The microvascular endothelium in scleroderma

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Vascular endothelial injury in SSc leads to a host of pathological changes in the blood vessels that adversely impact the physiology of many organ systems and eventually results in a state of chronic tissue ischaemia. Current hypotheses in SSc vascular disease pathogenesis suggest a possible infectious or chemical trigger(s) that activates both cellular and humoral immunity. Products of immune activation may lead to vascular injury possibly through the production of autoantibodies and the release of products of activated T cells that can directly damage the endothelium. Knowledge of the initial trigger of immune activation in SSc may offer an opportunity to develop a multiple step strategy for therapeutic intervention.

**Key words:** Scleroderma, Scleroderma vascular disease, Endothelial cells, Raynaud’s phenomenon, Endothelial apoptosis, Cytomegalovirus, Anti-endothelial antibodies.

**Introduction**

The endothelium normally exists as a continuous monolayer connected with its closely apposed basal lamina. It is functionally remarkable in regulating coagulation and fibrinolysis, permeability, vasoreactivity and cellular metabolism and nutrition.

The microvascular endothelium in SSc is severely damaged, basal laminae are usually thickened and reduplicated, and a vast number of capillaries are missing and obliterated with remarkable absence of new vessel formation. Microvascular endothelial cell (MVEC) injury and apoptosis is a central event in the pathogenesis of SSc vasculopathy that leads to microcirculatory dysfunction and eventual organ failure. Microvascular dysfunction is prominent in the early stages of the disease and progressively worsens as the disease progresses. The dysfunction is manifested by increased permeability and dysregulated control of vascular tone that is clinically best illustrated by the puffy hand stage and by RP. An imbalance in endothelial vascular signals with increased endothelium production and impaired nitric oxide and prostacyclin release mediates the vasospasm and contribute to intimal proliferation and vascular fibrosis and stiffness of the vessel wall. Platelet activation and enhanced coagulation with reduced fibrinolysis lead to fibrin deposits and contribute to the intimal proliferation and luminal narrowing.

MVEC apoptosis may also activate the immune-inflammatory system by dendritic cells and macrophage presentation of self-antigen present in the apoptotic debris to CD8+ T cells [1], and by the direct activation of the alternate complement and coagulation cascades leading to microvascular thrombosis and further vessel compromise [2]. MVEC apoptosis may also confer a state of resistance to apoptosis by the surrounding fibroblasts that may lead to myofibroblast differentiation and tissue fibrotic changes that follow [3]. The mechanism of MVEC apoptosis is not known; however, experimental studies identified multiple pathways that may lead to apoptosis including viral agents, cytotoxic T cells, antibody-dependent cellular cytotoxicity, anti-endothelial antibodies and ischaemia–reperfusion injury (Table 1).

**Viral triggers**

A human Cytomegalovirus (hCMV) instigated process that may lead to MVEC apoptosis is suspected in SSc because of increased levels of antibodies to this virus and because of its known association with vascular intimal proliferation and vasculopathy in graft rejection and coronary artery bypass restenosis. The evidence suggests that in SSc, some anti-topoisomerase I antibodies recognize the epitope—VTLLGAGIWLPP—contained within the hCMV-derived UL94 protein, which is homologous to the highly expressed MVEC surface protein NAG-2 (tetraspan novel antigen-2). Moreover, affinity-purified anti-UL94 peptide antibodies are shown to induce MVEC apoptosis in vitro, suggesting a molecular mimicry mechanism involvement in MVEC apoptosis [4, 5].

