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REVIEW

Pathophysiology of systemic sclerosis: current understanding and new insights

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ABSTRACT

Introduction: Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease characterized by chronic and progressive tissue and organ fibrosis with broad patient-to-patient variability.

Some risk factors are known and include combination of persistent Raynaud's phenomenon, steroid hormone imbalance, selected chemicals, thermal, or other injuries. Endogenous and/or exogenous environmental trigger/risk factors promote epigenetic mechanisms in genetically primed subjects.

Disease pathogenesis presents early microvascular changes with endothelial cell dysfunction, followed by the activation of mechanisms promoting their transition into myofibroblasts. A complex autoimmune response, involving innate and adaptive immunity with specific/functional autoantibody production, characterizes the disease. Progressive fibrosis and ischemia involve skin and visceral organs resulting in their irreversible damage/failure. Progenitor circulating cells (monocytes, fibrocytes), together with growth factors and cytokines participate in disease diffusion and evolution. Epigenetic, vascular and immunologic mechanisms implicated in systemic fibrosis, represent major targets for incoming disease modifying therapeutic approaches.

Areas covered: This review discusses current understanding and new insights of SSc pathogenesis, through an overview of the most relevant advancements to present aspects and mechanisms involved in disease pathogenesis.

Expert opinion: Considering SSc intricacy/heterogeneity, early combination therapy with vasodilators, immunosuppressive and antifibrotic drugs should successfully downregulate the disease progression, especially if started from the beginning.

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1. Introduction

Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease characterized by chronic and progressive tissue and organ fibrosis. Triggers for SSc remain only partially defined, whereas different risk factors have been identified and include combination of long-lasting Raynaud's phenomenon (RP), selected chemicals and silicone breast implants, frequent thermal or other mechanical injuries, mainly at acral regions of the body, and steroid hormone imbalance/stress [1,2]. Both endogenous and/or exogenous environmental trigger/risk factors are important promoters for epigenetic mechanisms in genetically primed subjects.

The pathophysiology of SSc is a progressive self-amplifying process, which first implicates the microvascular damage, followed by the autoimmune response and inflammation, and diffuse fibrosis (Figure 1) [3,4]. In conclusion, the different pathophysiological steps are finally associated with a progressive fibrotic involvement of skin and internal organs (Figure 1).

Current understanding of SSc has permitted the production of new biological drugs against disease-specific molecular targets to actuate disease-modifying therapeutic approaches. In this review, we summarize and discuss the current understanding and new insights into the pathogenesis of SSc.

2. The microvascular pathophysiology: current understanding

It is known that RP is a common episodic color change of the extremities in response to cold exposure, which is highly prevalent in patients with SSc (over 95% of patients have RP) [5]. Patients with primary RP progress in almost 15% of cases to secondary RP associated with SSc [6]. In fact, the initial but reversible microvascular damage induced by primary RP in the presence of other risks factors and enhanced immune response, progress to secondary RP, with irreversible microvessel deletion, capillary destruction, and subsequent increase in tissue fibrosis.

Secondary RP (SRP) is very often the first manifestation of SSc and may precede other organ disease signals by years. Almost every SSc patient experiences frequent ischemic events typical of RP and is associated with severe events, which are pronounced in the extremities i.e. in the fingers with recurrent digital ulcers in 25–50% of cases [5].

Evidence suggests that SRP in SSc results from a microvasculopathy involving all layers of the peripheral blood vessels and in part it is caused by the dysfunction of the endothelium [7]. The endothelium is a metabolically active tissue that, under normal circumstances, regulates

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Article highlights

- Systemic sclerosis (SSc) is a complex autoimmune connective tissue disease characterized by a chronic and progressive tissue and organ fibrosis.
- The pathophysiology of SSc is a progressive self-amplifying process, that first implicates the microvascular/endothelial damage, followed by the autoimmune response and inflammation, and finally characterized by diffuse fibrosis.
- The progressive microvascular pathophysiology in SSc is associated with the clinical disease progression: from the Raynaud's phenomenon to the microvessel leak with hemorrhages, capillary collapses/loss (both visible on capillaroscopy) and tissue ischemia.
- New insights on the microvascular SSc pathophysiology include VEGF isoforms (antiangiogenic) and impaired functioning of the endothelial progenitor cells (EPC) that are involved in the angiogenesis.
- Epigenetic studies have identified intrinsic alterations in SSc fibroblasts resulting from epigenetic changes, as well as altered microRNA expression (in particular miR-29, miR-155) that might underlie the persistent activation phenotype of these cells.
- The immune response in SSc is complex and involves both the innate and adaptive immunity with prevalence of Th2 cytokines.
- The macrophage (M) polarization versus a prevalence of hybrid M1/M2 circulating progenitor cells and versus the M2 phenotype in the fibrotic tissues, it is particularly evident in presence of SSc lung involvement.
- The production of specific autoantibodies in SSc seems associated with different clinical aspects, in addition several functional autoantibodies seem implicated in the pathophysiology of the disease.
- The fibrotic process in SSc is characterized by progressive tissue accumulation of extracellular matrix (ECM) protein like collagens, elastin, glycosaminoglycans, tenascin and fibronectin isoforms in skin and multiple organs.
- The endothelial(epitelial)-to-mesenchymal cell transition (EndoMT) process is a source of myofibroblasts in SSc and can be induced by TGF-β together with endothelin-1 (ET-1): both seem to be pivotal soluble players in the development of fibrosis.
- In SSc, myofibroblasts seem to originate also from adiponectin-positive intradermal progenitor cells: the process was confirmed by adipocyte phenotype investigations and is termed adipocyte-myofibroblast transition (AMT).

regional blood flow, transportation of nutrients, coagulation and fibrinolysis, and migration of blood cells while maintaining an antithrombotic lining in the vasculature.

