, Chatelus E, Sibilia J. Incidence and prevalence of systemic sclerosis: a systematic literature review. *Semin Arthritis Rheum* 2008; 37: 223-35.
2. Mayes MD, Lacey JV, Jr, Beebe-Dimmer J, *et al*. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. *Arthritis Rheum* 2003; 48: 2246-55.
3. LeRoy EC, Black C, Fleischmajer R, *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
4. Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007; 66: 940-4.
5. Szekanecz E, Szamosi S, Horvath A, *et al*. Malignancies associated with systemic sclerosis. *Autoimmun Rev* 2012 Mar 5.
6. Arnett FC, Cho M, Chatterjee S, Aguilar MB, Reveille JD, Mayes MD. Familial occurrence frequencies and relative risks for systemic sclerosis (scleroderma) in three United States cohorts. *Arthritis Rheum* 2001; 44: 1359-62.
7. Mora GF. Systemic sclerosis: environmental factors. *J Rheumatol* 2009; 36: 2383-96.
8. Lunardi C, Dolcino M, Peterlana D, *et al*. Antibodies against human cytomegalovirus in the pathogenesis of systemic sclerosis: a gene array approach. *PLoS Med* 2006; 3: e2.
9. Muller-Ladner U, Distler O, Ibba-Manneschi L, Neumann E, Gay S. Mechanisms of vascular damage in systemic sclerosis. *Autoimmunity* 2009; 42: 587-95.
10. Liakouli V, Cipriani P, Marrelli A, Alvaro S, Ruscitti P, Giacomelli R. Angiogenic cytokines and growth factors in systemic sclerosis. *Autoimmun Rev* 2011; 10: 590-4.
11. Del Papa N, Quirici N, Soligo D, *et al*. Bone marrow endothelial progenitors are defective in systemic sclerosis. *Arthritis Rheum* 2006; 54: 2605-15.
12. Chizzolini C, Brembilla NC, Montanari E, Truchetet ME. Fibrosis and immune dysregulation in systemic sclerosis. *Autoimmun Rev* 2011; 10: 276-81.
13. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest* 2007; 117: 557-67.
14. Fleischmajer R, Perlish JS, Reeves JR. Cellular infiltrates in scleroderma skin. *Arthritis Rheum* 1977; 20: 975-84.
15. Giacomelli R, Matucci-Cerinic M, Cipriani P, *et al*. Circulating Vdelta1+ T cells are activated and accumulate in the skin of systemic sclerosis patients. *Arthritis Rheum* 1998; 41: 327-34.
16. Prescott RJ, Freemont AJ, Jones CJ, Hoyland J, Fielding P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. *J Pathol* 1992; 166: 255-63.
17. Roumm AD, Whiteside TL, Medsger TA, Jr, Rodnan GP. Lymphocytes in the skin of patients with progressive systemic sclerosis. Quantification, subtyping, and clinical correlations. *Arthritis Rheum* 1984; 27: 645-53.
18. Kahari VM, Sandberg M, Kalimo H, Vuorio T, Vuorio E. Identification of fibroblasts responsible for increased collagen production in localized scleroderma by in situ hybridization. *J Invest Dermatol* 1988; 90: 664-70.
19. Scharffetter K, Lankat-Buttgereit B, Krieg T. Localization of collagen mRNA in normal and scleroderma skin by in-situ hybridization. *Eur J Clin Invest* 1988; 18: 9-17.
20. 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.
21. Sondergaard K, Stengaard-Pedersen K, Zachariae H, Heickendorff L, Deleuran M, Deleuran B. Soluble intercellular adhesion molecule-1 (sICAM-1) and soluble interleukin-2 receptors (sIL-2R) in scleroderma skin. *Br J Rheumatol* 1998; 37: 304-10.
22. Hussein MR, Hassan HI, Hofny ER, *et al*. Alterations of mononuclear inflammatory cells, CD4/CD8+ T cells, interleukin 1beta, and tumour necrosis factor alpha in the bronchoalveolar lavage fluid, peripheral blood, and skin of patients with systemic sclerosis. *J Clin Pathol* 2005; 58: 178-84.
23. Ferrarini M, Steen V, Medsger TA, Jr, Whiteside TL. Functional phenotypic analysis of T lymphocytes cloned from the skin of patients with systemic sclerosis. *Clin Exp Immunol* 1990; 79: 346-52.
24. Parel Y, Aurrand-Lions M, Scheja A, Dayer JM, 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.
25. Sakkas LI, Xu B, Artlett CM, Lu S, Jimenez SA, Platsoucas CD. Oligoclonal T cell expansion in the skin of patients with systemic sclerosis. *J Immunol* 2002; 168: 3649-59.
26. Scaletti C, Vultaggio A, Bonifacio S, *et al*. Th2-oriented profile of male offspring T cells present in women with systemic sclerosis and reactive with maternal major histocompatibility complex antigens. *Arthritis Rheum* 2002; 46: 445-50.
27. Kuwana M, Medsger TA, Jr, Wright TM. T and B cell collaboration is essential for the autoantibody response to DNA topoisomerase I in systemic sclerosis. *J Immunol* 1995; 155: 2703-14.
28. Hamaguchi Y. Autoantibody profiles in systemic sclerosis: predictive value for clinical evaluation and prognosis. *J Dermatol* 2010; 37: 42-53.
29. Arnett FC. Is scleroderma an autoantibody mediated disease? *Curr Opin Rheumatol* 2006; 18: 579-81.
30. Chizzolini C, Raschi E, Rezzonico R, *et al*. Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. *Arthritis Rheum* 2002; 46: 1602-13.
31. Fineschi S, Cozzi F, Burger D, Dayer JM, Meroni PL, Chizzolini C. Anti-fibroblast antibodies detected by cell-based ELISA in systemic sclerosis enhance the collagenolytic activity and matrix metalloproteinase-1 production in dermal fibroblasts. Rheumatology (Oxford)2007 ;46:1779-85.
32. Fineschi S, Goffin L, Rezzonico R, *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.
33. Bhattacharyya S, Kelley K, Melichian DS, *et al*. Toll-like Receptor 4 Signaling Augments Transforming Growth Factor-β Responses:

