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A Unifying Hypothesis for Scleroderma: Identifying a Target Cell for Scleroderma

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Abstract

We propose that a recent change in the conception of the role of type 1 interferon and the identification of adventitial stem cells suggests a unifying hypothesis for scleroderma. This hypothesis begins with vasospasm. Vasospasm is fully reversible unless, as proposed here, the resulting ischemia leads to apoptosis and activation of type 1 interferon. The interferon, we propose, initiates immune amplification, including characteristic scleroderma-specific antibodies. We propose that the interferon also acts on adventitial stem cells, producing myofibroblasts, rarefaction, and intimal hyperplasia—three morphologic changes that characterize this disease. Regulator of G-protein signaling 5 (RGS5), a regulator of vasoactive G-protein-coupled receptors, is a cell type-specific marker of pericytes and scleroderma myofibroblasts. RGS5 may provide a key link between initial hyperplasia and fibrosis in this disease.

Keywords

Type 1 interferon; Vasculopathy; Fibrosis; Adventitial stem cell; RGS5

Introduction

Despite promising results from recent clinical trials examining the response of patients to bone marrow stem cell replacement [1], there is no evidence that scleroderma (SSc) is the result of an immune response to the endothelial cells (ECs) or smooth muscle cells (SMCs) that comprise blood vessel walls. However, in this review, we summarize the evidence implicating vessel wall cells in the origins of this disease, and present a hypothesis that unites the vascular and immune phenotypes of SSc.

Multiple types of evidence support the hypothesis that SSc develops in response to an initial vascular insult:

1. Initial appearance as vasospasm. Vasospasm (Raynaud's phenomenon) is a nearly universal initial symptom of SSc.
2. Diffuse arterial intimal hyperplasia. Intimal hyperplasia, similar to the arteriopathy (transplant arteriosclerosis [TPA]), is the characteristic arterial pathology of SSc. TPA is associated with late-term loss of transplanted organs. Intriguingly, TPA is relatively unresponsive to anti-inflammatory or immunosuppressive approaches, and intimal hyperplasia is a known cause of vasospasm.
3. Capillary malformations. Another major pathological feature of vessels in SSc is capillary malformation and rarefaction. Capillary malformations are a common feature of ischemic tissue, representing the production of vascular endothelial growth factor (VEGF), an angiogenic factor, in response to hypoxia. Rarefaction is a relatively uncommon vascular pathology, although it has been observed in hypertension, cardiac failure, and diabetes [2–5]. As we review below, rarefaction in SSc is fully reversible following stem cell transplantation [6•, 7].

In summary, the pathological presentation of SSc suggests a primary vascular etiology, while the response to stem cell transplantation suggests that maintenance and progression of the disease is an immunologic or inflammatory process. The chronicity of the immune response is critical to SSc. This is significant given that the immune response is mediated by positive feedback loops, apparently representing processes that evolved to contain, suppress, and correct injuries induced by foreign agents.

Our concept of chronic fibrosis, as a result of a feedback response relevant to SSc, is tied to a hypothesis suggested by Nakanishi et al. [8•] and Erwig and Henson [9]. Henson and collaborators demonstrated that cells attracted to sites of acute or chronic inflammation by bacteria or the products of cell necrosis are themselves harmful to the surrounding healthy tissues. The activated inflammatory cells, including T-helper type 1 (Th1) lymphocytes, produce cytokines and proteolytic enzymes that are very destructive. Therefore, the activated inflammatory cells must be removed to limit damage to surrounding tissue and to allow regeneration or fibrosis to occur. Henson and collaborators presented evidence that this stage of inflammation is controlled by the response of Toll-like receptors to apoptotic debris produced as the neutrophils and macrophages undergo programmed cell death [8•].

For example, the characteristic cytokine of the Th1 response, interferon (IFN)- γ , increases the production of interleukin (IL)-12 by dendritic cells and macrophages. IL-12 stimulates the production of IFN- γ in Th cells, thereby promoting the Th1 response. IFN- γ also inhibits the production of additional cytokines, including IL-4. In contrast, IL-4 induces its own expression and thus stimulates additional T cells from the Th1 response to the new pathway. Relevant to SSc, it is important to realize that the profibrotic cytokine transforming growth factor (TGF)- β is also part of the lymphocytic cytokine profile. Both regulatory T cells and Th3 cells produce TGF- β and IL-10. TGF- β is especially relevant because its expression is characteristic of sclerotic tissues, including SSc [10]. What may be less known to readers is that IL-4, like TGF- β , is profibrotic. This has been studied most for IL-13, a homologue of IL-4 that binds through the IL-4 receptor. Most of the biological effects of the IL-4 receptor are linked to a single transcription factor: signal transducer and activator of transcription 6.