These important biologic functions are achieved through production of a complex array of molecules including vasodilators (i.e. nitric oxide (NO) and prostacyclin), vasoconstrictors (i.e. endothelin-1 (ET-1) and platelet activating factor), and cell adhesion molecules (i.e. selectins and integrins) [8].

An abnormal function of the endothelium results in an imbalance of vasoactive factors including overproduction of the vasoconstrictor ET-1 in skin, lung tissue and serum and underproduction of the vasodilator NO and prostacyclin in SSc patients.

In particular, ET-1 plays a prominent role in vascular tone regulation through its receptors ET_A and ET_B. ET_A receptor predominates on vascular smooth muscle and mediates vasoconstriction, whereas the ET_B receptor subtype, when presents on vascular endothelium, mediates vasodilation through NO release [9–11].

The frequent and sustained alteration of the microvascular tone is a noxious trigger to the endothelial barrier leading to opening of the endothelial junctions, further inflammatory cells homing, increased microvessel permeability and continuous vascular leak (Figure 2).

The phenomenon of progressive microvascular leak causes microhemorrhages and local edema (Figure 2) [12].

Platelet activation is demonstrated in SSc leading to the release of thromboxane, a potent vasoconstrictor. In addition, activated endothelial cells show an increased expression of the adhesion molecules vascular cell adhesion protein 1 (VCAM1), intercellular adhesion molecule (ICAM) and E-selectin, resulting in recruitment of inflammatory cells from the circulation (Figure 2) [12].

The cutaneous disease of SSc is also associated with a decreased release from sensory nerves of vasodilatory neuropeptides, such as calcitonin gene-related peptide (CGRP) and serotonin [13,14], further triggering vasospasm already with little provocation. Finally, there is evidence of upregulation of the vascular smooth muscle α2c adrenoceptor (α2c-AR) that can enhance vasoconstrictive responses to stress or cold stimuli [5].

Severe vasospasm of digital arteries and cutaneous thermoregulatory vessels is observed in SSc with repeated bouts of vasoconstriction that potentially may cause severe obstacle to the microcirculation and ischemia-reperfusion injury of tissues [15].

Together with the prolonged ischemia-reperfusion induced by the RP, other toxic stimuli may induce a state of persistent endothelial activation resulting in apoptosis, cell detachment, and microvascular damage (Figure 2).

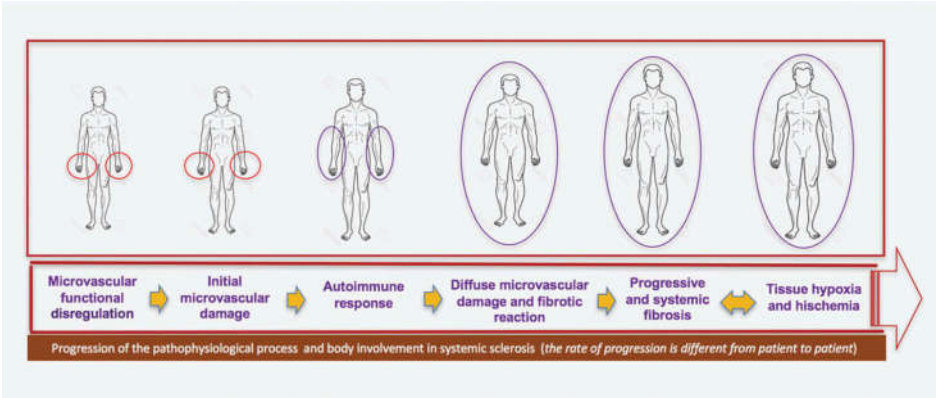

Figure 1. The figure reports the progression of the pathophysiological process and the corresponding clinical body involvement in systemic sclerosis (the rate of progression is different from patient to patient).

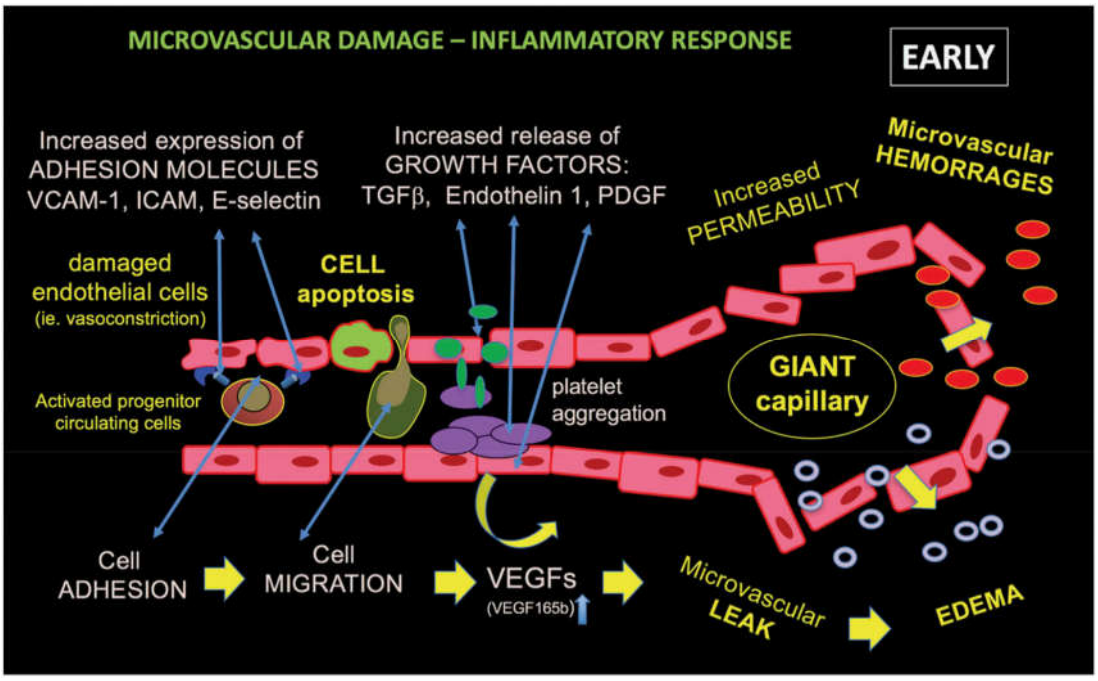

Figure 2. The figure describes the early steps on the systemic sclerosis pathophysiology involving the microvascular endothelium. The frequent and sustained alteration of the microvascular tone and cell apoptosis, are noxious trigger to the endothelial barrier leading to opening of the endothelial junctions, further inflammatory cells migration and homing, increased microvessel permeability and progressive vascular leak.

