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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.

Key Indexing Terms

Autoimmune disease; SLE: Systemic Lupus Erythematosus; Endothelial dysfunction; Type I interferons; Endothelial nitric oxide synthase; Lupus

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

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secrete specialized paracrine and autocrine chemical mediators preventing immune cell adhesion, vascular permeability and the production of chemotactic and pro-coagulant 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 al⁶ 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 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- κ B) 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 LDL¹⁴ 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 CD40L²³ 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 activity²⁵ 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 complications³¹ 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 mice³⁴ 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 treatment in LDLr^{-/-} mice, a phenomenon possibly due to enhanced lipolysis.⁴¹ Moreover, New Zealand Mixed mice (lupus-prone) Apoe^{-/-} IFNAR^{-/-} mice have improved aortic ring relaxation, less platelet aggregation, decreased T lymphocyte and macrophage plaque infiltration and declines in the thrombosis level compared with animals with the IFN- α receptor intact.³⁷ Surprisingly, rat aortic rings treated with high dose IFN- α show reductions in relaxation, which may be an indication of impairments in endothelial cell function.⁴² These combined studies show effects of IFN- α on multiple stages of atherosclerosis and vascular complications in mouse models, thereby providing further evidence for type I IFN-specific mechanisms involved in the increased incidence of CVD.

In addition to changes in Framingham risk factors and vasodilation, IFN- α also alters levels of circulating endothelial progenitor cell (cEPCs), which serves as an additional marker of VED. Both patients with Framingham risk factors and patients with SLE have reduced levels of cEPCs, leading to loss of vascular repair and potentiated vessel damage.⁴³

Previous studies indicate that eNOS-derived NO regulates cEPC mobilization and functions in response to VEGF,⁴⁴ shear stress, estrogen⁴⁵ and exercise.⁴⁶ In support of these findings, studies in mice show that VEGF-mediated NO production leads to release of EPCs from the bone marrow after enhanced matrix metalloproteinase-9 (MMP-9) secretion from stromal cells.⁴⁷ Similar to conventional CVD risk factors, type I IFNs associate with attenuated mobilization and function of EPCs in patients with SLE. Moreover, SLE EPCs mature abnormally and display reductions in VEGF and human growth factor gene expression *in vitro*. In addition, these abnormalities are restored with the addition of anti-IFN- α neutralizing monoclonal antibodies.⁴⁸ Patients with lupus with significant reductions in peripheral blood EPCs display increases in the peripheral blood mononuclear cell expression of MX1, a reporter gene for type I IFN. Patients with elevated MX1 levels also exhibit declines in vascular reactivity and endothelial dysfunction as measured by peripheral arterial plethysmography.³⁸ Studies associating a type I IFN signature with endothelial dysfunction are limited in patients with SLE; however, examples of this phenomenon are demonstrated in other nonautoimmune populations such as patients with hepatitis C.

Early studies in patients with chronic hepatitis C demonstrate a role of IFN- α in exacerbating cardiovascular complications and endothelial dysfunction. A number of case reports in patients with hepatitis C receiving IFN- α 2b therapy showed mild adverse effects. Individual patients experienced atrioventricular block,⁴⁹ myocardial disease,⁵⁰ ischemic heart disease⁵¹ and severe sinus bradycardia⁵² in the absence of traditional cardiovascular risk factors during therapy administration. However, on discontinuation of IFN treatment, patients improved, suggesting that IFN- α treatment effects were transient rather than permanent.⁵³

Reductions in the NO-dependent flow-mediated dilation were observed in a hepatitis C cohort study after administration of IFN- α 2b therapy,⁵⁴ whereas others showed impairments in exercise tolerance and diminished heart rate variability.⁵⁵ Accordingly,

identification of increases in VED serological markers including VCAM-1, monocyte chemoattractant protein-1 and fibrinogen were also observed after administration of IFN- α 2b therapy in hepatitis C populations who were nonresponsive to therapy.⁵⁶ The above findings demonstrate that high serum levels of type I IFN directly mitigate proper endothelial function and counteract pathways normally regulated by NO. However, controversy remains as to whether or not the effects of IFN- α in therapeutic settings is cause for concern because some studies suggest only modest effects of IFN- α on vascular and cardiac toxicity.^{53,57}