IL-13 has been implicated in multiple fibrotic pathologies, including schistosomiasis and pulmonary fibrosis [11].

We suggest that recent observations of type 1 IFN in SSc may correlate the vascular phenotype of SSc with a chronic immune response. As with Henson's hypothesis that apoptosis induces cytokines associated with the Th2 response, type 1 IFN in SSc is induced by apoptotic debris [12•]. Stetson and Medzhitov [13] suggested that the IFN pathway evolved as part of the response to circulating membrane/nucleotide complexes—the same apoptotic blebs studied by Henson. The relevance of the Stetson and Medzhitov [13] hypothesis is further supported by genetic association studies implicating the IFN response pathway and the apoptotic pathways in systemic lupus erythematosus (SLE) [14], and recently in SSc [15, 16]. Peripheral blood monocytes and lymphocytes in SLE and SSc show expression patterns characteristic of stimulation by type 1 IFNs [17, 18].

Finally, when SSc patients are treated by bone marrow stem cell transplantation, capillary regeneration, as well as a decrease in fibrosis, is correlated with a loss of expression of type 1 IFN in the skin [6•, 19]. Although the possible role of this cytokine in any vasculopathy remains speculative, it is clear that type 1 IFNs can elicit at least part of the SSc phenotype. We propose that SSc is mediated by VEGF and type 1 IFNs, the aberrant properties of which result in fibrosis and vasculopathy (Fig. 1).

Evidence of a Mechanism for Vasospasm in Scleroderma from Autopsy: Intimal Hyperplasia

Arteries have three layers, the innermost of which is called the intima. Intimal hyperplasia occurs when mesenchymal cells accumulate in the intima, and hyperplasia occurs normally during development. In addition, intimal hyperplasia is a near-universal response to arterial injury; it occurs in response to mechanical denudation of arterial endothelium, application of cytokines to the adventitia, exposure to radiation, and the placement of a cuff around the vessel [20]. Finally, in humans, atherosclerotic lesions localize to sites in which intimal hyperplasia occurs normally, possibly explaining the fact that the plaque SMCs are monoclonal [21].

SSc has two very different phenotypic subsets: limited SSc and diffuse SSc. These can be identified by differences in clinical presentation and by the temporal relationship to vasospasm (Table 1). Limited SSc is so named because skin involvement is limited to the hands and face, while a wider extent of skin involvement is observed in diffuse SSc. Whereas rarefaction and ischemia are most obvious in the fingers of limited SSc patients, diffuse SSc patients exhibit more widespread ischemia. Importantly, intimal hyperplasia has been documented at all levels of the arterial tree, from large muscular arteries down to precapillary arterioles. The best evidence for intimal hyperplasia in the entire arterial tree was found in a remarkable autopsy series reported by D'Angelo et al. [22] and recently reviewed by the present authors [7]. The D'Angelo et al. [22] study identified the central role of the occlusive arteriopathy in three of the four main causes of death from SSc: pulmonary hypertension, heart failure, and kidney crisis [23].

Functionally, intimal hyperplasia may be a major cause of vasospasm. Any increase in wall mass will greatly amplify the effect of vasoconstriction on restriction of blood flow [24]. For example, intimal hyperplasia precedes the vasospasm required for closure of the ductus arteriosus [25]. Shimokawa and Takeshita [26] have also shown that intimal hyperplasia amplifies the spastic response to vaso-constrictors implicated in vasospasm. The ability of a rho-kinase inhibitor to block intimal hyperplasia and prevent vasospasm may be relevant to SSc. Finally, the possibility that the clinical equivalent of this pathology may be Raynaud's phenomenon is supported by evidence that intimal hyperplasia is also an early event in the natural history of SSc; however, the mechanism of this characteristic feature of SSc has not been elucidated.

Does Capillary Rarefaction Imply Endothelial Cell Death?

