MINIREVIEW

Nitric Oxide: A Novel Mediator of Inflammation (43927AA)

ROBERT M. CLANCY AND STEVEN B. ABRAMSON[1]

Division of Rheumatology, Hospital for Joint Disease, and Department of Medicine, Division of Rheumatology, New York University Medical Center, New York, New York 10003

Nitric oxide (NO), first identified as an endothelium-derived relaxation factor, is now recognized to regulate the functions of many mammalian cells and tissues. Nitric oxide is synthesized via the oxidation of arginine by a family of nitric oxide synthases (NOS), which are either constitutive and calcium dependent (cNOS) or inducible and calcium independent (iNOS). The endogenous production of nitric oxide plays a vital role in the regulation of physiological processes (e.g., blood vessel tone, neurotransmission) as well as in host defense and immunity. There is increasing evidence that nitric oxide also plays a complex role in the modulation of the inflammatory response. The induction of nitric oxide synthase (iNOS) in tissues can lead to the sustained production of high concentrations of nitric oxide which may exert pro-inflammatory effects including vasodilation, edema, cytotoxicity, and the mediation of cytokine dependent processes. Conversely, the production of NO by endothelial cell cNOS may serve a protective, or anti-inflammatory, function by preventing the adhesion and release of oxidants by activated neutrophils in the microvasculature. In this review we describe the multifaceted role of nitric oxide in inflammation, particularly focusing on the regulation of inflammatory cells and the repertoire of molecular processes targeted by NO that control cellular functions.

Biosynthesis of Nitric Oxide

Nitric oxide is synthesized via L-arginine oxidation by a family of nitric oxide synthases (NOS). Isomeric forms of nitric oxide synthase, representing at least three distinct gene products, have been cloned in bovine, rat, mice, and human tissues (1–5) (Table I). The three isoforms of the enzyme vary in calcium dependence, kinetics, and regulation. However, all nitric oxide synthases are flavoproteins which require NADPH and tetrahydrobiopterin as co-factors. The amino acid sequences deduced from the cDNA of the endothelial and neuronal cNOSs and iNOS have been reported (1–3, 6). The various NOS proteins show similar domains/motifs in their structure. For example, cNOS and iNOS share sequence homology of the N-terminal region which may be the arginine binding and catalytic site (7, 8). Moreover, the C-terminal region of endothelial cNOS and iNOS show significant homology to FAD, FMN, and NADPH binding regions of cytochrome P450 reductase from rat liver (1, 3, 6). By modeling analysis, those C-terminal regions share multistranded β sheet and surrounding α helices that are similar to portions of the nucleotide binding domains of the crystallized ferredoxin NADPH reductase (2).

The isoforms of nitric oxide synthase have distinct properties. In general, NOS isoforms are either calcium dependent and constitutively expressed (cNOS; e.g., neurons, endothelium) or calcium independent and inducible (iNOS; e.g., macrophage, hepatocytes, chondrocytes). The subcellular localization among the isoforms also varies: for example, endothelial cNOS is mostly membrane bound, whereas the neuronal cNOS has been identified in the cytosol of central and peripheral neurons (9). NO derived from the cNOS isoforms acts as a physiologic regulator by relaxing vascular smooth muscle or by functioning as a neurotransmitter. Constitutively expressed NO synthases produce low (pico-to nanomoles) amounts of NO for short periods in a calcium/calmodulin-dependent manner in response to receptor stimulation (e.g., acetylcholine, bradykinin). In contrast, inducible NOS must

[1] To whom requests for reprints should be addressed at Hospital for Joint Diseases, 301 E 17th Street, New York, NY 10003.