3. The microvascular pathophysiology: new insights

In SSc, the microvascular damage is paralleled by an increased production of pro-angiogenic factors (e.g. VEGF-A, ET-1) and on the other hand defective response of the damaged endothelial cells (Figure 2) [16].

However, despite the increase of VEGF-A in SSc skin and serum, a clear evidence of an insufficient angiogenic response persists [17].

New insights showed the existence of a pro-angiogenic VEGF165 isoform and an antiangiogenic VEGF165b isoform generated by alternative splicing in VEGF-A pre-mRNA [18,19].

The anti-angiogenic VEGF165b isoform V appears overexpressed in SSc dermis in different cell types, including endothelial cells, perivascular mononuclear inflammatory cells, and fibroblasts.

In addition, the increased VEGF165b serum levels correlate with degree of nailfold capillary altered array and loss and SSc dermal microvascular endothelial cells (MVECs) express and release elevated levels of VEGF165b.

A recent study showed that, in SSc, platelets store high concentrations of VEGF165b and therefore they may be a major source of circulating VEGF165b especially following their activation on contact with the damaged SSc endothelium [20].

Interestingly, binding of VEGF165 to vascular endothelial growth factor receptor (VEGFR)-2 triggers endothelial cell proliferation, survival, and migration, which are fundamental for the process of angiogenesis [21]. On the contrary, when VEGF165b binds to VEGFR-2 with the same affinity as VEGF165, phosphorylation/activation of VEGFR-2 generates an incomplete downstream signaling.

A very recent investigation has shown that increased $VEGF_{165}b$ expression and secretion by macrophages induce an anti-angiogenic M1-like phenotype that directly impairs angiogenesis [22]. This finding, up to now, is limited to the atherosclerotic occlusions and decrease blood flow to the lower limbs, causing ischemia and tissue loss in patients with peripheral artery disease (PAD) [22].

In particular, VEGFR1 inhibition by $VEGF_{165}b$ results in S100A8/S100A9-mediated calcium influx to induce an M1-like phenotype that impairs ischemic muscle revascularization and perfusion recovery (Figure 2).

In SSc, a new emerging link between microvascular damage, endothelial dysfunction, and development of dermal fibrosis is represented by the endothelial-to-mesenchymal transition (EndoMT) process. EndoMT is a trans-differentiation process by which endothelial cells lose the ability to express their phenotype markers as well as their morphology and express mesenchymal cell products, such as α-smooth muscle actin (α-SMA) and collagen type I (COL-1), acquiring mesenchymal/myofibroblast features [23,24]. The EndoMT process seems to be mediated by vasoconstrictor molecules and growth factors, including ET-1 and transforming growth factor (TGF), probably as a consequence of the imbalance of vasoactive factors due to the abnormal function of the endothelium [25]. The EndoMT process might be responsible for the presence of an abnormal microvascular architecture, which may determine the capillary loss in SSc patients characterized by an active disease.

Further insights concern the angiogenic potential of endothelial cell (EC)-like mesenchymal stromal cells that was found to be reduced after being stimulated with VEGF and stromal cell-derived factor-1 *in vitro*, suggesting that endothelial repair may be affected in SSc starting from the bone marrow (BM) [26]. In this context, the pathologic endothelium,

via the altered cross-talk between ECs, mesenchymal stem cells (MSCs) and pericytes impairs the angiogenic process and modulate the production of profibrotic molecules in MSCs, promoting a switch of these multipotent perivascular cells toward a profibrotic myofibroblast phenotype [25]. MSCs from SSc patients showed intrinsic differentiation abnormalities in response to several profibrotic molecules, such as TGF-β1, and a disease-associated microenvironment, favoring a phenotypic switch toward myofibroblasts [27]. As a matter of fact, the role of the EndoMT, the cross-talk between ECs and pericytes, as well as the commitment of mesenchymal stem cells toward a profibrotic phenotype represent an important contribution the pathophysiology of SSc [28,29].

Endothelial progenitor cells (EPC) normally have the ability to develop into fully mature endothelial cells and contribute to neovascularization by targeting sites of endothelial injury [30]. They represent a heterogeneous cell population originating from a single multipotent progenitor cell (within the BM) and consist of cells at different stages of maturation, including early $CD133^+/VEGFR2^+$ or more mature $CD34^+/VEGFR2^+$ phenotypes [31]. Impaired functioning of the EPC has been thought to be involved in the pathogenesis and angiogenesis in SSc [32,33].

4. The immune system response in SSc

Microvascular endothelial cell injury and apoptosis is a central event in the pathogenesis of SSc vasculopathy that leads to immune system activation [34].

In human fibrotic skin diseases, apoptotic endothelial cells could only be detected in early inflammatory disease stages of SSc and localized SSc [35].

The alteration of both innate and adaptive immune response plays a prominent role in early SSc pathophysiology and includes the increased presence and altered functions of inflammatory cells and products in target tissues, such as the skin and lungs, together with a prominent type I interferon (IFN) signature. Polymorphisms in IFN-regulatory factors confer an increased risk of SSc, and IFN excess is evident in the blood and skin of a large percentage of SSc patients [36].