Surprisingly, there is no reliable evidence for cell death in SSc. In support of the hypothesis that a systemic injury to the endothelium initiates the SSc response, we have found evidence of a decrease in the number of capillaries in clinically involved and uninvolved skin of patients with SSc [6•]. Interestingly, capillary rarefaction also has been described in additional cardiovascular diseases, including hypertension, diabetes, and heart failure [2–5].

Although it may seem obvious that rarefaction implies cell death, and several reports have claimed that SSc patients show increased numbers of circulating ECs, this is not direct evidence that cells are actually dying. Circulating cells may be dead cells, endothelial precursor cells, or ECs being lost into the circulation at sites of angiogenesis. Objective evidence of endothelial cell death in vivo has not been demonstrated using classic methods of apoptosis, including TUNEL staining, the presence of apoptotic morphology, or electron microscopic analysis. Failure to observe cell death can be misleading. A clinically significant rate of EC death in SSc could be too low to observe by conventional methods. The normal rate of turnover in endothelium is estimated at only 1 cell per 1,000–10,000 per day. Thus, substantial losses of ECs could occur without ever being detected histologically.

Absence of objective markers of cell death does not imply that the endothelium in SSc is normal. ECs in SSc are morphologically abnormal. The SSc endothelium has characteristics of inflammatory endothelium, including a swollen, heaped up appearance; an activated hypochromic nucleus; and expression of CD123 [7]. Moreover, we determined that vascular endothelial (VE)-cadherin, the lineage-specific marker for endothelium and a junction protein required to form endothelial tubes, is downregulated in the ECs of SSc patients [6•, 27]. As VE-cadherin is required to assemble ECs into tubes, we suspect that angiogenesis is impaired in affected tissues of SSc patients, even though levels of VEGF are quite high [6•].

Relationship of Vasculopathy to Fibrosis

Hypoxia promotes angiogenic and fibrotic signaling pathways. Moreover, while the major clinical manifestations of SSc are attributed to fibrosis, these are associated with intimal hyperplasia in the affected organs, including the skin, lung, heart, and kidney [23]. Does the vasculopathy produce fibrosis or vice versa?

Fibrosis can be produced by several experimental or genetic diseases used as models of SSc. These fibrotic models include TGF- β overexpression, inhalation of bleomycin, and the graft-versus-host disease (cGVHD). The tight skin murine model of SSc is an extremely fibrotic model, but there is no evidence of vascular injury [28]. Other models currently being studied have shown evidence of endothelial damage or intimal hyperplasia, but not both [29]. A particularly attractive model for SSc is cGVHD. Like SSc, cGVHD has an immunologic basis, but it progresses apparently independently of immunologic injury [30]. Endothelial cell death is not obvious in these models, and we have not found such evidence, or evidence of rarefaction, in our own unpublished studies.

One intriguing model arises from an analysis of progressive fibrosis in a genetic model in birds [31]. In contrast to endothelium in human SSc, ECs in these birds show evidence of injury and death in the very early stages of disease (ie, TUNEL⁺ and von Willebrand factor [vWF]⁺). The birds go on to develop severe fibrotic dermal disease, as well as progressive fibrosis of the internal organs resembling SSc, even though evidence of endothelial injury subsides. It is intriguing to hypothesize whether a transient endothelial event, perhaps due to vasospasm, could play a similar role in humans.

Adventitial Cell: A New Player in Vascular Remodeling

A new player in vascular biology, the adventitial stem cell (ASC), may be critical to defining the target cell of SSc. These cells, similar to pericytes, play potential roles in intimal hyperplasia; rarefaction; and, as we discuss, fibrosis.

ASCs have recently received more attention from the vascular biology community. Based on cell kinetic studies conducted 30 years ago, the common wisdom has been that intima arises from medial SMCs [32]. However, Wilcox and colleagues [33] demonstrated that following balloon angioplasty, the major source of intimal cells was the adventitia, not the media. Since that seminal study, the origin of at least some intimal cells from the adventitia, especially in TPA, is becoming an accepted fact.

