Role of Interferon Alpha in Endothelial Dysfunction: Insights Into Endothelial Nitric Oxide Synthase–Related Mechanisms

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Abstract: Systemic lupus erythematosus (SLE) is an autoimmune disease that is characterized by the production of autoantibodies against nuclear antigens such as double-stranded DNA. Lupus predominantly affects women (ratio, 9:1). Moreover, premenopausal women with SLE are 50 times more likely to have a myocardial infarction. Although specific risk factors for advanced cardiovascular complications have not been identified in this patient population, endothelial dysfunction is highly prevalent. Recent studies show that the type I interferon signature gene expression coincides with impaired brachial artery flow–mediated dilation and diminished endothelial progenitor cell circulation, both markers of impaired endothelial function. Although many factors promote the development of vascular endothelial dysfunction, all pathways converge on the diminished activity of endothelial nitric oxide synthase (eNOS) and loss of nitric oxide (NO) bioavailability. Studies examining the effects of type I interferons on eNOS and NO in SLE are missing. This literature review examines the current literature regarding the role of type I interferons in cardiovascular disease and its known effects on regulators of eNOS and NO bioavailability that are important for proper endothelial cell function.

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Atherosclerosis is now recognized as a chronic inflammatory disease.[1] Traditional risk factors of cardiovascular disease (CVD) including elevated low-density lipoproteins (LDL) and free radicals from smoking have been associated with endothelial cell injury and dysfunction.[2] Proper endothelial cell function is physiologically important for blood filtration, vasodilation and vasoconstriction.[3] To aid in these processes, endothelial cells secrete specialized paracrine and autocrine chemical mediators preventing immune cell adhesion, vascular permeability and the production of chemotactic and procoagulant molecules that initiate and support the inflammatory process. Inflammatory mediators circulating in the blood of systemic lupus erythematosus (SLE) patients may potentiate endothelial cell activation, resulting in vascular endothelial dysfunction (VED). Substantial evidence suggests that VED is due to an initial decline in the endothelial nitric oxide synthase (eNOS) expression, activation and/or nitric oxide (NO) bioavailability.[4] Thus, it is important to understand the significance of SLE-specific inflammatory mediators that lead to VED through the suppression of eNOS.

SLE is an autoimmune disease characterized by the production of autoantibodies against nuclear antigens such as double-stranded DNA and single-stranded RNA. Moreover, type I interferons are thought to play an important role in the pathogenesis and activity of the disease. Because of the chronic activation of inflammatory pathways and disproportionate number of cardiovascular events in this patient population, SLE has been characterized as an independent risk factor for VED. However, specific inflammatory mediators involved in the pathogenesis of VED in SLE are poorly understood.

Abnormal endothelial function is associated with several comorbidities observed in patients with SLE, including neuropsychiatric SLE, lupus nephritis, and cardiovascular complications. Although current treatments have improved the 20-year survival rate to 80%,[5] premenopausal women are 52 times more likely to have a myocardial infarction compared with Framingham risk-matched controls. In 1976, early clinical observations by Urowitz et al[6] revealed bimodalities in SLE death patterns, with early deaths attributed to uncontrolled disease or infection and late deaths due to premature micro- and macro cardiovascular complications. Moreover, 30% to 40% of patients with SLE have carotid plaque or myocardial malfunctions,[7] whereas patients with SLE are 17 times more likely to die from coronary heart diseases. Thus, a better understanding of the cellular and molecular basis for disease is important for the development of preclinical biomarkers.

This review focuses on the cellular and molecular mechanisms that are thought to contribute to the development of SLE-induced vascular disease, and in particular, the possible links between Type I interferons and changes in eNOS, NO production and NO bioactivity.

VASCULAR ENDOTHELIAL DYSFUNCTION

In humans, both invasive and noninvasive measures of vasodilator and flow-mediated vasodilation have been applied to better understand the pathophysiology of coronary artery disease. In addition to vasodilation, increases in platelet aggregation, fibrinolysis, soluble E-selectin and soluble intracellular adhesion molecule-1, circulating endothelial cells and von Willebrand factor are also enhanced in VED.[8] Other measurements associated with VED include arterial stiffness, intima/media thickness of the carotid artery and carotid plaque deposition.[9] Thus, endothelial dysfunction results in characteristic changes in ultrasonic and serological measures. These alterations in the function accelerate the progression of in micro- and macrovascular pathologies. Thus, improving endothelial cell

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function may prevent disease morbidity and mortality in SLE populations.

Endothelial Dysfunction in Systemic Lupus Erythematosus

Cardiovascular complications and coronary heart disease are among the major causes of morbidity and mortality in women with SLE. However, this phenomenon cannot be fully explained by conventional risk factors such as obesity, diabetes, hypertension, family history of CVD or elevated LDL levels. Although current biomarkers that predict CVD events in patients with SLE are missing, SLE populations have markedly increased VED. To make progress in therapies and biomarker development in SLE-associated CVD, one must understand the role of inflammatory mediators that are responsible for accelerated vascular diseases in SLE. VED is characterized as reduced vasodilation and a shift toward a more proinflammatory, prothrombotic state. Surprisingly, patients with SLE with more pronounced VED have comparable carotid plaque and intima media thickness scores with sex- and age-matched controls,[10] which supports the hypothesis that VED precedes the development of vascular abnormalities. In studies conducted among patients with lupus, the intima media thickness negatively correlated with VED, whereas others have proposed that VED is associated with lupus activity index.[11] Several additional factors including Raynaud's syndrome, elevated systolic blood pressure, plasma fibrinogen levels and larger daily dose of prednisolone were present in patients with SLE with markedly abnormal endothelial function.