Interestingly, among T cells ($CD4^+$ T cells), the type 2 T helper (TH2) cells – characterized by secretion of IL-4 and IL-13 – are more expressed in SSc than TH1 cells, which primarily secrete anti-fibrotic IFNγ (Figure 3) [37]. In the $CD4^+$ Tcell population, T regulatory (Treg) cells, which represent 5–15% of them, have started being studied during the last decade in order to understand their possible role in the development of SSc. In the medical literature, the decreased functional capacity as well as the low number of circulating Treg cells in SSc patients, usually associated with a decrease in FOXP3 expression, have been described [38,39].

Otherwise, SSc patients with an active disease may have an increase in the number of these circulating cells. Of note, recent findings demonstrated that Treg cells can contribute to SSc development by their transformation to pathogenic effector T cells [38].

In addition, the conversion of circulating Treg into Th17 cells as well as skin-resident Treg to Th2 cells producing inflammatory and profibrotic cytokines respectively, has been demonstrated in SSc [38,40]. Other than regulatory T cells, angiogenic T cells were observed to be increased in peripheral blood of SSc patients, primarily in those patients with digital ulcers compared to patients without digital ulcers and in patients showing

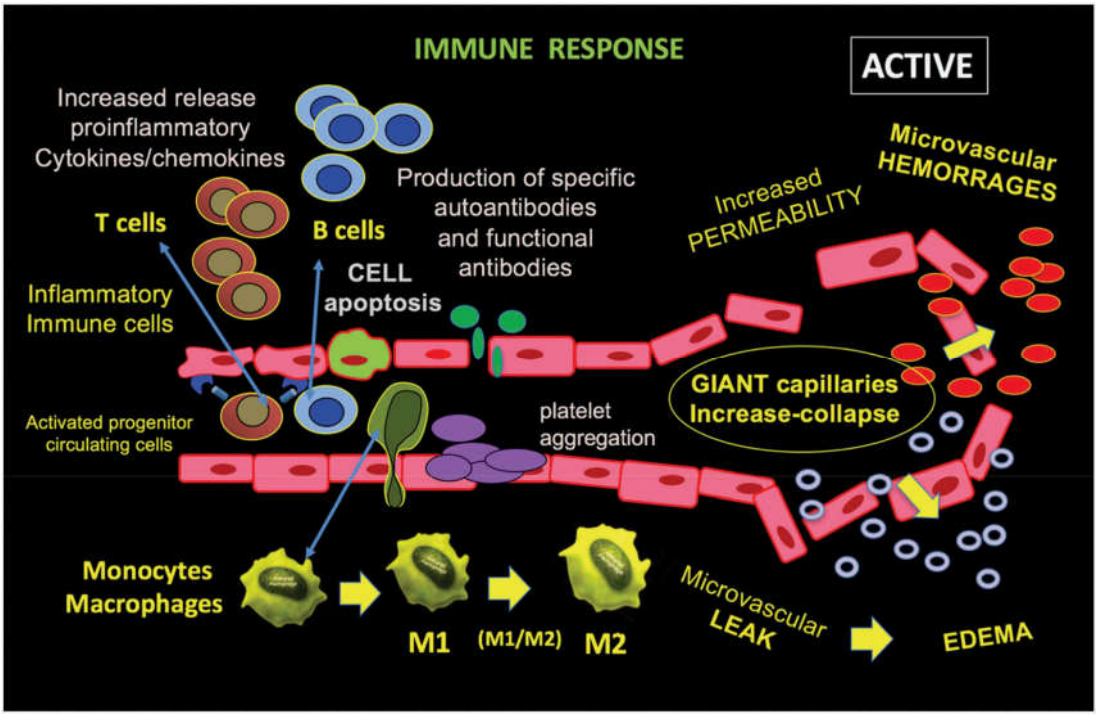

Figure 3. The early homing of the immune/inflammatory cells within the perivascular area (T cells, B cells, monocytes/macrophages) and platelets, further create in systemic sclerosis a toxic milieu for the microcirculation, together with the high production of cytokines/chemokines and specific or functional autoantibodies. The number of capillaries decreases and the fibrotic reaction starts.

a late nailfold videocapillaroscopy (NVC) patter compared to those with early/active NVC patterns [41].

Despite these results, the functional role in the pathogenesis of SSc and the possible involvement in fibrosis need to be understood and clarified.

High levels of IL-6 in SSc correlate with the extent of skin involvement and may anticipate poor long-term outcomes [42].

Chemokines also play important roles in angiogenesis and fibrosis. Serum and tissue levels of C-C motif chemokine 2 (CCL2; also known as MCP1), CCL3 (also known as MIP1α), IL-8 and CCL18 are increased in SSc patients and correlate with disease severity and progression (Figure 3) [43].

Plasmacytoid dendritic cells infiltrate the skin of SSc patients and are chronically activated, leading to secretion of interferon-α (IFN-α) and of the small chemokine platelet factor 4 (PF4; also known as CXCL4) which are both hallmarks of the disease [44].

On the other hands, an evident B cell activation produces several autoantibodies (AAb) targeting a variety of nuclear, cytoplasmatic, and extracellular autoantigens are the hallmark of SSc and are observed at the time of first diagnosis in more than 95% of patients (Figure 3) [45]. Some of these AAbs are highly specific for SSc [46].

Many of the AAbs found in SSc do not directly contribute to disease pathogenesis but are used as routine markers for diagnosis and prognosis of organ involvement [47].

In diffuse cutaneous SSc (dcSSc) anti-topoisomerase AAbs (ATA), formerly known as anti-Scl70 AAbs, are more prevalent, whereas anticentromere AAbs (ACA) are more frequent in limited cutaneous SSc (lcSSc).

A possible pathogenic role of ATA in SSc has been suggested following its binding to fibroblasts and induced adhesion and activation of cocultured monocytes [48]. This observation might provide an explanation for the amplification of the fibrogenic cascade in ATA-positive SSc patients.

In patients with isolated Raynaud syndrome, the presence of ACA has been reported to predict the likelihood that these patients will progress to SSc [49].

