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Review

# Vascular hypothesis revisited: Role of stimulating antibodies against angiotensin and endothelin receptors in the pathogenesis of systemic sclerosis☆

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ABSTRACT

Systemic sclerosis (SSc) is a connective tissue disorder of unknown etiology characterized by the presence of multiple autoantibodies, including those against angiotensin and endothelin receptors. Patients with SSc can develop heterogeneous clinical manifestations including microvascular damage, the dysregulation of innate and adaptive immunity, and generalized fibrosis of multiple organs. Autoantibodies against angiotensin II type I receptor ($AT_1R$) and endothelin-1 type A receptor ($ET_AR$) play important roles in the pathogenesis of SSc. These autoantibodies regulate physiological processes ranging from production of collagen by skin fibroblasts to angiogenesis modulation. Understanding the mechanisms behind autoantibodies against $AT_1R$ and $ET_AR$ could provide insight to future novel therapies for SSc patients. In this review, we focus on elucidating the immunopathological mechanisms triggered by anti-$AT_1R$ and anti-$ET_AR$ autoantibodies to summarize current knowledge about vascular abnormalities resulting in progressive damage of organs seen in patients with SSc.



**Contents**



## 1. Vascular abnormalities in systemic sclerosis

Systemic sclerosis (SSc) is an autoimmune disorder characterized by the pathogenetic triad of microvascular damage, the dysregulation of innate and adaptive immunity, and generalized fibrosis in multiple organs [1]. SSc can be classified into limited and diffuse cutaneous forms according to the skin involvement [2]. Clinically, one of the first signs of vascular involvement in SSc is the Raynaud's phenomenon, an abnormal reactivity of blood vessels to cold and other stimuli [3]. Despite all the treatments currently available, vasculopathies are the leading cause of Raynaud's phenomenon, digital ulcers, renal failure, and pulmonary arterial hypertension in SSc patients. These complications result in increased morbidity and mortality rates of patients with SSc [4].

The systemic manifestations of SSc were aptly explained by the vascular hypothesis introduced by Campbell and LeRoy in 1975 [5].

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They were the first to propose that the clinical manifestations of SSc are due to vascular lesions developed at varying rates and degrees across multiple organs. Campbell and LeRoy postulated that a cascade of events, including dysregulated inflammatory responses and persistent activation of fibroblasts, leads to decreased blood flow and eventual fibrosis of involved organs.

Capillary and small vessels, which are the main regulators of blood flow resistance in the circulation, are most commonly involved in SSc vascular defects. Abnormalities seen in these vessels comprise gaps, vacuolization, and eventual destruction of endothelial cells. Other vessel abnormalities include reduplication of the basal lamina, fibroblasts, and pericyte enlargement followed by perivascular fibrosis, and perivascular infiltration of immune cells consisting mainly of macrophages, T cells, and B cells [6,7].

In the last decades a variety of functional autoantibodies have been identified in patients with SSc [8]. Among them, increased concentrations of stimulating autoantibodies against both angiotensin II type 1 receptor ($AT_1R$) and endothelin-1 type A receptor ($ET_AR$) have been reported in SSc [9]. Two important observations led us to hypothesize that autoantibodies against $AT_1R$ and $ET_AR$ could be involved in the vascular abnormalities in SSc. First, the detrimental effects caused by autoimmune agonistic antibody-mediated $AT_1R$ stimulation were found in severe vasculopathies associated with allograft rejection and preeclampsia [10,11] Second, the vasoconstrictor as well as proinflammatory, proliferative, and profibrotic effects of angiotensin II (Ang II) and endothelin-1 (ET-1) mediated by the pleiotropic role of $AT_1R$ and $ET_AR$ implicated these molecules in the pathogenesis of SSc [12,13].

In this review, we focus on elucidating the immunopathological mechanisms triggered by anti-$AT_1R$ and anti-$ET_AR$-stimulating autoantibodies to summarize current knowledge about vascular abnormalities resulting in systemic progressive damage of organs seen in patients with SSc.

## 2. Anti-AT1R and anti-ETAR antibodies are markers of disease manifestations in systemic sclerosis and other vascular and fibrotic diseases

About 85% of SSc patients have increased levels of anti-$AT_1R$ and anti-$ET_AR$ antibodies compared to healthy donors. In patients with SSc, the antibody levels strongly correlate with each other and show cross-reactivity for both receptors [9]. The antibodies are mainly associated with vascular symptoms of SSc such as pulmonary arterial hypertension (PAH), digital ulcers, and renal crisis. However, increased concentrations of both antibodies are also associated with the diffuse SSc subtypes as well as with lung fibrosis. High concentrations of both anti-$AT_1R$ as well as anti-$ET_AR$ antibodies predict SSc-related mortality, PAH, and response to PAH therapy as well as immunosuppressive therapy [9]. High levels of anti-$ET_AR$ antibodies also predict incidental digital ulcers [14]. Finally, anti-$AT_1R$ and anti-$ET_AR$ antibodies were also found in other diseases and were linked to obliterative vasculopathy and fibrosis (Table 1). These data suggest a possible role of the antibodies in disease mechanisms.