Vascular endothelial cells are important for maintaining vascular homeostasis through the secretion of paracrine and autocrine signaling molecules with endothelial-derived NO being a key factor. Vascular NO serves multiple functions as a vasodilator, immune system modulator and antiatherogenic molecule. NO dilates blood vessels by stimulating the heme group of soluble guanylyl cyclase, which leads to cyclic guanosine monophosphate production. Binding of a vascular adhesion molecule-1 (VCAM-1)–specific nuclear factor kappa B (NF-κB) activation site by NO prevents the expression of VCAM-1 on the endothelial cell surface.[12] Similarly, NO halts DNA binding of specificity protein-1 (Sp1) and activator protein-1 (AP-1) to the intracellular adhesion molecule-1 promoter region.[13] NO can also inhibit leukocyte adhesion molecule CD11/CD18 expression and interfere with the leukocyte CD11/CD18 binding to the endothelial cell surface. In preventing atherogenesis, NO reacts with alkoxyl radicals to prevent oxidation of lipids and LDL[14] and counteracts platelet aggregation under pathophysiologic conditions by blocking adenosine diphosphate and collagen conformational changes in platelet gpIIb/IIIa receptors.[15] Furthermore, NO prevents smooth muscle proliferation that is important for fibrous cap formation in atheroma. It is clear that the release of endothelium derived NO under normal and pathophysiologic conditions is protective against atherogenesis and VED. However, NO produced in excess can be detrimental to the endothelial milieu.[16] Thus, tight regulation of eNOS is necessary for the maintenance of vascular homeostasis.

Genetic abnormalities are important for determining lupus susceptibility and may play a role in severity of disease. eNOS polymorphisms were shown to contribute to increased propensity toward VED-related diseases in patients with SLE. Genetic studies in patients with SLE from Crete, Greece, showed an increased prevalence of a/b substitution in the eNOS gene intron 4a/b (a 27-base pair variable number tandem repeat), which is important for determining the risk for development of glomerulonephritis in patients with SLE but not the susceptibility to disease.[17] Turkish patients with SLE presenting with the 27-bp variable number tandem repeat polymorphism on intron 4 of eNOS are more susceptible to disease.[18] Furthermore, T-776C and E298D variants were found to be associated with reduced NO synthesis in SLE.[19] Future studies are required to determine the functional role of these polymorphisms in SLE disease onset and severity.

In addition to genetic abnormalities present in patients with SLE, several SLE-specific circulatory factors associated with chronic inflammation may promote abnormal eNOS function. These mediators include tumor necrosis factor alpha (TNF-α),[20] interleukin-17,[21] interferon gamma,[22] CD40L[23] and C-reactive protein.[24] To date, the presence of these factors has not been associated with premature VED in patients with lupus.

Studies in eNOS-knockout mouse models provide clues regarding the importance of eNOS in preventing CVD.[25] eNOS-knockout mice display hypertension, deficient vasorelaxing activity[25] and heart failure.[26] They also have abnormal aortic valve function and altered wound-healing capacity,[27] resulting from deficient response to vascular endothelial growth factor (VEGF)-stimulated angiogenesis.[28] Physiological stress exacerbates renal injury in these animals.[29] In addition, diabetic NOS 3 $(eNOS)^{-/-}$ mice develop accelerated retinopathy,[30] more pronounced glomerular capillary complications[31] and increases in insulin resistance and hyperlipidemia.[32] Furthermore, atherosclerotic NOS 3-deficient mice exhibit larger atherosclerotic plaque lesions.[33] Recent studies from our laboratory showed that in MRL/MpJ-*Tnfrsf6*^{lpr}/J mice[34] developing spontaneous lupus, the lack of NOS 3 resulted in increased aortic lipid deposition,[35] and increases in clinically significant crescentic and necrotic glomerulonephritis.[34] These studies demonstrated that eNOS is a significant regulator of oxidative stress, which can in turn signal inflammation.

In conclusion, it is important to note that functional eNOS or NOS 3 is important for blood vessel maintenance and endothelial function. Inflammatory mediators and genetic polymorphisms may lead to alterations in the endothelial cell health and vascular homeostasis, and eNOS dysfunction can, in turn, lead to inflammation from VED. To date, mechanisms outlining the pathogenesis of VED in patients with SLE are unclear. Thus, further studies teasing out inflammatory mediators involved in endothelial dysfunction are necessary for improved therapeutics and mortality rates.

Systemic Lupus Erythematosus and Type I Interferons

A growing body of literature has suggested a role of type I interferons in the loss of endothelial function in SLE animal models and patient populations.[36–38] Type I interferons, normally produced by plasmacytoid dendritic cells and polymorphonuclear leukocytes in response to viral infections, increase during SLE flares as a result of increased immune complex–mediated activation of Toll-like receptors 7 and 9 (TLR7/9). As a result, more than 50% of patients with SLE express type I signature genes in their peripheral blood mononuclear cells.[39] In nonautoimmune populations, prolonged exposure to interferon alpha (IFN-α) is now recognized as a major risk factor for the development of clinical manifestations of SLE.[40] Collectively, these studies suggest a role of type I IFNs in the development of SLE in addition to its impacts on flare and remission patterns.

Endothelial Dysfunction and Type I Interferons

Type I interferons accelerate atherosclerosis in both mice and humans. Atherosclerotic plaque area, triglyceride levels and serum cholesterol levels were increased after low dose IFN-α