In general, ACA positive patients have a better prognosis and show lower mortality than SSc patients with other antinuclear AAbs (ANA); however, 50% will eventually die from pulmonary arterial hypertension (PAH) [45].

Similarly to ATA, also anti-RNA polymerase I/III AAbs (anti-RNAP) are typically associated with a more rapidly progressive dcSSc. Interestingly, the presence of anti-RNAP, especially the presence of anti-RNAP III, is strongly associated with renal crisis and malignancy [46,47,50].

Other AAbs that are highly specific for SSc include anti-Th/To ribonucleoprotein (RNP) AAbs; anti-fibrillarin/U3RNP and anti-U11/U12RNP AAbs, and anti- U1RNP AAbs [45].

5. The functional autoantibodies in SSc: new insights

There is increasing evidence that generally functional AAbs exhibit pathogenic features by binding to their target molecule, where they induce abnormal activation or inhibition of crucial cell functions directly related to disease pathogenesis.

Functional AAbs have been observed also in SSc patients and are directed against G protein-coupled receptor (GPCR), tyrosine kinase receptors, or other proteins like matrix metalloproteinases (Figure 3) [51].

AAbs binding the platelet-derived growth factor receptor (PDGFR) have been the first functional AAbs described in SSc and have been shown to contribute to the pathogenesis of the diseases via activation of fibroblasts and fibroblast-like cells [52].

However, heterogeneous set of anti-PDGFR AAbs involved in SSc pathogenesis seems to show distinctive functional properties with opposite effects.

AAbs against angiotensin II type 1 receptor (AT1R) and endothelin type A receptor (ETAR) are GPCRs that are widely expressed on cells of the vascular system and have been detected in sera of SSc patients [53].

Anti-AT1R and anti-ETAR AAbs were found in nearly all SSc patients and high levels are associated with severe SSc manifestations such as digital ulcers, PAH, and LF: predicting cardiovascular complications and mortality, especially in SSc-related PAH [54].

Both AT1R and ETAR act agonistically as demonstrated by the induction of ERK 1/2 phosphorylation and increased TGFβ messenger RNA (mRNA) expression in ECs [53].

Finally, anti-endothelial cell Abs (AECA) are detected in 25–85% of patients with SSc and were associated with severe vascular involvement [29]. In a recent review, Mihai C and colleagues described how the ubiquitous nuclear protein CENP-B is the main target of AECA in patients with lcSSc and AECA from patients with dcSSc, with or without anti-topoisomerase I (anti-Scl-70) antibodies, bind to endothelial cell topoisomerase I, suggesting that classical autoantibodies such as CENP-B and topoisomerase I could act as AECA [55].

An increased ability of AECA to adhere to ECs and to induce EC activation, AAb-dependent cell-mediated cytotoxicity, and endothelial apoptosis has been demonstrated in several *in vitro* studies [56].

These effects can be linked to SSc pathogenesis, especially to EC altered function. The existence of functional AAbs among the heterogeneous AECA group has been demonstrated, in particular AECA targeting ICAM-1 that have been shown to induce the inflammatory activation at the level of ECs [57].

One important aspect is that the detection and identification of functional AAbs together their molecular targets might represent a possible way of specific treatments, especially during the early step of SSc, before the overt manifestations of disease progression.

6. The macrophage M1/M2 polarization and the fibrotic lung in SSc: new insights

In early SSc, circulating progenitor cells, such as monocytes recruited from the bone marrow, migrate in the tissues together with T cells and macrophage precursors, and create a perivascular infiltrate with a changed phenotype (Figure 3) [58].

Macrophages, after differentiation from monocytes, can generate different phenotypes of cells distinguished on the basis of different surface markers as classically activated (M1) and/or alternatively activated (M2) macrophages [58].

Generally, M1 macrophages are effector phagocytes with an increased microbicidal or tumoricidal capacity and produce proinflammatory cytokines like TNF-alpha, IL-6, IL-1, whereas M2-polarized macrophages produce anti-inflammatory cytokines, mostly IL-4, IL-13 and IL-10 [59].

During the tissues wound healing or at the peak of the profibrotic late immune response, M2 macrophages are considered as inducers of tissue fibrosis in SSc. Therefore, M2 macrophages partially suppress M1 responses (by their polarization) and promote extracellular matrix (ECM) protein synthesis, including profibrotic cytokine release as well as potentiate the antiinflammatory response by inducing Th2 effector activities [59].

Interestingly, ET-1 seems to induce the M2 polarization in cultured human macrophages, a process apparently contrasted by the action of the ET-1 receptor antagonism (ETA/B RA), suggesting possible clinical implications in those fibrotic diseases characterized by increased ET-1 concentrations, such as SSc but also type 2 diabetes [60].

Therefore, in SSc the persistency of tissue damage is not efficiently repaired, especially due to the immune system activation. The increased and sustained release of cytokines, and growth factors, especially from Th2 cells and M2 macrophages, induces in SSc a progressive fibrotic state that involves tissues and organs [61].

Importantly, major gene signatures related to phenotype, activation and migration of macrophages have been shown to be significant for the progressive pulmonary fibrosis, pointing to macrophages as key players in SSc lung involvement [62,63].

Intriguingly, imbalance in macrophage phenotype and macrophage activation, have been lately considered essential for the development of inflammatory-autoimmune, fibrotic, infective and neoplastic disorders all characterized by lung involvement [64–69].

As mentioned, macrophages have been initially categorized as M1 or M2, mirroring T cells categories. M1 macrophages express specific phenotype markers, including toll-like receptors (i.e. TLR2 and TLR4) and the co-stimulatory molecules CD80 and CD86, and are involved in triggering intensive inflammation and tissue damage [70].

On the contrary, M2 macrophages primarily express the mannose receptor-1 (CD206) and macrophage scavenger receptors (CD204 and CD163), and they are associated with T helper (Th) 2 response, tissue repair as well as fibrosis [71,72].

However, classifications based on a wider spectrum of phenotypes of which M1 and M2 subsets would constitute the two extremes have been reported [73].

