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The role of endothelial cells in autoimmune rheumatic disease

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Vasculopathy is a generic feature of autoimmune rheumatic disease and there is substantial evidence that endothelial cell dysfunction has a role in pathogenesis and clinical manifestations of this challenging group of diseases. Endothelial cells (EC) are a target for injury and through their essential functional role in vascular homoeostasis, this has significant impact. In addition, the emerging recognition that EC are important regulators of other cell types and can differentiate into other relevant cell types has direct relevance. These aspects are reviewed with a focus on recent published evidence regarding the importance of EC in development, progression and treatment of autoimmune rheumatic disease. The potential role of the adaptive and innate immune system in causing endothelial cell damage, including anti-endothelial cell autoantibodies, will be reviewed. Recent advances in understanding how EC may differentiate into mesenchymal lineages and the interplay between physiological roles in healing or tissue repair and dysfunctional responses in acquired connective tissue disease will be reviewed.

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Introduction

Endothelial cells (EC) are central to the pathobiology of maintaining vessel and tissue haemostasis. Systemic autoimmune rheumatic diseases, in particular the connective tissue diseases, have aberrant pathways in the function of EC, leading to inflammation, fibrosis and vasculopathy. In this review, we consider the importance of endothelial cell dysfunction in the autoimmune rheumatic diseases — in particular, systemic sclerosis (SSc), systemic lupus erythematosus (SLE), antiphospholipid syndrome (APLS) and vasculitis. We describe the interaction of EC with other key cell types and molecular mechanisms contributing to immune dysregulation. We also explore the recent literature and translational research data looking at potential therapeutic targets and pharmacological treatments that aim to modify the dysregulated state of endothelial function in disease.

Endothelial cell dysfunction in autoimmune rheumatic disease pathobiology and pathogenesis

There are many ways in which EC dysfunction is important in autoimmune disease pathogenesis. This includes the role of EC regulating vascular tone and in inflammation as gatekeepers of the quality and quantity of leucocyte trafficking during immune regulation. The endothelium is a semipermeable single-cell layer lining the vasculature and lymphatic system. ECs act as a physical barrier and first line of defence from pathogens [1].

A generic feature of many autoimmune rheumatic diseases is tissue inflammation and immune activation. Initially, neutrophils and monocytes enter tissue from the bloodstream. Activated macrophages and parenchymal cells produce pro-inflammatory cytokines and chemokines. TNF-alpha and IL-1 are potent cytokines activating ECs resulting in a dysfunctional endothelium. Downstream signalling cascades then activate secretion of GM-CSF, IL-1, IL-6 and monocyte chemoattractant protein [2]. ECs lining the blood vessels are activated producing cell adhesion molecules (ICAM-1, VCAM-1 and E-selectin) that attract circulating leucocytes to sites of inflammation (i.e. skin, kidney, joint, etc.), which is also an important aspect in the development of atherosclerosis and plaque growth [3].

Another pathogenic mechanism is that of the dysregulated humoral immune response. Herein, EC dysfunction produces antibodies that form immune complexes (ICs) with autoantigens such as microparticles and apoptotic bodies. This occurs in both the micro- and macrovasculature of SLE ECs. Therefore, MPs and MP-ICs could be a possible therapeutic target to avoid EC injury in SLE patients. [4] Vascular integrity is maintained by endothelial progenitor cells (EPCs), a heterogeneous population of cells. Once injury or an

environmental insult occurs, EPCs mobilise to replace damaged ECs. In the peripheral blood, EPCs are made up of endothelial cell-forming cells (ECFCs), which are the true endothelial cell precursors and myeloid angiogenic cells (MACs) act to sustain angiogenesis. Both ECFCs and MAC are downregulated in SLE causing vasculopathy [5].