A Novel Mechanism for Maintaining and Amplifying Fibrosis in Scleroderma. *Am J Pathol* 2013; 182: 192-205. Epub 2012 Nov 7

34. van Lieshout AW, Vonk MC, Bredie SJ, *et al*. Enhanced interleukin-10 production by dendritic cells upon stimulation with Toll-like receptor 4 agonists in systemic sclerosis that is possibly implicated in CCL18 secretion. *Scand J Rheumatol* 2009; 38: 282-90.

35. Eloranta ML, Franck-Larsson K, Lovgren T, *et al*. Type I interferon system activation and association with disease manifestations in systemic sclerosis. *Ann Rheum Dis* 2010; 69: 1396-402.

36. Kim D, Peck A, Santer D, *et al*. Induction of interferon-alpha by scleroderma sera containing autoantibodies to topoisomerase I: association of higher interferon-alpha activity with lung fibrosis. *Arthritis Rheum* 2008; 58: 2163-73.

37. Assassi S, Mayes MD, Arnett FC, *et al*. Systemic sclerosis and lupus: points in an interferon-mediated continuum. *Arthritis Rheum* 2010; 62: 589-98.

38. Agarwal SK, Wu M, Livingston CK, *et al*. Toll-like receptor 3 upregulation by type I interferon in healthy and scleroderma dermal fibroblasts. *Arthritis Res Ther* 2011; 13: R3.

39. Farina GA, York MR, Di Marzio M, *et al*. Poly(I:C) drives type I IFN- and TGFbeta-mediated inflammation and dermal fibrosis simulating altered gene expression in systemic sclerosis. *J Invest Dermatol* 2010; 130: 2583-93.

40. Netea MG, Nold-Petry CA, Nold MF, *et al*. Differential requirement for the activation of the inflammasome for processing and release of IL-1beta in monocytes and macrophages. *Blood* 2009; 113: 2324-35.

41. Romano E, Manetti M, Guiducci S, Ceccarelli C, Allanore Y, Matucci-Cerinic M. The genetics of systemic sclerosis: an update. Clin Exp Rheumatol 2011 Mar-;29(2 Suppl 65):S75-86.

42. Dieude P, Guedj M, Wipff J, *et al*. Association between the IRF5 rs 2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. *Arthritis Rheum* 2009; 60: 225-33.