Endothelial Nitric Oxide Synthase and Type I Interferons

It is now understood that inflammatory production of type I IFNs and their therapeutic use may cause changes in the vascular endothelial function. Thus, a focus on the mechanisms whereby IFN- α may alter eNOS and NO production is justified. The question therefore arises as to how and if type I interferons can affect eNOS function and NO production. To date, no studies have published the impact of IFN- α on eNOS enzymatic function and NO production. However, type I IFNs have been reported to have direct and indirect effects on eNOS-specific transcription factors, cofactors important for eNOS regulation, kinases and phosphatases that are important for enzyme regulation, and oxidative stress pathways.

eNOS expression is regulated on various levels, including transcription. Although controversy remains as to whether changes in transcription are important for endothelial-derived NO production, it's important to recognize that changes in transcription rates could potentially impact endothelial function. Modulation of transcription factor binding to the eNOS promoter is affected by humoral and physical factors in (patho) physiologic states. Regulatory transcription factors were shown to include the followings: Sp1/3 and AP-1 and 2-transcription factors. Reductions in the binding of Sp1/3-containing complexes with the eNOS promoter leads to declines in eNOS expression in TNF- α -treated bovine aortic endothelial cells.²⁰ Like TNF- α , IFN- α reduces Sp1/3 activation, leading to decreases in the VEGF transcript levels in pancreatic carcinomas.⁵⁸ IFN- α also alters Sp1 binding in primary human hepatocytes, leading to decreased c-Met-mediated cell proliferation.⁵⁹ eNOS promoter activity also depends on AP-1 binding. Previous studies showed that porcine aortic endothelial cell exposure to hydrogen peroxide (H₂O₂) lead to declines in eNOS expression as a result of impaired AP-1 eNOS promoter binding.⁶⁰ Likewise, transfection of IFN- α adenovirus into 253J BV cells blocks AP-1 activation in addition to other nuclear proangiogenic signals.⁶¹ Thus, IFN- α may reduce eNOS transcription by affecting the expression of transcription factors that are essential for eNOS promoter activation.

eNOS is activated by phosphorylation at serine sites located within the reductase domain, including serine 1177, serine 633 and serine 615 and dephosphorylation at serine 114, threonine 495 and tyrosine 657.⁴ Phosphatase 2A (PP2A) blunts phosphorylation of the enzyme at the activation sites in C-reactive protein-primed human aortic endothelial cells.⁶² PP2A activation occurs on stimulation by intracellular enzymes, including the IFN response gene protein kinase R (PKR).⁶³ Furthermore, PKR stimulates other kinases known for preventing eNOS-S1177 activation, including Jun N-terminal kinase and extracellular signal-regulated kinase1/2 (ERK1/2) activation.⁶⁴ Alternatively, it has been demonstrated

that PKR promotes p38 activation, a kinase previously shown to phosphorylate eNOS-S1177 in human vascular endothelial cells in response to insulin.⁶⁵ eNOS-specific kinases are activated by extracellular stimuli such as VEGF. IFN- α , given as a treatment for liver cancer, suppresses VEGF mRNA transcription, VEGF plasma levels and microvessel densities.⁶⁶ Moreover, human umbilical vein endothelial cells treated with IFN- α and 5-fluorouracil had reduced VEGF transcription and secretion.⁶⁷ Collectively, these studies suggest a possible role of IFN- α -induced changes in eNOS phosphorylation through the downregulation of VEGF and loss of kinase activation (Figure 1).

Type I Interferons and Tetrahydrobiopterin (BH₄)

BH₄ is essential for proper eNOS coupling and the flow of electrons from the reductase domain to L-arginine and subsequent NO production. Proper BH₄ levels are important for endothelial cell maintenance, where 60% of all BH₄ has been identified in the vascular wall^{68,69} On eNOS activation, NADPH is oxidized to NADH and 2 electrons are transferred from flavin adenine dinucleotide to flavin mononucleotide. Calmodulin accelerates electron transfer from NADPH through the heme groups found at the center of the dimer. Electrons then flow to BH₄ and then to L-arginine, where oxidation of this substrate yields NO and L-citrulline.⁷⁰ Inadequate supply of BH₄ during oxidative stress can lead to eNOS uncoupling, either through inadequate synthesis by guanosine 5'-triphosphate cyclohydrolase 1 or oxidation of BH₄.⁷¹ Although the exact effects of IFN- α on BH₄ synthesis and oxidation remain unclear, both animal and human studies support a role of IFN- α in BH₄ depletion.