There is also clear evidence that ECs have a role in regulation and may be a target of the adaptive immune system. There is emerging recognition of the central role of the IL-33/ST2 axis in autoimmune disease and ECs may have a central role in perturbation of this pathway. Thus, ECs are one of the key cell populations that express IL-33, a pro-inflammatory nuclear factor that plays an important immunomodulatory role in both physiological and pathological inflammation [6]. It has been shown that under conditions of cellular injury or damage, IL-33 is released from ECs and various other cell types. The IL-33/ST2 axis stimulates several cells of in the innate immune system such as TNF-alpha, TLR4, CD80, CD40 and other chemokines [7]. As a target of the adaptive immune system, it has been shown over many years that antibodies targeting EC surface antigens occur in autoimmune rheumatic disease and may have a role in pathogenesis.

Role of endothelial cell dysfunction in individual diseases

Systemic sclerosis

SSc is a heterogeneous condition with the major drivers of SSc morbidity and mortality being tissue fibrosis as well as both structural and functional vasculopathy resulting in renal crisis, pulmonary artery hypertension (PAH), Raynaud phenomenon and digital ulcers [8]. EC injury is a crucial triggering event for vascular remodelling [9]. ECs have a central pathogenic effector role. The early changes are characterised by microvascular changes, EC apoptosis [10] and immune system activation [11].

Early EC injury causes vascular remodelling and anti-EC antibodies (AECAs) are effectors of EC activation/damage. AECAs are one of the drivers of endothelial damage in SSc [12]. Earlier studies [13] have shown that antibodies directed against antigen targets such as ICAM-1 on the surface of EC tissue result in pro-inflammatory activation.

AECAs have a positive correlation with microvascular abnormalities. Antibodies targeting functional G-protein-coupled receptors (GPCR) have been shown to have a both a physiological and pathological role across a range of autoimmune conditions [14,15]. These include chemokine GPCRs such as CXCR3 and CXCR4 that are overexpressed in SSc and correlate with worsening lung function [16]. ET-1 and AT1R antibodies (*see Therapeutics section*) target vascular GPCRs and are implicated in SSc vasculopathy. Endothelial PAR-1 antibodies cause downstream IL-6 activation and also offer an exciting therapeutic target [17].

The damage to ECs may precede the fibroblastic activation responsible for the fibrotic phenotype of SSc. There is substantial evidence of epigenetic alteration of EC in SSc [6], including DNA methylation and histone changes. Epigenetic modifications in SSc may offer therapeutic targets, for example, DNA methyltransferase inhibitors: azacitidine and decitabine show anti-fibrotic effects [18]. This builds upon a body of evidence implicating epigenetic mechanisms in maintaining the altered phenotype of microvascular EC isolated from SSc skin [19,20].

There is evidence of endothelial-to-mesenchymal transition (EndoMT) in dermal vessels of SSc murine models, leading to dysregulated ECs and their transformation to profibrotic myofibroblasts [21]. This is supported by several studies showing EndoMT in SSc tissues, including in the skin [21] and pulmonary circulation where it may have relevance to complications such as pulmonary arterial hypertension [22].

Systemic lupus erythematosus and antiphospholipid syndrome

There is considerable evidence of the importance of EC dysfunction in other autoimmune rheumatic diseases that have considerable vasculo-centric clinical manifestations including vasculitis. Antibodies to β2-glycoprotein I (β2GP-I)/LDL have been implicated in SLE-associated vascular disease. The APLS is associated with thrombosis and recurrent fetal loss. β2GP-I under oxidative stress activates dendritic cells causing downstream T-cell activation and subsequent IFN gamma production. [23]. Anti-β_2GPI antibodies also mediate EC activation [24]. In *in vitro* studies, modulation of downstream pathways includes activation of p38 MAPK and NF-κB [25,26] resulting in reduced nitric oxide synthesis and increased expression of tissue factor and cell adhesion molecules [27]. Production of type-1 interferons has been shown to mediate damage to ECs amongst other cell types, in the kidney in SLE [28]. SLE risk factors include high levels of cytokines (IL-6, VEGF), endothelial adhesion molecules, costimulatory molecules (CD40/CD40L), CRP and fibrinogen. IC deposition [4] in organs where ultrafiltration processes occur, tissue injury and a pro-coagulant state all result in compromise to the endothelium and microvascular injury.