0037-9727/95/2105-0000$10.50/0

Table I. Contrasting Properties of Constitutive and Inducible Forms of Nitric Oxide Synthase

	Constitutive forms	Inducible form
Present in:	Endothelium (ecNOS), Brain (ncNOS)	Macrophages, endothelial cells, chondrocytes, hepatocytes, synoviocytes, smooth muscle cells, etc.
Stimuli	Acetylcholine, ADP Thrombin Shear stress	Endotoxin γ-Interferon Interleukin-1, tumor necrosis factor-α
Calcium dependency	Yes	No
Amounts of nitric oxide produced	Low (pico, nanomolar)	High (nano,-micromolar)
Duration of production	Short	Long
Function	Physiological regulation, anti-inflammation (?)	Host defense, cytotoxicity, inflammation

Note. Depending on the cell type, endotoxins and certain cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-a), promote the expression of iNOS. Often, combinations of different cytokines are required for full induction. Various other cytokines, for example, transforming growth factor-β (TGFβ), agents such as glucocorticoids inhibit the induction of NOS.

be expressed following exposure to diverse stimuli, such as the inflammatory cytokines (e.g., interleukin-1 [IL-1], tumor necrosis factor [TNF]) and lipopolysaccharide (LPS); once expressed the inducible enzyme generates significantly larger and sustained amounts of NO than does the constitutive isoform.

It is important to note that while the expression of cNOS in endothelium and brain among species is similar, the expression of iNOS varies markedly. Human monocytes express iNOS only after exposure to granulocyte-stimulating factor plus TNF or γ-interferon INF-γ plus TNF or LPS (10–12). In contrast, murine monocyters express iNOS after LPS alone. In addition, the quantity of NO produced by human monocytes/macrophages, lymphocytes, and neutrophils is considerably less than that seen in the murine counterpart. The two human cell types that can be most readily induced to express iNOS *in vitro* are hepatocytes and chondrocytes. Therefore, one must exercise caution in the interpretation of observations made in animal systems since the expression of human iNOS, particularly in leukocytes, appears to be more tightly regulated than in other species.

Biochemical Properties of Nitric Oxide

Nitric oxide, a gaseous free radical, is labile and in the presence of oxygen is rapidly metabolized to nitrates and nitrites (13, 14). The chemistry of nitric oxide is complex (15, 16) and may involve interrelated redox forms (17). The most important reactions biologically are believed to be those with oxygen, with transitional metal ions and with free thiols (17). In addition, nitric oxide reacts rapidly with superoxide anion to yield peroxynitrite, which can rearrange into nitrate in a reaction that may produce the toxic hydroxyl radical (17). The chemistry and biological activity of peroxynitrite were recently reviewed by Pryor and Squadrito (18).

Reactions with Iron. The binding of NO to the heme group of soluble guanylate cyclase activates this enzyme, raising intracellular levels of cGMP, in many but not all types of cells (19, 20). There are several other important reactions of nitric oxide with the heme-associated iron. These include: (i) formation of met-hemoglobin *via* an interaction in the heme group between NO and O_2^- which concomitantly oxidizes the heme (oxyhemoglobin) iron from Fe^{2+} to Fe^{3+}, and (ii) the reversible inactivation of cytochrome P450 via a direct interaction between NO and iron (21). Nitric oxide also has a high affinity for nonheme iron. It binds to iron-sulfur clusters in aconitase and to complexes I and II of the mitochondrial respiratory chain thus inhibiting oxidative phosphorylation (22–25). Finally, the formation of stable, nitrosylated iron-containing protein derived from L-arginine can be demonstrated in cytokine-activated macrophages by electron paramagnetic resonance, suggesting a function for iron-nitrosyl complexes in cytotoxicity (26, 27).

Reactions with Thiols. Under aerobic conditions, free thiols react with nitric oxide forming S-nitrosothiol compounds (28, 29). This reaction requires oxygen: however, the reactive nitric oxide species is unknown (18, 30). Although peroxynitrite is a possible candidate, the studies of Wink and co-workers indicate that glutathione did not react with peroxynitrite to form an SNO adduct as monitored by UV/visible spectroscopy (30). Compared with nitric oxide, which is short-lived ($t_{1/2}$< 15 sec), these S-nitrosothiol derivatives are significantly more stable (e.g., $t_{1/2}$ > 2 hr for S-nitrosoglutathione and S-nitrosoalbumin) and retain nitric oxide-like properties (31). Indeed, it is generally agreed that endothelium-derived relaxing factor (EDRF), originally identified as nitric oxide, reflects the biological activity of more stable nitrosothiol derivatives as well as nitric oxide (29, 32). In plasma, the predominant redox forms of NO are S-nitrosothiols, the most abundant of which is S-nitrosoalbumin, present in micromolar concentrations in normal subjects (31). NO also exerts effects within cells by reacting with intracellular thiols, particularly glutathione, to

form stable bioactive intermediaries which affect oxidant production and glucose metabolism (33). S-nitrosothiols, unlike NO, do not readily react with superoxide anion (O_2^-) and other reactive oxygen species; this may limit the generation of toxic free radicals and thus protect from NO toxicity (17).

