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SHORT ANALYTICAL REVIEW

The biology of nitric oxide and other reactive intermediates in systemic lupus erythematosus

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Abstract Formation of reactive nitrogen and oxygen intermediates (RNI and ROI) is an essential part of the innate immune response. Markers of systemic RNI production are increased in the setting of systemic lupus erythematosus (SLE) activity. Several lines of evidence suggest mechanisms through which the activity of inducible nitric oxide synthase (iNOS) is pathogenic in SLE, including the ability of peroxynitrite ($ONOO^-$, a product of iNOS activity) to modify proteins, lipids, and DNA. These modifications can alter enzyme activity and may increase the immunogenicity of self antigens, leading to a break in immune tolerance. In humans, observational data suggest that overexpression of iNOS and increased production of $ONOO^-$ lead to glomerular and vascular pathology. Therapies designed to target iNOS activity or scavenge ROI and RNI are in development and may provide the means to reduce the pathogenic consequences of ROI and RNI in SLE.

Introduction

Systemic lupus erythematosus (SLE) is a classic autoimmune disease defined by the formation of immune complexes with autoantigens. However, the innate immune system plays an integral role in propagating inflammatory responses initiated by this acquired immune response. An important part of that innate immune response is the production of reactive nitrogen and oxygen intermediates (RNI and ROI). One of the most widely studied RNI, nitric oxide (NO), is overproduced in the setting of lupus activity. Its pathogenic potential in lupus or any other disease lies largely in the extent of its production and the proximity of its synthesis to ROI such as superoxide (SO). NO and SO react to form peroxynitrite ($ONOO^-$), a much more reactive and potentially pathogenic molecule. There is convincing evidence in murine lupus nephritis that inducible nitric oxide synthase (iNOS) activity increases with the progression of disease and leads to glomerular, joint, and dermal pathology. In addition, $ONOO^-$-mediated modifications of proteins and DNA may

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increase the immunogenicity of these self antigens, leading to a break in immune tolerance. Redox-sensitive signaling pathways can be activated by the production of ROI/RNI, leading to further transcription of inflammatory mediators. In humans, there are observational data suggesting that overexpression of iNOS and increased production of $ONOO^-$ lead to glomerular and vascular pathology. Therapies designed to target iNOS activity or scavenge ROI/RNI have not been tested in humans in part due to concerns over the specificity of many available compounds for their targets. However, several new compounds are in development that offer promise for human trials.

Biology of reactive nitrogen intermediates (RNI)

Free radicals are highly reactive molecules with unpaired electrons. They represent an important arm of host defense against a variety of pathogens [1]. Not only are reactive oxygen and nitrogen intermediates (RONI) directly toxic to invading pathogens, they activate redox-sensitive signaling pathways such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) that in turn regulate the transcription of proinflammatory proteins such as cytokines [2]. In systemic lupus erythematosus (SLE), overproduction of free radicals in the absence of infection may lead to a break in immune tolerance, increased tissue damage, and altered enzyme function. In this review, the discussion of reactive intermediates (RI) will be confined to reactive oxygen and nitrogen free radicals. Examples of ROI include superoxide (SO), hydrogen peroxide, and hydroxyl radicals, while nitric oxide (NO) and peroxynitrite ($ONOO^-$) are the RNI to be discussed. Reactive oxygen and nitrogen intermediates (RONI) play an important role in cellular signaling processes when produced at low levels. At higher levels, these molecules can cause direct toxicity to cells and induce modifications to lipids, amino acids, and DNA.