As noted earlier, EPCs have been implicated in the pathogenesis of SLE [5]. A recent review has provided an overview of the role of EPCs in SLE and the

potential for immunomodulatory agents, including anti-CD20 on B cells (rituximab), anti-IFN-alpha (anifrolumab) and anti-BAFF (belimumab) to restore EPC function [29].

Neutrophil extracellular traps (NETs) are protein scaffolds that contribute to host immune defences. Aberrant production or clearance of NETs and their release of autoantigens contribute to lupus pathogenesis through loss of immune tolerance [30]. A recent study showed that NET-bound RNA is taken up by ECs and can induce exacerbated pro-inflammatory responses in the endothelium in SLE [31]. ANCA and neutrophil activation are responsible for loss of EC integrity in granulomatosis with polyangiitis. Another recent study showed that the *in vitro* angiogenic capacity of ECFCs is impaired when they are treated with sera from patients with anti-proteinase-3-positive vasculitis [32].

Role of anti-endothelial cell antibodies

As described above, AECAs have been implicated in the pathogenesis of SSc. Evidence for their role in SLE and vasculitis, as for SSc, was provided over 20 years ago [33–36] with identification of putative EC self-antigen targets. More recently, two self-antigens from EC were identified in Takayasu arteritis [37]. The utility of these antibodies as potential biomarkers remains to be confirmed, but the presence of AECAs suggests widespread EC activation and damage.

Therapeutic targeting of endothelial cell in autoimmune rheumatic disease

ET-1 as a target for treating Connective Tissue Disease-associated vasculopathy

The endothelial cell-derived vasoconstrictor peptide ET-1 was first discovered in 1988 [38]. ETA and ETB are the two receptor subtypes [39] that are therapeutic targets in SSc-associated conditions — digital ulcers and PAH [40]. Stimulating antibodies for angiotensin (AT1R) and endothelin (ET1R) receptors are thought to be co-factors in the pathogenesis of SSc among other vasculopathies [41].

The RAPIDS-2 trial [45] found bosentan, a non-selective ETA/ETB receptor antagonist, showed a 30% reduction in the number of new digital ulcers in SSc patients. Rubens et al. [42] reported a 10-fold increase in ET-1 plasma concentrations in patients with PAH, which corresponded to disease activity and severity. Ambrisentan, a selective ETA receptor antagonist, is licensed for use in PAH as studied in the ARIES-1 and ARIES-2 trials [43]. Similarly, the SERAPHIN trial showed significant mortality and morbidity benefit with macitentan, a potent dual ETA/ETB receptor antagonist [44]. Thus, ERAs have shown clinical efficacy in trials and are now approved therapies for PAH.

Role for mammalian target of rapamycin inhibitor as therapeutic agent in vasculitis and systemic lupus erythematosus

The mammalian target of rapamycin (mTOR) complex, a serine/threonine kinase known for its immunosuppressive effects [46], modulates EC function. It also has downstream effects on Th1 and Th17 cell proliferation, Treg depletion, plasma cell differentiation, macrophage dysfunction and increased antibody and IC production. Pharmacological blockade of the mTOR pathway in pulmonary hypertension (Everolimus) targets the vascular endothelium and smooth muscle cells [47]. In Takayasu's arteritis, rapamycin significantly reduced IgG-induced EC viability and proliferation [48], thus reducing vascular intimal hyperplasia. Similarly, in SLE [49] and APS [49,50], rapamycin indirectly targets ECs with effector modulation of T cells, plasma cells and ICs. Thus, the mTOR pathway is a promising target in reducing vascular proliferation associated with SLE.