Nitric Oxide-Dependent ADP-Ribosylation. ADP-ribosylation, the covalent binding of ADP-ribose to acceptor amino acids, is an important post-translational modification of cellular proteins. It was first appreciated as an exogenous mechanism by which various bacterial toxins affect eukaryotic cell function. Among the most well characterized ADP-ribosylation reactions are those promoted by pertussis and botulinum C2 toxins which target Gαi and actin, respectively. The role of endogenous ADP-ribosylation in the regulation of cellular function is less well characterized. Lapetina and co-workers reported that nitric oxide stimulates the ADP-ribosylation of a 37-kDa protein in platelets and other cell types (34). This protein proved to be glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (34, 35). ADP-ribosylation of GAPDH by NO inhibits GAPDH activity and thereby decreases intracellular energy stores (36). We have reported that nitric oxide promotes the ADP-ribosylation of both GAPDH and G-actin in human neutrophils (37). This ADP-ribosylation of cytosolic actin requires the presence of a plasma membrane cofactor and is associated with the inhibition of actin polymerization and actin stress fiber formation. ADP-ribosylation may be an important mechanism by which nitric oxide regulates the state of actin polymerization at the inner leaflet of the plasma membrane and thereby regulates cytoskeletal functions such as cell adhesion, migration, and phagocytosis (38).

Pro-Inflammatory Properties of Nitric-Oxide

Host Defense and Cytotoxicity. Based largely on animal studies, there is increasing evidence that the excessive production of nitric oxide plays a role in phlogistic responses, promoting classical signs of inflammation as well as tissue injury (Table II). Teleological support for such a role is suggested by the fact that inflammatory cytokines such as interleukin-1 and tumor necrosis factor promote the expression of the inducible isoform of nitric oxide synthase and an increase in nitric oxide production (2, 5, 39–41). Nitric oxide formation may have originated as a first-line defense against invading microbial organisms including parasites, bacteria, and viruses (42). The cytotoxic effects of nitric oxide provide nonspecific immunity, not only for invading organisms, but also for the killing of tumor cells (22, 42). The biochemical basis for the cytotoxicity and cytostasis induced by nitric oxide is most likely due to its capacity to react with iron-containing enzymes of the respiratory cycle (eg., aconitase and complexes I and II of the mitochondrial respiratory chain) and in the synthesis of DNA (22–25, 43, 44). In addition, NO reacts with and depletes intracellular glutathione to increase susceptibility to oxidant stress (33). Finally, while nitric oxide itself is cytotoxic, NO can react with other free radicals to generate molecules such as peroxynitrite which enhance its cytotoxicity (17). It is critical to note that the cellular regulation of NO synthesis is a key determinant of cytotoxicity: the picomolar concentrations of NO produced by cNOS are nontoxic and sufficient for intracellular signaling, while the high concentrations generated by iNOS are potentially pro-inflammatory, damaging the surrounding cells and tissues (2, 5, 39–41).

Table II. Evidence That Nitric Oxide Modulates Inflammation

Pro-inflammatory properties
• Promotes vasodilation (edema, erythema)
• Elevated in inflammatory synovial fluids
• Elevated in sepsis
• Activates PGH synthase
• Inhibitors of NO synthesis ameliorate experimental models of arthritis
Anti-inflammatory properties
• NO inhibits leukocyte and platelet adhesion to endothelium
• NO inhibits lymphocyte proliferation
• NO inhibits oxidant production by phagocytes
• Inhibitors of NO synthesis increase vascular injury in models of endotoxemia