43. Nash RA, McSweeney PA, Crofford LJ, *et al*. High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for severe systemic sclerosis: long-term follow-up of the US multicenter pilot study. *Blood* 2007; 110: 1388-96.

44. Burt RK, Shah SJ, Dill K, *et al*. Autologous non-myeloablative haemopoietic stem-cell transplantation compared with pulse cyclophosphamide once per month for systemic sclerosis (ASSIST): an open-label, randomised phase 2 trial. Lancet 2011 ;378(9790):498-506.

45. 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.

46. Del Galdo F, Jimenez SA. T cells expressing allograft inflammatory factor 1 display increased chemotaxis and induce a profibrotic phenotype in normal fibroblasts in vitro. *Arthritis Rheum* 2007; 56: 3478-88.

47. Salmon-Ehr V, Serpier H, Nawrocki B, *et al*. Expression of interleukin-4 in scleroderma skin specimens and scleroderma fibroblast cultures. Potential role in fibrosis. Arch Dermatol 1996 ; 132:802-6.

48. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 2004 ;4:583-94.

49. Mavalia C, Scaletti C, Romagnani P, *et al*. Type 2 helper T-cell predominance and high CD30 expression in systemic sclerosis. *Am J Pathol* 1997; 151: 1751-8.

50. Truchetet ME, Brembilla NC, Montanari E, Allanore Y, Chizzolini C. Increased frequency of circulating Th22 in addition to Th17 and Th2 lymphocytes in systemic sclerosis: association with interstitial lung disease. *Arthritis Res Ther* 2011; 13: R166.

51. 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.

52. Granel B, Allanore Y, Chevillard C, *et al*. IL13RA2 gene polymorphisms are associated with systemic sclerosis. *J Rheumatol* 2006; 33: 2015-9.

53. Medsger TA, Jr, Ivanco DE, Kardava L, Morel PA, Lucas MR, Fuschiotti P. GATA-3 up-regulation in CD8+ T cells as a biomarker of immune dysfunction in systemic sclerosis, resulting in excessive interleukin-13 production. *Arthritis Rheum* 2011; 63: 1738-47.

54. Gourh P, Agarwal SK, Divecha D, *et al*. Polymorphisms in TBX21 and STAT4 increase the risk of systemic sclerosis: evidence of possible gene-gene interaction and alterations in Th1/Th2 cytokines. *Arthritis Rheum* 2009; 60: 3794-806.

55. 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.

56. Scala E, Pallotta S, Frezzolini A, *et al*. Cytokine and chemokine levels in systemic sclerosis: relationship with cutaneous and internal organ involvement. *Clin Exp Immunol* 2004; 138: 540-6.

57. Radstake TR, van Bon L, Broen J, *et al*. The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular expression of TGFbeta and IFNgamma distinguishes SSc phenotypes. *PLoS One* 2009; 4: e5903.

58. 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(5–6): 239-46.

59. Denton CP, Abraham DJ. Transforming growth factor-beta and connective tissue growth factor: key cytokines in scleroderma pathogenesis. *Curr Opin Rheumatol* 2001; 13: 505-11.

60. 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.

61. Hasegawa M, Fujimoto M, Kikuchi K, Takehara K. Elevated serum levels of interleukin 4 (IL-4), IL-10, and IL-13 in patients with systemic sclerosis. *J Rheumatol* 1997; 24: 328-32.

62. Gruschwitz MS, Vieth G. Up-regulation of class II major histocompatibility complex and intercellular adhesion molecule 1 expression on scleroderma fibroblasts and endothelial cells by interferon-gamma and tumor necrosis factor alpha in the early disease stage. *Arthritis Rheum* 1997; 40: 540-50.

63. Rodriguez-Reyna TS, Furuzawa-Carballeda J, Cabiedes J, *et al.* Th17 peripheral cells are increased in diffuse cutaneous systemic sclerosis compared with limited illness: a cross-sectional study. *Rheumatol Int* 2011; 32: 2653-60.

64. Fujii H, Hasegawa M, Takehara K, Mukaida N, Sato S. Abnormal expression of intracellular cytokines and chemokine receptors in peripheral blood T lymphocytes from patients with systemic sclerosis. *Clin Exp Immunol* 2002; 130: 548-56.