Several other EC-targeting therapeutics are being evaluated in SLE. Existing therapeutics have shown favourable outcomes in SLE. For example, hydroxychloroquine has an immunomodulator effect on ECs in animal models [51]. A recent trial showed that Ramipril improved endothelial function and increased the number of EPCs and VEGFs in the absence of cardiovascular risk factors [52]. *In vitro* and murine models have shown anti-type-I IFN alpha and anti-BAFF reverse the reduction of circulating EPC numbers and their function. [29]. B-cell-depleting therapies such as Rituximab showed a significant reduction in EC activation markers such as VEGF, ICAM-1 and IL8 [53].

IFN-β is upregulated in the ECs of skin in dermatomyositis (DM). The increased amount of inactivated PPAR-γ [54], and adhesion molecules in EC versus healthy control skin in DM, is thought to increase the recruitment of inflammatory cells via leucocyte–endothelial interaction. This is notable as previous studies [55,56] have found decreased PPAR-γ resulting in increased inflammatory cell recruitment. These novel findings in DM may offer new targets for potential therapies on the horizon, for example, PPAR-γ agonists such as Lenabasum. Like in SSc, the adhesion molecules expressed on ECs are important for the attraction of immune cells to sites of inflammation, for example, the skin, joints and kidneys. A recent meta-analysis and systematic review of dysregulated EC markers in SLE recently showed a positive, albeit weak correlation between these EC markers and disease activity in SLE [57] (Table 1).

Table 1

Overview of EC dysfunction in autoimmune rheumatic disease.

	Mechanism	Reference (s)
Target of autoimmune damage	AECA	[12,13,33–37•]
	Autoantibody-mediated effect (GPCRs)	[14–16]
	Complement-mediated effect	[2,17]
Direct role in pathogenesis	ECs in PAH	[14,43–45]
	EPCs	[5•,27–29•]
	EndoMT	[21,22]
	Thrombogenesis	[32•,52•]
	EC apoptosis	[10]
Indirect role through regulation of other cell types	Regulator of autoimmunity	[53,47–50]
	Antigen presentation	[4,7]
	Fibroblast regulation	[54–56]
	Leucocyte trafficking	[3,26,57•]
	Epigenetic modifications	[18–20]
	Neutrophils	[30,31•]

Conclusion

This review highlights the importance of EC dysfunction in autoimmune rheumatic disease. It is clear that EC dysfunction is important for pathogenesis and relevant to understanding disease mechanisms that could be broadly relevant in autoimmune and vascular disease. In addition, ECs represent an important therapeutic target, implicated in the pharmacodynamic effects of current and emerging therapeutics and as a potential specific target in future to alleviate vascular manifestations and attenuate inflammation that leads to irreversible tissue damage and organ dysfunction. But there remain many outstanding gaps in knowledge. For example, the mechanisms and role of EPC and importance of endoMT, as well as the extent to which autoantibodies modulate EC function and whether ECs are an important target for autoimmune disease. Although the role of EPCs has been well-characterised in *in vitro* studies, challenges remain in standardising methodologies in ECFC culture and flow cytometry techniques. Thus, our advancing understanding of the pathogenic mechanisms by which ECs can cause autoimmune and microvascular dysfunction paves the way for exciting new therapeutic targets and development of prognostic biomarkers in connective tissue diseases.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

CPD has received research grants to the institution from Servier, Horizon, Arxx Therapeutics and GlaxoSmithKline, consulting fees from Arxx Therapeutics, Roche, Janssen, GlaxoSmithKline, Bayer, Sanofi, Galapagos, Boehringer Ingelheim, CSL Behring and Acceleron, and honoraria from Janssen, Boehringer Ingelheim and Corbus.

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There is the heterogeneity of EC markers in SLE with certain markers having a range of functions and some also have the potential to be novel biomarkers.