Moreover, it was observed that the majority of alveolar macrophages combine M1 and M2 features in steady state and that the mixed M1/M2 phenotype can be altered by HIV infection [74].

Interestingly, a recent preliminary study demonstrated higher percentages of circulating mixed M1/M2 monocytes/macrophages in SSc patients compared to healthy subjects (Figure 3) [75]. In addition, a very recent study showed for the first time, that higher circulating mixed M1/M2 monocyte/macrophage cell percentages are associated with interstitial ling disease (ILD), systolic pulmonary artery pressure (sPAP) and anti-Scl-70 positivity in SSc patients, opening the path for research on their possible role as pathogenic or biomarker elements for SSc lung involvement [76].

7. Players for the fibrotic process in SSc: current understanding

The fibrotic tissue is characterized by the large presence of α-SMA positive cells, apoptosis-resistant myofibroblasts that secrete not only ECM proteins but also TGF-β and other profibrotic mediators (Figure 4) [77].

Monocyte-derived circulating mesenchymal progenitor cells (fibrocytes), as well as tissue-specific trans-differentiation from pericytes and ECs all contribute to the expansion of the myofibroblasts (Figure 4).

Fibrosis with progressive tissue accumulation of ECM proteins like collagens, elastin, glycosaminoglycans, tenascin and fibronectin in skin and multiple organs, is a prominent pathological finding and distinguishing hallmark of clinically overt SSc (limited cutaneous and diffuse cutaneous SSc).

Interestingly, increased concentrations of alternatively spliced isoforms of ECM proteins such as fibronectin (fibronectin-EDA (FnEDA)) and tenascin-C have been found to be accumulated within fibrotic tissues like it happen in advanced hyperplastic synovial tissue in rheumatoid arthritis (RA) [78–81].

In fact, in RA and SSc, it has been shown that at least tenascin-C isoform can directly bind to TLR4 on stromal cells and induce fibroblast activation and myofibroblast differentiation [82].

The progressive and increased deposition of ECM proteins, especially at the beginning fibronectin and tenascin followed by increased COL-1 and less fibrillin with their crosslinkings, increases skin and organ stiffness and reduces their elasticity, resulting in a real mechanical stress. The tissue mechanical stress that arises in SSc progression, further maintain fibroblast activation and further intensify the progression of the fibrotic process [83].

Fibroblasts, are differentiated in activated myofibroblasts as principal effector cells during the progression of the disease, in a profibrotic cellular milieu produced by the early immune/inflammatory reaction and formed by growth factors of different origin and selected cytokines (i.e. IL13, IL-6), developmental pathways, ET-1 and other soluble mediators (Figure 1) [84].

Beyond chronic fibroblast activation, SSc fibrosis represents a failure to terminate the normal tissue repair to the immune/inflammatory stimulus.

As matter of fact, the early innate immune system contribution via Toll-like receptors signaling, matrix-generated biomechanical stress via integrins signaling, hypoxia and oxidative cellular stress with associated cell apoptosis, is implicated from the beginning and is crucial in perpetuating the SSc fibrotic process [85].

In addition, studies have identified intrinsic alterations in SSc fibroblasts resulting from epigenetic changes, as well as altered microRNA expression (in particular miR-29, miR-155) that might underlie the persistent activation phenotype of the cells [86,87].

Interestingly, miR-155 was found significantly increased in SSc (including dermal fibroblasts) and the highest expressing miRNA in lung fibroblasts [88].

Its expression was dependent on inflammasome activation as miR-155 expression could be blocked when inflammasome

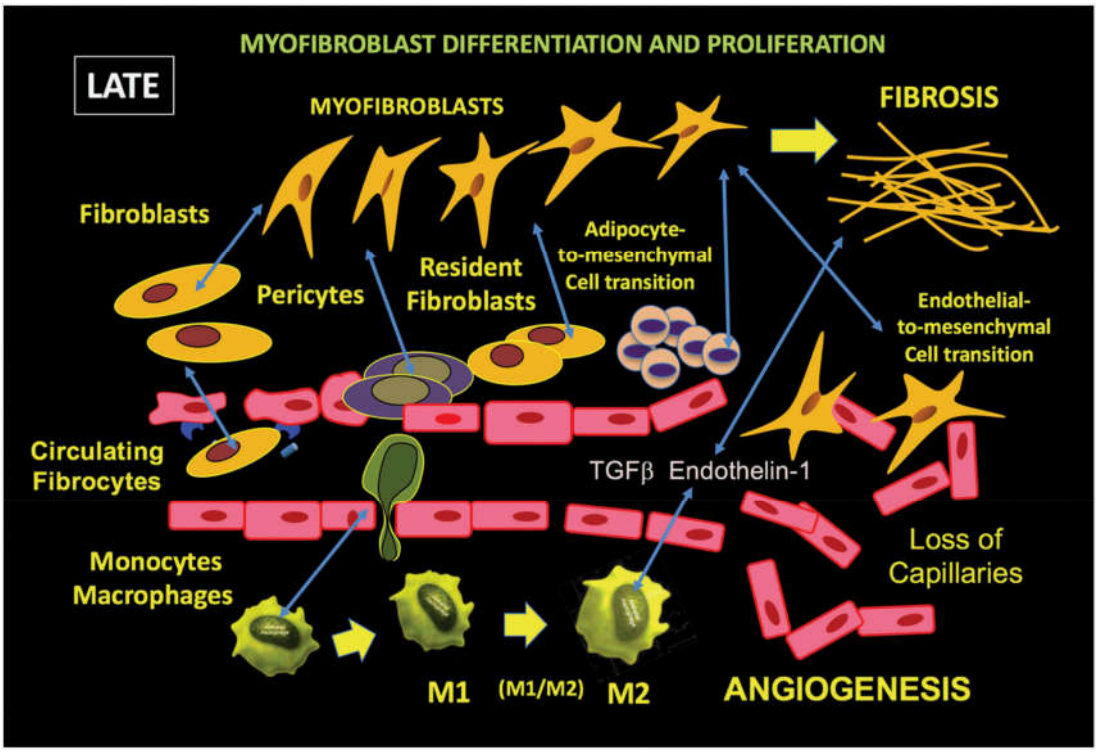

Figure 4. The scleroderma fibrotic tissue is characterized by the large presence of α-smooth muscle actin-positive myofibroblasts that secrete not only extracellular matrix proteins but also TGFβ and other profibrotic mediators, like endothelin-1 (ET-1). Monocyte-derived circulating mesenchymal progenitor cells (fibrocytes), as well as tissue-specific transdifferentiation from pericytes, adipocytes, and endothelial cells, all contribute to the expansion of the myofibroblasts.