The error in the original model was the assumption that the vessel wall had only two compartments: the intima and the media. More recently, a variety of evidence has supported the origin of intimal cells from a previously poorly defined cell, the adventitial fibroblast [33, 34]. It now appears that arterial adventitial cells comprise a stem cell population, formally termed ASCs. These cells express characteristic markers of stem cells, including Sca1 and CD34 [35••]. Moreover, at least in the myocardium, maintenance of the stem cell state is essential to vascular stability. Lavine and colleagues [36••] demonstrated that adventitial cells express *patched*, a marker of stimulation by Hedgehog (Hh) [36••]. ASCs are maintained in that state by Hh. However, when Hh is blocked, ASCs differentiate, and myocardial capillaries undergo rarefaction. These studies offer the first mechanistic insight into a cause of vascular rarefaction. In summary, vessel walls consist of three cell types: ECs, SMCs, and adventitial cells. The adventitial cell has stem cell properties and may be the major source of intimal cells.

While blood vessels are defined by their endothelial linings, the development of vascular networks and pruning of these networks depends on interactions of the ECs with cells in the

surrounding mesenchyme—the perivascular cells. The development of complex branched vascular networks, and especially stability of these networks, is a natural event that occurs during development, tissue repair, and response to vascular injury [37•, 38]. These processes are mediated primarily by hypoxia, with the resulting stimulation of hypoxia-induced factor-1 α , VEGF, and platelet-derived growth factor (PDGF)-B [39, 40]. Immature endothelial tubes that have yet to become encoated by perivascular cells exist in a “plasticity window.” In this plastic state, cell junctions, presumably mediated by VE-cadherin (see above) and other components of the cell junction, are poorly formed. Consequently, ECs are susceptible to detrimental stimuli that would not affect mature ECs. The adventitial cell supports the SMC layers of the vessel wall.

During vascular remodeling, perivascular cells are attracted by PDGF-B produced by the endothelium [39]. Once vessels mature, perivascular cells encoat and stabilize the vessel. Pericytes, like SMCs, express smooth muscle α -actin (SMA), but they also express other markers, including, NG2; PDGF receptor- β ; and, as we discuss below, regulator of G-protein signaling 5 (RGS5) [41•]. Detachment of pericytes in response to excess PDGF destabilizes endothelial tubes, resulting in vessels that are vulnerable to pruning or rarefaction [5, 40]. For example, this process may occur in the uterus of women with preeclampsia who also experience rarefaction of capillaries, apparently due to the profound inhibition of VEGF expression [42]. Similarly, loss of adventitial cells in the coronary arteries of mice results in rarefaction [36•]. As already noted, current evidence suggests that neointima, including possibly in the thickened intima characteristic of SSc, originates from the adventitial cells [33, 34]. Thus, the ASCs could be a critical player in rarefaction and intimal hyperplasia.

RGS5 is a Marker for Pericytes and Scleroderma Myofibroblasts

In healthy adults, RGS5 is a marker of arteries and arterioles [43]. During development, however, RGS5 expression correlates with coating of the newly formed capillary spouts by pericytes [44]. This coincides with the cessation of branching morphogenesis [45]. Furthermore, RGS5 has been implicated in the stabilization of vessels in cancer [46•]. Finally, we demonstrated that SSc patients are characterized by an abundance of RGS5⁺ myofibroblasts [6•]. Therefore, the involvement of RGS5 in pericyte–EC interactions during vessel remodeling seems critical.

The observation of RGS5 expression in myofibroblasts of SSc patients and its control of vascular stability raises an obvious question: what is the function of RGS5? RGS proteins act as guanosine triphosphate (GTP)ase-accelerating proteins for the G α proteins. Members of the RGS-R4 subfamily of RGS proteins (RGS1, 2, 3, 4, 8, 13, 16, and 18) are specific for the G α_q and G α_i small G-proteins [47]. When agonists bind to G-protein–coupled receptors (GPCRs), the receptors release G α_{GTP} , leading to activation of down-stream signaling pathways. Presence of an RGS targeted to the specific G α is believed to accelerate the hydrolysis of bound GTP, returning G α to the inactive, guanosine diphosphate–bound state (G α_{GDP}). In turn, G α_{GDP} returns to the GPCR. Therefore, it is likely that any cell with elevated RGS5 will show decreased responsiveness to agonists of receptors that use these small G proteins, including angiotensin, endothelin, and sphingosine-1-phosphate. Perhaps

related to this, our group [48•] and others [49] have demonstrated that RGS5-null mice are hypotensive. In summary, RGS5, a likely candidate for artery-specific regulation of GPCR function, is a marker of pericytes and is associated with maintaining arterial stability.