**Cytotoxic T cell**

Cytotoxic T-cell involvement in MVEC apoptosis is suggested by histological and experimental findings in the disease. Thus, it is known that MVEC apoptosis can result from their interaction with cytotoxic T cells either by Fas or granzymes/perforin-related mechanisms. For example, CD4+ T cells can mediate MVEC apoptosis by a Fas-related mechanism as seen in cytolytic T cells killing of vascular endothelium in the rejection reaction, whereas the granzyme/perforin system mediates apoptosis by the major cytotoxic T cells, the CD8+ T cells, NK and LAK cells. Granzymes gain access to the cells following cellular membrane damage by perforin. Involvement of cytotoxic T cells in SSc is suggested by the presence of a 60 kDa protein in SSc sera that was described as an endothelial cytotoxic factor. This factor was characterized as the granular enzyme, and was detected in the perivascular spaces in SSc skin biopsies.

**Antibody-dependent cellular cytotoxicity**

Antibody-dependent cellular cytotoxicity of vascular endothelium is reported in up to 40% of the SSc patients. The effector cells express Fc receptors and are both non-T cells and non-adherent T lymphocytes, while the antibody is an IgG with MVEC specificity that mediate MVEC cytotoxicity via the Fas pathway.

**Anti-endothelial antibodies**

Anti-endothelial cells antibodies (AECA) are present in 40–50% of the SSc sera and are mostly of the IgG1 isotype. The antibody titres correlate negatively with pulmonary diffusion capacity and positively with pulmonary hypertension and with digital ischaemic ulcers, suggesting a pathological role in the development of the vascular disease. Some AECA are reported to induce MVEC apoptosis independent of the fas–fas ligand pathway. This is clearly shown in the chicken model of SSc [UCD-200], where serum transfer into normal chicken embryos results in binding of antibodies to the microvascular in the choroida allantoic membrane in association with endothelial apoptosis [6]. The exact
identity of the endothelial antigen is not known; however, a topoisomerase 1 specificity for some AECAs has been suggested. Moreover, SSc sera containing ACAs or anti-topoisomerase I antibodies can induce MVEC apoptosis in association with increased gene expression of caspase 3 and the SSc autoantigen fibrillin 1 [7].

The only published proteomic analysis of endothelial antigen(s) recognized by AECAs identified 53 proteins consisting of cytoskeleton proteins, proteins involved in cellular mobility, regulation of apoptosis and senescence as well as proteins implicated in clotting and antigen presentation [8].

Ischaemia–reperfusion injury

Ischaemia–reperfusion injury is an inflammatory process that results from interaction between humoral and cellular components including the complement, cytokine and the contact-activated cascades. In general, soon after the start of reperfusion, endothelial dysfunction of ischaemic vascular bed develops. The initial endothelial dysfunction appears to be related to adhesion molecule expression and the recruitment of neutrophils and platelets. MVEC injury is believed to be mediated by superoxide radicals formed by endothelial cells and by neutrophils, perhaps via the hypoxanthine–xanthine oxidase pathway. Superoxide inhibits the release of nitric oxide, prostacyclin, tissue plasminogen activator, protein S and xanthine oxidase pathway. Superoxide inhibits the release of nitric oxide, perhaps via the hypoxanthine–xanthine oxidase pathway. Superoxide inhibits the release of nitric oxide, prostacyclin, tissue plasminogen activator, protein S and xanthine oxidase pathway.

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Conclusions

The aetiology and pathogenesis of SSc remain unknown. Nonetheless, signs of vascular injury and devascularization of involved organs in association with evidence of profound endothelial dysfunction are well documented. The fact that the vascular tree, particularly the microcirculation, is the target tissue in this disease is now well established. It is likely that the immune process is aimed at the destruction of microvessels leading to the clinically recognized state of chronic organ ischaemia. Identification of the initial vascular trigger of immune stimulation is fundamental to our understanding of the disease. The impact of vasculopathy on disease complications is clearly demonstrated by the fact that most of the successful therapeutic interventions in SSc are directed at the vascular disease (Table 2). Still, countless central issues in the pathogenic process of SSc remain poorly understood. Issues related to the initial trigger in the disease, the nature of immune activation, mechanisms of intimal proliferation and the relationship of vascular injury to tissue fibrosis are some of the unresolved essential questions.

References