signaling was inhibited. In the absence of miR-155, inflammasome-mediated collagen synthesis could not be induced but was restored when miR-155 was expressed in miR-155-deficient fibroblasts (murine model) [88].

Among growth factors and cytokines implicated in SSc, TGF-β (a pleiotropic cytokine) is considered the main modulator of fibrosis and is produced from macrophages and other cells as inactive precursor, is accumulated within the ECM and is converted to its biologically active form via integrin-mediated activation [89].

In detail, TGF-β signaling involves sequential phosphorylation of the type I TGF-β receptor (TGFR1), activation of Small mother against decapentaplegic (SMAD, namely SMAD2–SMAD3) dependent and independent intracellular signaling pathways and their binding to a consensus SMAD-binding element found in TGF-β -inducible profibrotic genes [90,91].

In addition, further intracellular mediators of the TGF-β signaling pathways, implicated in SSc pathogenesis, involve the activation of the kinase cascade including MAPK1 and MAPK3 (mitogen-activated protein kinases originally called as ERK2 and ERK1, extracellular signal-regulated kinases) [92].

8. The fibrotic process: new insights

Basically, ET-1 is a potent vasoconstrictor that in humans is encoded by the *EDN1* gene and produced by vascular endothelial cells.

In particular, it was found that ET-1 produced by activated ECs contributes to myofibroblast activation using TGF-β machinery via an ET-1/TGF-β receptor complex in SSc [93].

However, it is now evident that activated fibroblasts produce ET-1, that is able to increase fibronectin synthesis in normal and SSc human skin fibroblasts, suggesting further important roles also for ET-1 in the pathogenesis of the SSc fibrotizing process (Figure 4) [94–96].

Inhibitors of ET-1/2 receptors (bosentan, macitentan) have been found to interfere with the profibrotic action of TGF-β, blocking in detail the ET-1 receptor portion of the ET-1/TGF-β receptor complex [97].

In fact, an important study has shown that ET-1 induces an increased expression of fibronectin mRNA in cultured peritoneal mesothelial cells and contributes to the ability of TGF-β to promote a profibrotic phenotype in human fibroblasts [98].

In conclusion, both the early prolonged vasoconstrictor activity (i.e. RP in 90–98% of SSc patients) exerted by ET-1 and the later profibrotic effect by ET-1 on fibroblasts, seem to contribute to the pathogenesis of SSc.

The first sign occurring in of patients with SSc is RP, an abnormal reactivity of digital microvasculature under cold and other stimuli which highlights the central role of microvascular damage before the fibrotic process in the pathogenesis of SSc [99,100].

Recently, epithelial-to-mesenchymal transition (EMT) and EndoMT represented a newly recognized types of cellular trans-differentiation, and these processes have emerged as further possible sources of tissue myofibroblasts in presence of damage [101].

Despite evidence suggesting that EndoMT is involved in not only pathological but also physiological conditions such as normal wound healing, the underlying molecular mechanisms involved in this process are now better recognized in SSc [102–104].

EndoMT can be induced by TGF-β and together with ET-1, both seem to be pivotal players in the development of SSc fibrosis [105].

As matter of fact, it is well documented that the activation of TGF-β intracellular transcription factors is responsible for the production of other profibrotic molecules, such as indeed ET-1 [106].

Moreover, TGF-β-induced ET-1 release has been associated with the fibrotic response of skin and lung SSc fibroblasts, and in clinical management of SSc patients the inhibition of the both ET-1 receptors with antagonist drugs bosentan and macitentan, was recently found to downregulate the EndoMT in cultures of normal and SSc fibroblasts [107,108].

In addition, TGF-β contributed to myofibroblast increase in the damaged tissues also promoting the transition of pericytes, a process that seems to involve the activation of several molecules such as ADAM12, a protein highly expressed in skin, mesenchymal stem cells and fibroblasts of SSc patients, and its inhibition may contribute to attenuate the profibrotic activity of all these cells [28].

Together with a fibroblast-myofibroblast transition (FMT) involved in the profibrotic process, it has been recently reported limited to animal models, that myofibroblasts are produced from adiponectin-positive intradermal progenitor cells and the process was confirmed by adipocyte phenotype investigations and termed adipocyte-myofibroblast transition (AMT) [109].

This pathway demonstrates very rapid dynamics and in 24 hours after stimulation with TGF-β1, dermal adipocytes were in a transition state and expressed the myofibroblast α-SMA marker. Interestingly in animal models cell fat mapping showed that the majority of the myofibroblasts accumulated in dermal fibrosis originate from dermal adipocytes [109].

The phenomenon was first showed long time ago, when was demonstrated that an acute thermic or mechanical injury to adipose tissue, induces a fast phenotypic transformation of the mature adipocytes into fibroblast-like cells with a primitive phenotype [110].

Interesting, high levels of miR-155 suppress adipogenic differentiation and keep preadipocytes in an undifferentiated state thus inhibiting adipogenesis [111,112].

In conclusion, adipocytes from interfacial local white adipose tissue (WAT) adjacent to the fibrotic area in SSc are phenotypically different from bulk adipocytes and seem involved in pathogenesis of SSc [113].