NO is a membrane-permeable free radical molecule synthesized by nitric oxide synthase (NOS) using arginine and oxygen as substrates. Three isoforms of NOS are transcribed from three separate genes. All isoforms dimerize in the presence of cofactors to become active. Each monomer contains a reductase and oxygenase domain. The reductase domain catalyzes the transfer of two electrons to heme iron in the oxygenase domain. Calmodulin, nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are required cofactors for the reductase domain. Electrons from the reductase domain are transferred to the oxygenase domain of the adjacent monomer, where heme and tetrahydrobiopterin (BH_4) act as cofactors. Here, a reaction between O_2 and L-arginine is catalyzed, resulting in formation of NO and citrulline (Fig. 1). Two isoforms (endothelial or eNOS and neuronal or nNOS) are generally constitutively expressed and are dependent on sufficient concentrations of calcium for activity. In the vascular system, NO produced by eNOS is a potent vasodilator and regulator of vascular tone in response to shear stress. Nitroglycerin mimics the activity of eNOS by acting as a donor of NO [3]. The beneficial effect of NO produced by the constitutively expressed NOS isoforms is blunted when NO is produced in an environment high in ROI as discussed later.

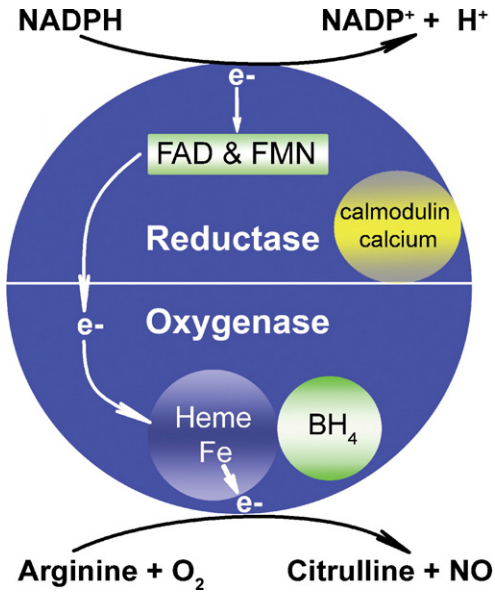

Figure 1 NO synthesis from arginine by iNOS and cofactors. Electrons (e−) are donated by NADPH to FAD and FMN in the reductase domain. This step requires Ca^{2+} (much higher levels for eNOS and nNOS than for iNOS) and calmodulin. Two cycles of electrons are then transferred by these carriers to heme iron in the oxygenase domain of the adjacent dimer. This reaction is similar to that in P450 enzymes. The role of tetrahydrobiopterin (BH_4) in this process is unclear, but it may assist in the coupling of NADPH oxidation and NO formation, thus preventing SO formation. With arginine and O_2 as substrates, donated electrons then catalyze two reaction steps, the formation of N^{ω}-hydroxy-L-arginine (NHA) followed by conversion of NHA to NO and citrulline. SO is formed when L-arginine substrate is limited, and electrons from the reductase domain react directly with oxygen [3]. This figure is reprinted from [77].

A third NOS gene (NOS2) produces an inducible isoform (iNOS) that is primarily expressed in immune cells, most notably macrophages and macrophage-derived cells. INOS is expressed in response to inflammatory stimuli that are well characterized in murine cells. Among these stimuli are several cytokines and toll-like receptor ligands such as lipopolysaccharide, interleukin-6 (IL6), interferon-γ (IFNγ), IL1β, and tumor necrosis factor-α (TNFα). In human cells, complex mixtures of cytokines are necessary for induction. In most cells, signaling pathways converge on the janus kinase/signal transducer and activator of transcription (JAK/STAT) and/or the nuclear factor-kappa B (NF-κB) pathways [4]. Nuclear hormone receptors may play a role in regulation of iNOS induction. There is evidence to support a role for estrogen as an inducer [5] and PPARγ ligands as inhibitors [6] of iNOS induction in response to IFNγ or IFNγ + LPS stimulation respectively in murine cells. iNOS is expressed during pathologic states in human endothelial cells, synovial fibroblasts, polymorphonuclear cells, lymphocytes, and natural killer cells [7]. In normal human tissue, expression is strong in myocytes, skeletal muscle, and Purkinje cells [8].