IFN- α (105 units/kg) injections in rat amygdala and raphe areas lead to abatement of BH₄.⁷² Moreover, IFN- α -treated hepatitis C subjects have low levels of BH₄ and higher levels of its inactive oxidized form dihydrobiopterin (BH₂).⁷³ Thus, IFN- α may serve as a potential mediator of eNOS uncoupling and oxidative stress in vascular endothelial dysfunction by promoting depletion of eNOS cofactors. Collectively, these studies suggest that exposure to IFN- α may lead to BH₄ depletion through oxidation.

When BH₄ levels are low and eNOS remains uncoupled, the enzyme can act as an NADPH oxidase, producing reactive oxygen species (ROS) in excess. Superoxide (SO) scavenges NO through a direct chemical reaction that yields peroxynitrite (ONOO⁻) production within the vascular wall. It is important to note that peroxynitrite has multiple proatherogenic effects. First, it oxidizes the eNOS cofactor BH₄ to BH₃ radical. This oxidation, in turn, uncouples NOS and reduces NO production, while simultaneously increasing SO production. Second, it causes inhibition of cellular L-arginine transport that is important for NO production.⁷⁴ Third, ONOO⁻ stimulates release of thromboxane A₂, a known contributor of eNOS dysfunction.⁷⁵ Fourth, ONOO⁻ activates the production of PP2A, leading to dephosphorylation of eNOS and endothelial dysfunction. Thus, ONOO⁻ causes eNOS uncoupling and inactivity in addition to perpetual SO production.

SO has multiple indirect effects on eNOS function. For example, it oxidizes LDL to oxLDL. OxLDL, in turn, binds to LOX-1 expressed on the endothelial cell surface, which on activation, can inhibit eNOS activation. OxLDL also stimulates xanthine oxidase and NADPH oxidase (NOX) to produce SO anions, which likely promotes eNOS deactivation. Collectively, these studies suggest that SO negatively impacts the endothelium by reducing

the availability of physiologic NO and promoting a continuous cycle of oxidative stress and vascular damage.

Type I Interferons, Arginase, and L-Arginine

Endothelial cells express arginase I/II that competes with eNOS for the substrate L-arginine essential for NO production. The expression and activity of arginase II were shown to be elevated in placental vessels from women with intrauterine growth restriction complications, whereas eNOS protein expression was reduced.⁷⁶ Similarly, upregulation of arginase in aged aortic mice coincided with decreased NO production and increased ROS bioavailability due to the resulting eNOS uncoupling.⁷⁷ Further supporting these studies was the finding that arginase inhibition restored endothelial-dependent vasorelaxation in response to acetylcholine, eNOS recoupling and subsequently reduced ROS levels in aged rats.⁷⁸ Thus, depletion of L-arginine resulting from excessive arginase activity could explain the loss of NO bioavailability and eNOS uncoupling that perpetuate endothelial dysfunction. Studies showing enhanced arginase II expression in SLE are missing.

Studies examining the role of interferon on L-arginine availability are also limited. However, a study conducted in patients with high-risk melanoma showed that pegylated IFN- α caused reductions in arginine availability and overall declines in NO production.⁷⁹ In addition, IFN- α induces NO synthase 2 in various cell types, resulting in depletion of extra- and intracellular L-arginine stores.⁸⁰ Thus, IFN- α -mediated depletion of L-arginine may result in eNOS uncoupling, ultimately leading to VED observed in patient populations including SLE.

Type I Interferons, Reactive Oxygen Species and Endothelial Dysfunction

Imbalances in L-arginine and other cofactors essential for proper eNOS regulation can lead to the phenomenon known as oxidative stress. Oxidative stress is characterized as an imbalance in the production of ROS and antioxidants, which leads to dysfunctional endothelial cells. ROS impacting endothelial cell signaling mechanisms include free radicals such as SO, ONOO⁻ (peroxynitrite) and OH $\cdot$ (hydroxyl radical), and non-radicals including H₂O₂. Conventional risk factors including hypertension, diabetes mellitus and hypercholesterolemia dramatically potentiate ROS levels in the vascular wall, promoting oxidative stress.

The magnitude of oxidative stress depends on levels of SO within the cell which itself depends on the rate of synthesis and the availability of antioxidants to reduce these molecules. In addition to uncoupled NO synthases (NOSs), a number of enzyme systems produce ROS in endothelial cells including NADPH oxidases (NOX),⁸¹ xanthine oxidases, arachidonic-acid metabolizing systems, the mitochondrial electron transport chain and the cytochrome P450 enzyme. Conversely, 3 superoxide dismutase enzymes are important for counteracting superoxide production including copper zinc SOD, manganese SOD and endothelial cell SOD. However, H₂O₂ degrades in the presence of glutathione peroxidase and catalase.