Inflammation and Tissue Injury. The recognition of the nonspecific effector role of NO in host defense mechanisms was shortly followed by an appreciation of its importance in inflammation and autoimmune tissue injury. NO production is increased in a variety of diseases and several classical signs of inflammation are reversed by NOS inhibitors (Table III). Kubes and coworkers studied the effect of two inhibitors of NO synthase, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) on a carrageenin-induced model of vascular permeability in rat skin. Both L-NAME and L-NMMA inhibited the increase in vascular permeability and edema formation provoked by carrageenan (45, 46). Mulligan *et al.* (47) used a rat model of immune complex lung injury to assess the involvement of nitric oxide. Inhibitors of NO synthesis were protective in this model, whereas L-arginine, a precursor of NO, exacerbated tissue injury. Bronchoalveolar lavage fluids from these rats contained the metabolites of NO, nitrite and nitrate. However, in the presence of NOS inhibitors, these products were significantly diminished. The authors speculated that the reaction of NO with superoxide anion (O_2^-) to form peroxynitrite played a significant role in the tissue injury observed in this experimental model. Similarly, NO has been implicated in the inflammation and tissue destruction of

Table III. Diseases Associated with Excessive Production of Nitric Oxide

Disease	NOS inhibitor in animal models	
Rheumatoid arthritis (57, 43)	Improves	Adjuvant arthritis, streptococcal cell-wall arthritis (58–60)
Osteoarthritis (61)	Not determined	
SLE (62)	Improves	MRL/lpr model of lupus (63)
Psoriasis (55)	Not determined	
Inflammatory bowel disease (53)	Improves	(54)
Septic shock (50)	Worsens	Vascular injury (82, 87)
	Improves	Hypotension (51, 52)
Ischemia/reperfusion injury (32, 82)	Worsens	(81, 84, 85)
	Improves	(83)

streptozocin-induced diabetes in the mouse: L-NAME and NMMA, inhibitors of NOS, protect islet cells from destruction and suppress the onset of diabetes (48).

There is increasing evidence that NO plays a role in a variety of human diseases. Excessive production has been observed in patients with sepsis (49–52), ulcerative colitis (53, 54), psoriasis (55), arthritis (43, 56–61), allograft rejection (49), and systemic lupus erythematosus (62, 63) (Table III). Potential sources of nitric oxide in these inflammatory processes are unclear, but could include vascular endothelium, vascular smooth muscle, hepatocytes, macrophages, and chondrocytes; the inducible form of NOS has been detected in each of these cell types (49). However, differences between species regarding iNOS expression make it difficult to predict, based upon animal models, the precise cellular source of NO in human disease. For example, in psoriatic skin lesions, which are infiltrated with leukocytes and keratinocytes, the expression of iNOS was confined to the vascular endothelium as indicated by immunohistochemical staining (55).

Nitric Oxide in Arthritis. The importance of nitric oxide in the development of arthritis is increasingly appreciated (43, 57–59). In a recent report (60) by Stefanovic-Racic, adjuvant-induced arthritis (AIA) was suppressed by the nitric oxide synthase inhibitor N^G-monomethylarginine (L-NMA). In the AIA animals, the onset of symptoms was preceded by elevated production of nitrates and nitrites. N^G-monomethyl-L-arginine acetate (L-NMA), which inhibits both iNOS and cNOS blocked (i) NO biosynthesis, (ii) paw swelling, and (iii) histopathological changes in ankle joints. The protective effect of NOS inhibitors was reversed by administration of the NOS substrate L-arginine. In human arthritis, increased concentrations of nitrites have recently been demonstrated within the joint fluids of patients with both osteoarthritis and rheumatoid arthritis (56). There is evidence that a significant source of NO in joints are chondrocytes which express iNOS following exposure to inflammatory cytokines (40). While iNOS is not expressed in human chondrocytes obtained from nonarthritic cartilage, chondrocytes obtained from osteoarthritic cartilage express iNOS by immunoblot and spontaneously produce NO in culture for up to 72 hr (61). Nitric oxide produced by chondrocytes may mediate the effects of IL-1. In rabbit chondrocytes, treatment with NOS inhibitors, blocks the capacity of IL-1 to inhibit proteoglycan synthesis (64, 65) (Fig. 1). Similarly, in bovine chondrocytes, IL-1-dependent susceptibility to oxidant injury is completely reversed by NOS inhibitors (66).