9. Conclusions

The progression of the mechanisms implicated in the complex pathophysiology of SSc are clinically mirrored by the patient complications, and partially morphologically reflected by the safe observation of the microvascular alterations that are classified/scored by nailfold videocapillaroscopy as progressive 'early', 'active' and 'late' patterns (Figure 5) [114–118].

The complex nature of SSc suggest and early and combined treatment of the patient with different approaches [119].

10. Expert opinion

The pathogenesis of SSc include epigenetic/genetic, vascular and immunologic mechanisms, that are all implicated in the progressive fibrosis that mainly characterize the overt disease, and all are major targets for the actual and/or incoming new disease modifying therapeutic (DMARD) approaches.

Of note, the heterogeneity of clinical manifestations in SSc patients and the current limited therapeutic approaches represent important aspects and, at the same time, limitations in the study of SSc pathogenesis that further highlight the complexity of the disease management.

As matter of fact, the majority of the actual and incoming therapies for SSc, exert the final action to contrast the progression of fibrosis, even if the drugs have been originally planned for a different activity, for example vasodilation or immunosuppression.

The use of vasodilators in SSc is a reality from several years, acting on different targets but showing sometimes unexpected actions, as recently shown for instance by the use of the prostacyclin receptor agonist selexipag, synthesized for the treatment of pulmonary arterial hypertension (PAH) in SSc.

Selexipag and mainly its active metabolite (ACT-333,679), have been found for the first time to potentially interfere with

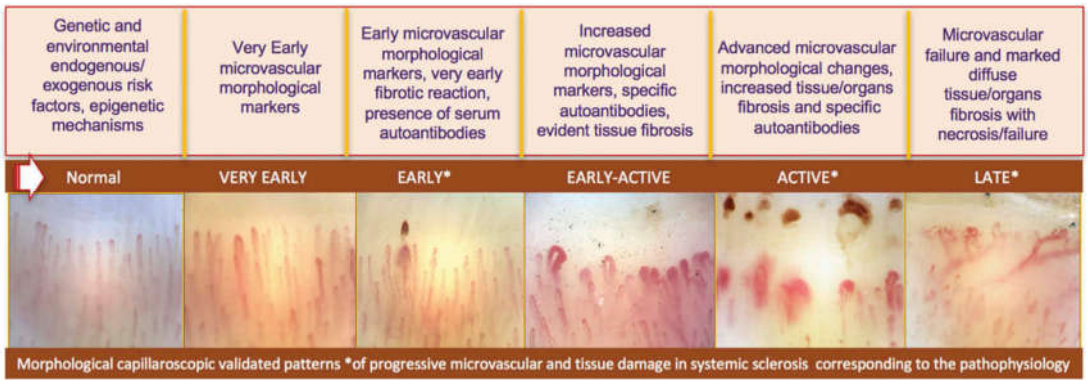

Figure 5. The progression of the mechanisms implicated in the complex pathophysiology of SSc are clinically mirrored by the observation of the microvascular alterations that are classified/scored by the safe nailfold videocapillaroscopy as progressive 'early', 'active' and 'late' patterns.

the profibrotic activity of cultured SSc fibroblasts/myofibroblasts at least *in vitro*, through the downregulation of fibrogenic Erk1/2 and Akt kinase intracellular signaling [120]. This effect is recognized as complementary/secondary to the original supposed vasodilatory main action.

The ET-1 receptor (ETA and ETB) antagonists (sitaxsentan, macitentan, and bosentan) represent another kind of therapy programmed originally to treat PAH in SSc, and that was also found to exert antifibrotic actions by reducing the synthesis of COL-1, fibronectin and fibrillin-1 in primary cultures of skin fibroblasts from SSc patients [97,121].

In addition, and even more relevant, both ETR antagonists (bosentan and macitentan) were found to inhibit the EndoMT, a complex biological process in which in SSc, endothelial cells lose their specific markers and acquire a mesenchymal or myofibroblastic phenotype, that mean fibrosis [108].

A further example, of unexpected antifibrotic activity, arises from the use of CTLA-4Ig in SSc in order to achieve an immunosuppressive action by interacting/blocking the costimulatory molecules (CD80/CD86) expressed on activated immune cells [122].

As matter of fact, a couple of studies showed unexpected antifibrotic actions on experimental dermal fibrosis and on cultured circulating fibrocytes and fibroblasts from SSc patients after treatment with CTLA-4Ig [123,124].

Keeping the immunosuppressive treatment as fundamental in autoimmune diseases like SSc, the use of rituximab (B-cell depleting drug), has been shown again to exert antifibrotic actions by reducing the skin myofibroblasts infiltration in treated SSc patients [125].

In addition, a 2-treatment course (months 0/6) with rituximab, appeared to be well tolerated and seemed to have potential efficacy for skin disease and stabilization of internal organ status in early diffuse cutaneous SSc [126].

A further remodeling effect on microvasculature was signaled following the treatment of SSc patients with rituximab for years, in fact a reduction of capillary loss was observed, as evaluated by nailfold capillaroscopy [127].

Similar reduction of microvascular damage, as evaluated by nailfold videocapillaroscopy, was previously described in SSc patients treated with cyclosporine A or cyclophosphamide, in order to obtain immunosuppression [128,129].

In conclusion, new target treatments are under evaluation for the management of fibrosis in SSc patients, in particular lung fibrosis, i.e., Nintedanib, a tyrosine kinase inhibitor that interact with several profibrotic pathways implicated in the pathogenesis at least of pulmonary SSc fibrosis and/or M2 macrophages.

Advances in translational research, including possible limitations, have undoubtedly open new horizons for the advanced and targeted therapy in SSc.

In the next years the systematic early combination therapy of SSc patients with vasodilators, immunosuppressive and antifibrotic drugs (with direct and indirect activity), should successfully downregulate the disease progression from the beginning and in particular the whole fibrotic process.

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