65. Valentini G, Baroni A, Esposito K, *et al*. Peripheral blood T lymphocytes from systemic sclerosis patients show both Th1 and Th2 activation. *J Clin Immunol* 2001; 21: 210-7.

66. Martelli-Junior H, Cotrim P, Graner E, Sauk JJ, Coletta RD. Effect of transforming growth factor-beta1, interleukin-6, and

interferon-gamma on the expression of type I collagen, heat shock protein 47, matrix metalloproteinase (MMP)-1 and MMP-2 by fibroblasts from normal gingiva and hereditary gingival fibromatosis. *J Periodontol* 2003; 74: 296-306.

67. Postlethwaite AE, Holness MA, Katai H, Raghow R. Human fibroblasts synthesize elevated levels of extracellular matrix proteins in response to interleukin 4. *J Clin Invest* 1992; 90: 1479-85.

68. Serpier H, Gillery P, Salmon-Ehr V, *et al*. Antagonistic effects of interferon-gamma and interleukin-4 on fibroblast cultures. *J Invest Dermatol* 1997; 109: 158-62.

69. Hoffmann KF, McCarty TC, Segal DH, *et al*. Disease fingerprinting with cDNA microarrays reveals distinct gene expression profiles in lethal type 1 and type 2 cytokine-mediated inflammatory reactions. *FASEB J* 2001; 15: 2545-7.

70. Sandler NG, Mentink-Kane MM, Cheever AW, Wynn TA. Global gene expression profiles during acute pathogen-induced pulmonary inflammation reveal divergent roles for Th1 and Th2 responses in tissue repair. *J Immunol* 2003; 171: 3655-67.

71. Aliprantis AO, Wang J, Fathman JW, *et al*. Transcription factor T-bet regulates skin sclerosis through its function in innate immunity and via IL-13. *Proc Natl Acad Sci U S A* 2007; 104: 2827-30.

72. Chizzolini C, Parel Y, De Luca C, *et al*. Systemic sclerosis Th2 cells inhibit collagen production by dermal fibroblasts via membrane-associated tumor necrosis factor alpha. *Arthritis Rheum* 2003; 48: 2593-604.

73. Chizzolini C, Rezzonico R, Ribbens C, Burger D, Wollheim FA, Dayer JM. Inhibition of type I collagen production by dermal fibroblasts upon contact with activated T cells: different sensitivity to inhibition between systemic sclerosis and control fibroblasts. *Arthritis Rheum* 1998; 41: 2039-47.

74. Chizzolini C, Parel Y, Scheja A, Dayer JM. Polarized subsets of human T-helper cells induce distinct patterns of chemokine production by normal and systemic sclerosis dermal fibroblasts. *Arthritis Res Ther* 2006; 8: R10.

75. Luzina IG, Atamas SP, Wise R, Wigley FM, Xiao HQ, White B. Gene expression in bronchoalveolar lavage cells from scleroderma patients. *Am J Respir Cell Mol Biol* 2002; 26: 549-57.

76. Ferreira AM, Takagawa S, Fresco R, Zhu X, Varga J, DiPietro LA. Diminished induction of skin fibrosis in mice with MCP-1 deficiency. *J Invest Dermatol* 2006; 126: 1900-8.

77. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; 27: 485-517.

78. Hemdan NY, Birkenmeier G, Wichmann G, *et al*. Interleukin-17-producing T helper cells in autoimmunity. *Autoimmun Rev* 2010; 9: 785-92.

79. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. *Nat Immunol* 2008; 9: 641-9.

80. Volpe E, Servant N, Zollinger R, *et al*. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat Immunol* 2008; 9: 650-7.

81. Yang L, Anderson DE, Baecher-Allan C, *et al*. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature* 2008; 454(7202): 350-2.

82. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007; 8: 942-9.

83. Wilson NJ, Boniface K, Chan JR, *et al*. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; 8: 950-7.

84. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, *et al*. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; 204: 1849-61.

85. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. *J Immunol* 2008; 180: 214-21.

86. Cosmi L, De Palma R, Santarlasci V, *et al*. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. *J Exp Med* 2008; 205: 1903-16.