The evidence implicating nitric oxide in human arthritis has led to significant interest in pharmacologic inhibitors of NO synthesis. Indeed, agents currently utilized in the treatment of arthritis appear to affect NO activity. Auranofin reduces the response of isolated rabbit aortae to NO (67), and glucocorticoids (68)

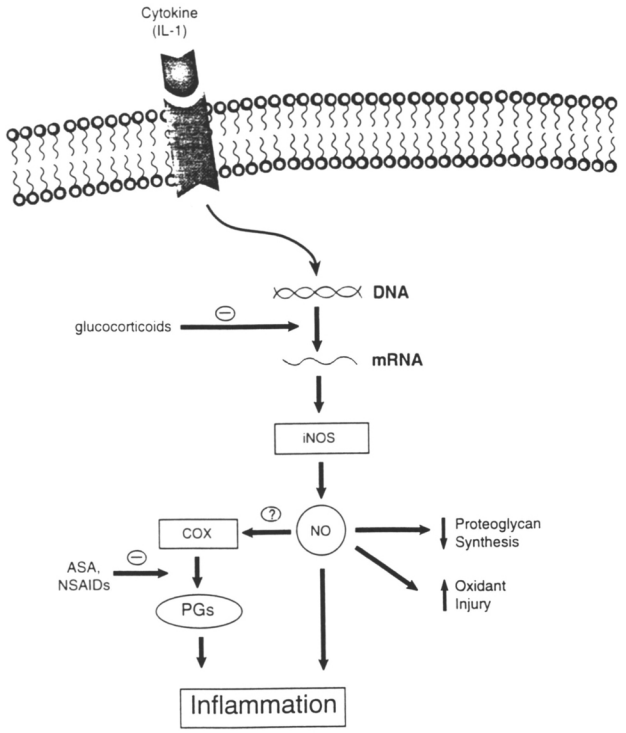

Figure 1. Tentative model, based on studies on animals, which illustrates that selected catabolic IL-1 effects in chondrocytes are mediated by the induction of iNOS. Nitric oxide in this model not only acts as an autacoid to mediate the action of cytokines such as IL-1 and modulate COX, but also can be released from the cell to exert inflammatory effects of its own.

inhibit the induction of iNOS. At therapeutic concentrations, aspirin inhibits iNOS expression in murine macrophages (manuscript submitted). In addition, methotrexate can inhibit dihydrofolate reductase which may interfere with the synthesis of tetrahydrobiopterin, a co-factor necessary for NOS activity (69).

Nitric Oxide and Prostaglandins. A complex relationship is emerging with regard to "cross-talk" between the nitric oxide and cycloxygenase (COX) pathways. Like NOS, COX has constitutive and inducible isoforms. COX-2 is the inducible form of this enzyme, the synthesis of which is triggered by those cytokines which also induce iNOS. Not unexpectedly, a variety of cells (e.g., endothelium, macrophages, chondrocytes) can produce NO and prostaglandins simultaneously in response to cytokines and other activators. The paracrine effects of these molecules are often similar and include the capacity to relax smooth muscle, inhibit platelet and neutrophil adhesion, and inhibit neutrophil oxidant production. Recent evidence indicates that these two pathways interact closely. Nitric oxide can stimulate COX activity, possibly via reaction with the heme component which binds to the active site of the COX enzyme (40, 70–72) (Fig. 1). Furthermore, NOS inhibitors decrease IL-1 induced release of PGE_2 in rat mesangial cells (73), indicating that endogenous NO production enhances COX activity in these cells. Conversely, endogenous PGE_2 decreased IL-1-stimulated iNOS mRNA induction in these same studies (73). Thus, nitric oxide and prostaglandin pathways appear to interact as "yin/yang" regulators of a variety of physiological and inflammatory processes.