SO production in the endothelium is mainly derived from NOX, xanthine oxidase and NOS enzymes. NOX 2 and 4 are the major producers of SO in the endothelium; however, other

isoforms of these enzymes can be expressed by vascular smooth muscle cells and infiltrating inflammatory cells that could ultimately impact NO production and availability along with endothelial function. Evidence for NOX activation in the vascular wall has been provided in human studies. In a clinical setting, NOX 2 was shown to be most important in producing cytotoxic levels of ROS in insulin-resistant endothelial cells.⁸² Moreover, studies in patients undergoing coronary artery bypass graft surgery showed NOX as the major source of ROS production resulting in VED.⁸³ In addition to NOX, others have shown that exogenous xanthine oxidase prevents VEGF-induced NO production and endothelial cell survival, whereas endogenous levels of NO are important for normal cell signaling mechanisms.⁸⁴ Uncoupled NOS is also a significant generator of SO and is reportedly involved in the pathogenesis of atherosclerosis.^{85–87}

Free radical reactions between SO and NO result in ONOO[•] synthesis and inactivation of NO. NO is not only important for blood vessel dilation and homeostasis but also binds and inhibits complex IV in the electron transport chain, impairing release of ROS. NO also impairs the activation of NOX enzymes within the cell, thus further regulating ROS production. NO also serves as a paracrine agent by modulating NOX 1 expression in mesangial cells and regulating the activity of NOX 2 through phosphorylation of the NOX 2 p47 subunit.⁸⁸ However, an imbalance in the NOX activation results in NO depletion and subsequent endothelial dysfunction. IFN- α induces NADPH oxidase activation and SO production in endothelial cells, promoting leukocyte adhesion to rat vessel walls, a phenomenon abrogated by SOD and other immune modulators.⁸⁹ Moreover, IFN- α induces NADPH oxidase activation in rat liver preneoplasia leading to apoptosis. However, studies are missing showing the downstream impacts of IFN- α -triggered NOX stimulation and loss of NO signaling (Figure 1).

CONCLUSIONS

Endothelial dysfunction is highly prevalent among patients with lupus and associates with multiple circulating factors including type I interferons. Studies in other tissues and disease states support a role of IFN- α in reducing transcription of NOS, reducing post-translational activation of eNOS, reducing eNOS cofactor availability leading to uncoupling, and increasing reactive oxygen production that can lead to scavenging of NO. However, studies regarding the role of type I interferons in eNOS dysfunction and loss of NO bioavailability both directly and indirectly in patients with lupus are missing. As detailed here, type I interferons may serve as novel rational targets for studies in preventing ED in patients with SLE.

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FIGURE 1.

In patients with SLE, the ability of the endothelium to maintain homeostasis and function are comprised, in part, by interferon alpha (IFN- α). To maintain vascular integrity, endothelial nitric oxide synthase (eNOS) must be properly regulated. On IFN- α production, the activation of the interferon alpha receptor-1 (IFNAR-1) on the endothelial cell surface results in activation of protein kinase R (PKR). PKR was previously shown to activate janus kinase (JNK), protein phosphatase 2A (PP2A), and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2). These enzymes are responsible for preventing activation of eNOS and subsequent NO production in the endothelium. Incidentally, loss of NO results in platelet aggregation, inflammatory cytokines like monocyte chemoattractant protein-1 (MCP-1) production, vascular adhesion molecule-1 (VCAM-1) transcription and ICAM-1 expression. Thus, these pathways may serve as one mechanism whereby IFN- α impairs NO production. Other factors regulated by IFN- α potentially involved in the loss of endothelial-derived NO are: negative regulation of vascular endothelial growth factor (VEGF) production important for eNOS activation; impaired activator protein-1 (AP-1) activation and specificity protein-1 and 3 (SP1/3) function, both responsible for nitric oxide synthase 3 (NOS 3) transcription. Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) transcription through NF- κ B and its activation are mediated by IFN- α and are important for superoxide ($O_2^{\cdot-}$) production contributing to peroxynitrite ($ONOO^-$) synthesis in the presence of NO. $ONOO^-$ has a number of detrimental effects on the endothelium including reduction of tetrahydrobiopterin (BH_4) to dihydropteridine (BH_2) resulting in eNOS uncoupling. The uncoupling of eNOS disturbs oxidative balance by increasing the production of $O_2^{\cdot-}$ within the cell, and may cause cellular dysfunction owing to an overwhelming amount of oxidative stress.