87. Pappu R, Ramirez-Carrozzi V, Sambandam A. The interleukin-17 cytokine family: critical players in host defence and inflammatory diseases. *Immunology* 2011; 134: 8-16.

88. Fenoglio D, Battaglia F, Parodi A, *et al*. Alteration of Th17 and Treg cell subpopulations co-exist in patients affected with systemic sclerosis. *Clin Immunol* 2011; 139: 249-57.

89. Papp G, Horvath IF, Barath S, *et al*. Altered T-cell and regulatory cell repertoire in patients with diffuse cutaneous systemic sclerosis. *Scand J Rheumatol* 2011; 40: 205-10.

90. Pene J, Chevalier S, Preisser L, *et al*. Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. *J Immunol* 2008; 180: 7423-30.

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

92. Murata M, Fujimoto M, Matsushita T, *et al*. Clinical association of serum interleukin-17 levels in systemic sclerosis: is systemic sclerosis a Th17 disease? *J Dermatol Sci* 2008; 50: 240-2.

93. 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.

94. Radstake TR, van Bon L, Broen J, *et al*. Increased frequency and compromised function of T regulatory cells in systemic sclerosis (SSc) is related to a diminished CD69 and TGFbeta expression. *PLoS One* 2009; 4: e5981.

95. 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.

96. 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.

97. Lin AM, Rubin CJ, Khandpur R, *et al*. Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. *J Immunol* ;187:490-500.

98. Hueber AJ, Asquith DL, Miller AM, *et al*. Mast cells express IL-17A in rheumatoid arthritis synovium. *J Immunol* 2010; 184: 3336-40.

99. Noordenbos T, Yeremenko N, Gofita I, *et al*. Interleukin-17-positive mast cells contribute to synovial inflammation in spondylarthritis. *Arthritis Rheum* 2012; 64: 99-109.

100. Duncan MR, Berman B. Stimulation of collagen and glycosaminoglycan production in cultured human adult dermal fibroblasts by recombinant human interleukin 6. *J Invest Dermatol* 1991; 97: 686-92.

101. Antiga E, Quaglino P, Bellandi S, *et al*. Regulatory T cells in the skin lesions and blood of patients with systemic sclerosis and morphoea. *Br J Dermatol* 2010; 162: 1056-63.

102. Banica L, Besliu A, Pistol G, *et al*. Quantification and molecular characterization of regulatory T cells in connective tissue diseases. *Autoimmunity* 2009; 42: 41-9.

103. Mathian A, Parizot C, Dorgham K, *et al*. Activated and resting regulatory T cell exhaustion concurs with high levels of interleukin-22 expression in systemic sclerosis lesions. *Ann Rheum Dis* 2012; 71: 1227-34.

104. Miyara M, Yoshioka Y, Kitoh A, *et al*. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity* 2009; 30: 899-911.

105. Deknuydt F, Bioley G, Valmori D, Ayyoub M. IL-1beta and IL-2 convert human Treg into T(H)17 cells. *Clin Immunol* 2009; 131: 298-307.

106. Komura K, Fujimoto M, Hasegawa M, *et al*. Increased serum interleukin 23 in patients with systemic sclerosis. *J Rheumatol* 2008; 35: 120-5.

107. Agarwal SK, Gourh P, Shete S, *et al*. Association of interleukin 23 receptor polymorphisms with anti-topoisomerase-I positivity and pulmonary hypertension in systemic sclerosis. *J Rheumatol* 2009; 36: 2715-23.

108. Mi S, Li Z, Yang HZ, *et al*. Blocking IL-17A promotes the resolution of pulmonary inflammation and fibrosis via TGF-beta1-dependent and -independent mechanisms. *J Immunol* 2011; 187: 3003-14.

109. Gasse P, Riteau N, Vacher R, *et al*. IL-1 and IL-23 mediate early IL-17A production in pulmonary inflammation leading to late fibrosis. *PLoS One* 2011; 6: e23185.

110. Okamoto Y, Hasegawa M, Matsushita T, *et al.* Potential roles of interleukin 17A in the development of skin fibrosis. *Arthritis Rheum* 2012 Jul 25:doi: 10.1002/art.34643.

111. Wilson MS, Madala SK, Ramalingam TR, *et al*. Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. *J Exp Med* 2010; 207: 535-52.