## 3. Role of stimulating antibodies against angiotensin and endothelin receptors

$AT_1R$ and $ET_AR$ are expressed on cells of both the vascular and immune system. These two molecules belong to the G-protein-coupled receptor (GPCR) family, which comprises the largest superfamily of diverse integral membrane proteins [15]. Binding of stimulating anti-$AT_1R$ and anti-$ET_AR$ autoantibodies to their receptors can trigger multiple cellular and systemic events, which are summarized in Fig. 1. In this context, stimulating anti-$AT_1R$ and anti-$ET_AR$ derived from SSc patients induces a variety of cellular responses such as production of TGF-β by human dermal microvascular endothelial cells [9]. They induce IL-8, vascular cell adhesion molecule-1 (VCAM-1), and diminish wound repair in human microvascular endothelial cells. In peripheral blood mononuclear cells, anti-$AT_1R$ and anti-$ET_AR$ antibodies induce T cell chemotaxis and secretion of interleukin (IL)-8 and the chemokine (C-C motif) ligand 18 (CCL18) specifically expressed by monocytes [16]. They induce type I collagen production by skin fibroblasts [17]. These induced effects are significantly reduced by addition of $AT_1R$ and $ET_AR$ blockers.

All of the above-mentioned cellular events are well known to be involved in SSc pathogenesis. For instance, increased levels of the inflammatory cytokine IL-8 are observed in patients with SSc and related to activation of mononuclear phagocytes, neutrophils, fibroblasts, and endothelial cells [18]. In accordance, enhanced levels of circulating IL-8 during inflammatory conditions can lead to tissue damage [19]. TGF-β is a pleiotropic cytokine that induces fibroblast activation, proliferation, and upregulates the synthesis of collagen and extracellular matrix leading to the fibrotic state seen in SSc lesions [20]. Increased production of the chemokine CCL18 in the bronchoalveolar lavage cells from patients with SSc reflects pulmonary fibrotic activity in SSc. SSc skin has an increased proadhesive phenotype that promotes leucocyte adhesion and infiltration via adhesion molecules such as VCAM-1 [21]. Despite all these findings, considering the pleiotropic roles of $ET_AR$ and $AT_1R$ [12,13], a variety of other functions in

**Table 1**
Diseases and mechanisms associated with stimulating autoantibodies against AT1R and $ET_AR$. SSc, systemic sclerosis; SLE, systemic lupus erythematosus; SMC, vascular smooth muscle cell; PASMC, pulmonary artery smooth muscle cell; HUVEC, human umbilical vein endothelial cell; PBMC, peripheral blood mononuclear cell; HMEC, human dermal microvascular endothelial cells; VSMC, vascular smooth muscle cells. OVCAR3, ovarian carcinoma cell line; Pai-1, plasminogen activator inhibitor-1; −, only clinical association but no experiment performed; Ref, reference.

| Diseases | Autoantibody recognizing | Mechanisms associated | Cell type investigated | Ref. |
|---|---|---|---|---|
| **SSc** | $ET_AR$ and $AT_1R$ | • ERK 1/2 phosphorylation, regulation of TGF-β transcription and wound repair; $Ca^{2+}$ release<br>• Chemotaxis, production of IL-8 and CCL18<br>• Cell migration<br>• Production of ROS<br>• Collagen production | • HMEC<br>• PBMC<br>• Neutrophil and T cell<br>• Neutrophil<br>• Fibroblast | [9,16,17] |
| SLE and Lupus nephritis | $ET_AR$ and $AT_1R$ | • Cell proliferation and permeability, production of 5-HTT, PDGFRβ, VEGF-A, and PDGF-B. | • PASMC and HUVEC | [39,40] |
| Renal-allograft rejection | $AT_1R$ | • ERK 1/2 phosphorylation, induction of AP-1, and NFkB activity | • VSMC and endothelial cells | [10] |
| Preeclampsia | $AT_1R$ | • Activation of NADPH oxidase and NFkB<br>• IL-6 and Pai-1 production | • VSMC and trophoblasts<br>• Mesangial cells | [30,41] |
| Hypertension | $AT_1R$ and $ET_AR$ | • Control of heart rate | • Cardiomyocytes | [42] |
| Cancer | $AT_1R$ | • Angiogenesis of the tumor, migration | • OVCAR3 | [43] |
| Diabetes | $AT_1R$ | • Left ventricular dilatation | • – | [44] |
| Graves' disease | $AT_1R$ | • Left ventricular dilatation | • – | [45] |
| Buerger's disease | $AT_1R$ and $ET_AR$ | • Thrombosis | • – | [46] |
| Cystic fibrosis | $AT_1R$ and $ET_AR$ | • Fibrosis | • – | [47] |