The stimulus for SSc myofibroblasts to express RGS5 could be PDGF [50, 51]. Baroni et al. [52] suggested that autoantibodies to PDGF, perhaps anti-idiotypic antibodies that are able to stimulate the PDGF receptor, could be the cause of SSc. Although these authors focused on PDGF as a potentially profibrotic molecule, the overlap of activation and blocking binding by a low-affinity ligand makes their interpretation problematic. Nonetheless, support for the critical role of PDGF comes from the observations already noted that insufficient PDGF expression prevents the encoachment and stabilization of vessels, while excess PDGF may disrupt the coating, leading to rarefaction. Therefore, in SSc, we propose that both the intimal cell and the myofibroblast may be derived from abnormally functioning ASCs.

What is the Role for Type 1 Interferons in Scleroderma?

We suggest that type 1 IFNs (IFN- α and IFN- β) might provide a bridge between the vascular pathology of SSc and the apparent role of the immune response. We demonstrated that peripheral blood monocytes and lymphocytes from patients with SSc show expression patterns characteristic of stimulation by type 1 IFNs [12•, 17], a pattern also observed in SLE [18].

It is important to distinguish between the different classes of IFNs. Most IFN-related studies in vascular biology refer to IFN- γ , not IFN- α or IFN- β . As described by Tellides and Pober [53], effects of IFN- γ on blood vessels in vivo may reflect the activation of macrophages and the increase in antigen presentation by IFN- γ . These activated cells of the immune system express cytokines that act upon SMCs. Tellides and Pober [53] inserted pig and human arteries into the aortas of immunodeficient mice and found that IFN- γ potentiated growth factor-induced mitogenesis and intimal thickening. Consistent with this, a recent study reported that IFN response factor 1 (*IRF1*), a gene regulated by stimulation of the IFN receptor, inhibits intimal formation [54•]. In contrast, we described type 1 IFN expression, and notably IFN- α , as being critical to SSc [6•, 17]. Shen and colleagues [55] found that type 1 and type 2 IFNs have very different effects on myofibroblasts from hepatic fibrosis [55]. In vitro, IFN- β and IFN- γ significantly inhibited myofibroblast proliferation, but IFN- α had no effect. Similarly, IFN- β and IFN- γ reduced SMA expression, while IFN- α did not affect SMA expression.

Our observation of an IFN response signature in the SSc monocytes [17] is also reminiscent of a report by Schirmer et al. [56•]. They recently examined the IFN response patterns in monocytes from patients with coronary artery disease, comparing those who successfully develop collaterals with those who fail to form collateral vessels [56•]. Monocytes, after stimulation with lipopolysaccharides, showed different transcription patterns depending on the number of collaterals formed. Furthermore, IFN- β and its downstream targets were increased in the absence of collateral formation. Type 1 IFNs have been proposed to inhibit endothelial migration and to promote endothelial death, and are therefore markedly

antiangiogenic [57]. Therefore, the failure to form vessels, and perhaps rarefaction, might be explained by the described antiangiogenic properties of type 1 IFNs.

A Unifying Hypothesis for Scleroderma

IFNs are implicated as critical mediators of SSc because we demonstrated that peripheral blood monocytes and lymphocytes from patients with SSc show expression patterns characteristic of stimulation by type 1 IFNs, a pattern also seen in other autoimmune diseases [12•, 58•]. In the case of SSc, IFN is probably generated in the sites of active SSc rather than at some systemic source, because efforts to identify type 1 IFNs in the serum have not yet been successful. In contrast, we found that the induction of IFN- α expression is a prominent feature of the affected skin of SSc patients [6••]. In other studies from our group, we found that apoptotic debris, combined with antibodies present in SSc, stimulated the production of type 1 IFN by plasmacytoid dendritic cells [12•].