112. Simonian PL, Roark CL, Wehrmann F, *et al*. Th17-polarized immune response in a murine model of hypersensitivity pneumonitis and lung fibrosis. *J Immunol* 2009; 182: 657-65.

113. Fossiez F, Djossou O, Chomarat P, *et al*. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J Exp Med* 1996; 183: 2593-603.

114. Yao Z, Painter SL, Fanslow WC, *et al*. Human IL-17: a novel cytokine derived from T cells. *J Immunol* 1995; 155: 5483-6.

115. Yamamoto T, Eckes B, Hartmann K, Krieg T. Expression of monocyte chemoattractant protein-1 in the lesional skin of systemic sclerosis. *J Dermatol Sci* 2001; 26: 133-9.

116. Furuse S, Fujii H, Kaburagi Y, *et al*. Serum concentrations of the CXC chemokines interleukin 8 and growth-regulated oncogene-alpha are elevated in patients with systemic sclerosis. *J Rheumatol* 2003; 30: 1524-8.

117. Keane MP, Arenberg DA, Lynch JP, 3rd, *et al*. The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. *J Immunol* 1997; 159: 1437-43.

118. Distler JH, Jungel A, Caretto D, *et al*. Monocyte chemoattractant protein 1 released from glycosaminoglycans mediates its profibrotic effects in systemic sclerosis via the release of interleukin-4 from T cells. *Arthritis Rheum* 2006; 54: 214-25.

119. Yamamoto T, Eckes B, Mauch C, Hartmann K, Krieg T. Monocyte chemoattractant protein-1 enhances gene expression and synthesis of matrix metalloproteinase-1 in human fibroblasts by an autocrine IL-1 alpha loop. *J Immunol* 2000; 164: 6174-9.

120. Agarwal S, Misra R, Aggarwal A. Interleukin 17 levels are increased in juvenile idiopathic arthritis synovial fluid and induce synovial fibroblasts to produce proinflammatory cytokines and matrix metalloproteinases. *J Rheumatol* 2008; 35: 515-9.

121. Cortez DM, Feldman MD, Mummidi S, *et al*. IL-17 stimulates MMP-1 expression in primary human cardiac fibroblasts via p38 MAPK- and ERK1/2-dependent C/EBP-beta, NF-kappaB, and AP-1 activation. *Am J Physiol Heart Circ Physiol* 2007; 293: H3356-65.

122. Zuo F, Kaminski N, Eugui E, *et al*. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. *Proc Natl Acad Sci U S A* 2002; 99: 6292-7.

123. Kuroda K, Shinkai H. Gene expression of types I and III collagen, decorin, matrix metalloproteinases and tissue inhibitors of metalloproteinases in skin fibroblasts from patients with systemic sclerosis. *Arch Dermatol Res* 1997; 289: 567-72.

124. Young-Min SA, Beeton C, Laughton R, *et al*. Serum TIMP-1, TIMP-2, and MMP-1 in patients with systemic sclerosis, primary Raynaud's phenomenon, and in normal controls. *Ann Rheum Dis* 2001; 60: 846-51.

125. Toubi E, Kessel A, Grushko G, Sabo E, Rozenbaum M, Rosner I. The association of serum matrix metalloproteinases and their tissue inhibitor levels with scleroderma disease severity. *Clin Exp Rheumatol* 2002; 20: 221-4.

126. Frost J, Ramsay M, Mia R, Moosa L, Musenge E, Tikly M. Differential gene expression of MMP-1, TIMP-1 and HGF in clinically involved and uninvolved skin in South Africans with SSc. *Rheumatology* (Oxford) 2012; 51: 1049-52.

127. Truchetet ME, Allanore Y, Montanari E, Chizzolini C, Brembilla NC. Prostaglandin I2 analogues enhance already exuberant Th17 cell responses in systemic sclerosis. *Ann Rheum Dis* 2012; 71: 2044-50.

128. Hsu HC, Yang P, Wang J, *et al*. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008; 9: 166-75.

129. Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol* 2009; 10: 857-63.

130. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat Immunol* 2009; 10: 864-71.

131. Yang XO, Panopoulos AD, Nurieva R, *et al*. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J Biol Chem* 2007; 282: 9358-63.

132. Veldhoen M, Hirota K, Westendorf AM, *et al*. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* 2008; 453(7191): 106-9.

133. Ramirez JM, Brembilla NC, Sorg O, *et al*. Activation of the aryl hydrocarbon receptor reveals distinct requirements for IL-22 and IL-17 production by human T helper cells. *Eur J Immunol* 2010; 40: 2450-9.

134. Volpe E, Touzot M, Servant N, *et al*. Multiparametric analysis of cytokine-driven human Th17 differentiation reveals a differential regulation of IL-17 and IL-22 production. *Blood* 2009; 114: 3610-4.

135. Wolk K, Witte E, Witte K, Warszawska K, Sabat R. Biology of interleukin-22. *Semin Immunopathol* 2010; 32: 17-31.

136. Zhang N, Pan HF, Ye DQ. Th22 in inflammatory and autoimmune disease: prospects for therapeutic intervention. *Mol Cell Biochem* 2011; 353(1–2): 41-6.

137. Hikami K, Ehara Y, Hasegawa M, *et al*. Association of IL-10 receptor 2 (IL10RB) SNP with systemic sclerosis. *Biochem Biophys Res Commun* 2008; 373: 403-7.

138. Sonnenberg GF, Nair MG, Kirn TJ, Zaph C, Fouser LA, Artis D. Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. *J Exp Med* 2010; 207: 1293-305.

139. Braun RK, Martin A, Shah S, *et al*. Inhibition of bleomycin-induced pulmonary fibrosis through pre-treatment with collagen type V. *J Heart Lung Transplant* 2010; 29: 873-80.

140. Simonian PL, Wehrmann F, Roark CL, Born WK, O'Brien RL, Fontenot AP. gammadelta T cells protect against lung fibrosis via IL-22. *J Exp Med* 2010; 207: 2239-53.

141. Furuzawa-Carballeda J, Ortiz-Avalos M, Lima G, Jurado-Santa Cruz F, Llorente L. Subcutaneous administration of polymerized type I collagen downregulates interleukin (IL)-17A, IL-22 and transforming growth factor-beta1 expression, and increases Foxp3-expressing cells in localized scleroderma. *Clin Exp Dermatol* 2012; 37: 599-609.

142. Eyerich S, Eyerich K, Pennino D, *et al*. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest* 2009; 119: 3573-85.

143. Giovannetti A, Rosato E, Renzi C, *et al*. Analyses of T cell phenotype and function reveal an altered T cell homeostasis in systemic sclerosis. Correlations with disease severity and phenotypes. *Clin Immunol* 2010; 137: 122-33.

144. Slobodin G, Ahmad MS, Rosner I, *et al*. Regulatory T cells (CD4(+)CD25(bright)FoxP3(+)) expansion in systemic sclerosis correlates with disease activity and severity. *Cell Immunol* 2010; 261: 77-80.

145. Broen JC, Wolvers-Tettero IL, Geurts-van Bon L, *et al*. Skewed X chromosomal inactivation impacts T regulatory cell function in systemic sclerosis. *Ann Rheum Dis* 2010; 69: 2213-6.

146. Koenen HJ, Smeets RL, Vink PM, van Rijssen E, Boots AM, Joosten I. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. *Blood* 2008; 112: 2340-52.

147. Osorio F, LeibundGut-Landmann S, Lochner M, *et al*. DC activated via dectin-1 convert Treg into IL-17 producers. *Eur J Immunol* 2008; 38: 3274-81.

148. Yang XO, Nurieva R, Martinez GJ, *et al*. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity* 2008; 29: 44-56.

149. Beriou G, Costantino CM, Ashley CW, *et al*. IL-17-producing human peripheral regulatory T cells retain suppressive function. *Blood* 2009; 113: 4240-9.

150. Truchetet ME. Brembilla NC, Montanari E, Lonati P, Raschi E, Zeni S, Fontao L, Meroni PL and Chizzolini C.Interleukin-17A+ cells are increased in systemic sclerosis skin and their number is inversely correlated to the extent of skin involvement. *Arthritis Rheum* 2013, under press.

151. Brembilla NC, Montanari E, Truchetet ME, Raschi E, Zeni S, Meroni PL and Chizzolini C. Th17 cells favor inflammatory responses and inhibit type I collagen deposition by scleroderma fibroblasts. In preparation.