This is consistent with the suggestion from Erwig and Henson [9] and Stetson and Medzhitov [13] that the production of IFN, in response to viral infection, may have evolved in chordates to mediate the anti-inflammatory response to apoptotic debris. Because an anti-inflammatory, profibrotic response follows apoptotic cell injury, a role for IFNs in response to death also suggests a functional relationship between type 1 IFNs, TGF- β , and possibly the IL-4 receptor. Type 1 IFNs, however, may also amplify the immune response by preventing apoptosis of expanding T cells and B cells [12•]. We hypothesize that the pathogenesis of SSc, as well as other autoimmune diseases, is initiated by apoptotic events that trigger expression of type 1 IFNs. This expression, as Stetson and Medzhitov [13] suggested, may be pathological for two reasons: 1) type 1 IFN expression itself promotes expression of type 1 IFN, and 2) the effect of type 1 IFN on the immune system may enhance expansion of clones that may include antibodies to apoptotic fragments. Importantly, this hypothesis does not require the initial event in SSc to be vascular injury. Rather, it suggests that action of type 1 IFNs upon cells of the vessel wall may result in SSc. We suggest that the ASC may be the critical cellular target (Fig. 1).

As we have discussed, ASCs play a critical role in intimal hyperplasia. Loss of the normal function of the adventitial cells would explain intimal hyperplasia. Intimal hyperplasia at the level of larger arteries would appear as vasospasm (Raynaud's phenomenon). Intimal hyperplasia at the level of small arterioles would cause hypoxia by increasing peripheral resistance. The resulting deficiency of flow would, of course, be exacerbated by vasospasm, resulting in hypoxia. Hypoxia stimulates expression of VEGF. Excess VEGF in this hypothesis causes ASCs/ pericytes/mural cells to detach from vessels. As a result, these small vessels become unstable and vulnerable to rarefaction. Finally, the expression of RGS5 by myofibro-blasts in SSc completes the hypothesis, suggesting that one or more vasoactive agonists could play a role in vasospasm and formation of the myofibroblast.

Conclusions

The following are key issues raised by the hypothesis:

1. The hypothesis suggests that fibrotic animal models would more closely resemble SSc if the models were augmented by the induction of ischemia and the production of type 1 IFN.
2. The hypothesis suggests that intimal hyperplasia may precede Raynaud's phenomenon in SSc.
3. It suggests that patients would benefit from drugs that block intimal hyperplasia. Fasudil, a rho-kinase inhibitor, is especially interesting because of its combined effects on hyperplasia and vasospasm [26].
4. The hypothesis suggests that type 1 IFN may prevent changes in ASCs that are required for normal angio-genesis and may play a critical role in differentiation of these cells into myofibroblasts.
5. RGS5, a key marker of pericyte formation and regulator of vasospastic GPCR agonists, may provide a key link between intimal hyperplasia and fibrosis in SSc.

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Fig. 1.

a Target cell for scleroderma (SSc): adventitial stem cell (ASC). Recent studies, largely from Lavine et al. [36••] and Passman et al. [35] (see text), have identified the ASC as critical in vessel stability. This cell surrounds the artery wall, may be related to pericytes, is likely the cell of origin for intimal hyperplasia, and is a potential precursor to the myofibroblast (arrow indicates the movement of the ASC both into the intima and out to form myofibroblasts). Our unifying hypothesis suggests that these cells may be the initial target cells for SSc. **b** A unifying hypothesis for SSc. We propose that the initial event in SSc is intimal hyperplasia. The neointima is prone to vaso-spasm, as seen as experimental animals and in Raynaud's phenomenon. Vasospasm is fully reversible unless, as proposed here, the resulting ischemia leads to apoptosis and activation of type 1 interferon (IFN). The IFN, we propose, initiates immune amplification that is characteristic of SSc, with SSc-specific antibodies reflecting the ischemic vascular injury. The IFN, we propose, also acts on ASCs,

producing both myofibroblasts and more intimal hyperplasia. Vascular endothelial cell growth factor (VEGF) induction, as a result of ischemia, stimulates angiogenesis, but rarefaction occurs because of the lack of normal pericyte functions required to stabilize the newly formed vessels

Table 1

Differences in vascular involvement in the two subtypes of scleroderma

Vascular change	Limited SSc	Diffuse SSc
Raynaud's phenomenon	Often precedes diagnosis	Concurrent with or after diagnosis
Capillary malformations	Early	Concurrent with diagnosis
Intimal hyperplasia	Often severe and widespread	Variable but commonly involves small arteries
Rarefaction	Not yet studied	Early
Cause of death	Pulmonary hypertension	Pulmonary fibrosis/heart disease

SSc scleroderma