Anti-Inflammatory Properties of Nitric Oxide

NO: An Endothelium-Derived Defensive Molecule. The continuous release of NO by vascular endothelium plays an important role in maintaining the integrity of the vascular endothelium under both physiological and pathological conditions. Nitric oxide helps to modulate blood flow to various tissues and regulates interactions between vascular endothelium and circulating inflammatory cells (Fig. 2). Nitric oxide can inhibit platelet aggregation and reduce platelet adhesion to endothelial monolayers (74, 75). Nitric oxide also inhibits homotypic neutrophil aggregation (76) and the adhesion of neutrophils to endothelium (77, 78), a requisite early event in the development of acute inflammatory process. *In vivo,* using intravital microscopy to study cat mesentery, Kubes has demonstrated that the inhibitor of NO synthesis, L-NAME, markedly increased neutrophil adherence and emigration in postcapilary venules, indicating that NO plays an important physiologic role in preventing leukocyte-endothelial adhesion (77). Moreover, L-NAME also caused an increase in vascular protein leakage and increased microvascular permeability, an early effect which was not dependent on increased neutrophil adherence. This may be secondary to the capacity of NO to help maintain endothelial shape since the cells contract following exposure to NOS inhibitors (79).

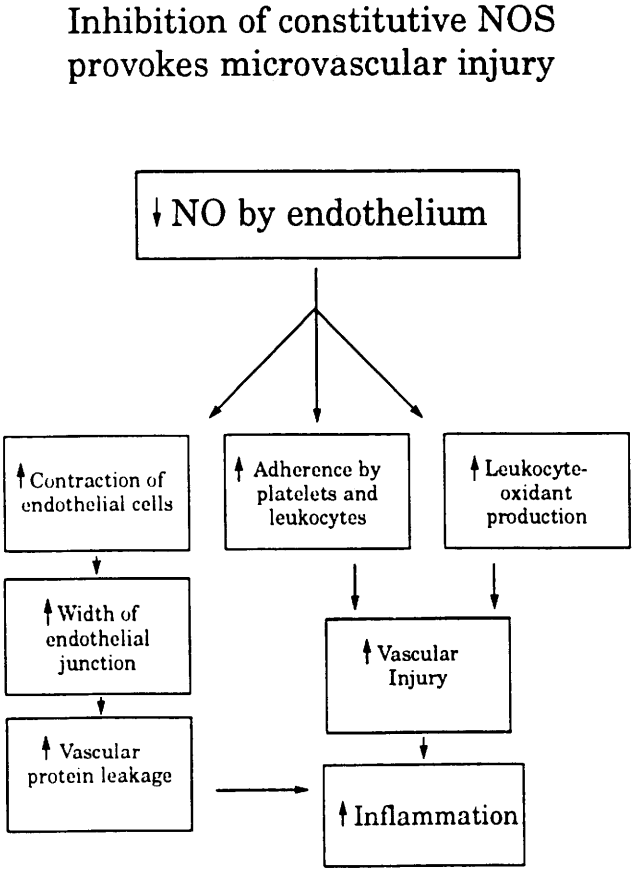

Figure 2. Diagram showing possible mechanisms involved in increased vascular inflammation elicited by inhibition of nitric oxide synthesis.

The adhesion of neutrophils to postcapillary venules depends upon a number of factors, including the expression of adhesion molecules on the surface of activated neutrophils (e.g., CD11b/18, L-selectin) and endothelium (e.g., ICAM-1, E-selectin). In the studies cited above, monoclonal antibodies to the common β subunit (CD18) of CD11b/18 completely blocked leukocyte adherence and emigration induced by L-NAME. The mechanism by which NO inhibits CD11b/18-dependent adherence is unknown. It is possible that the inhibition by NO of actin polymerization may result in a paucity of focal attachment points available to interact with the cytoplasmic tail of CD18 via the actin binding protein α-actinin (38, 80).

In addition to its role in preventing neutrophil adherence, nitric oxide also serves a defensive function by inhibiting the production of superoxide anion by activated neutrophils (76, 81). This may be particularly important at sites of inflammation where the endothelium must protect itself against toxic mediators re-

leased from chemoattractant-stimulated, emigrating neutrophils. Exposure of neutrophils to NO, which is unstable ($t_{1/2} < 15$ sec), is sufficient to inhibit O_2^- production for up to 40 min (76). This effect of NO is secondary to direct inhibition of a membrane component of the NADPH oxidase, possible via the iron nitrosylation of cytochrome b_{558} (76).

The capacity of NOS inhibitors to increase leukocyte adherence and for NO donors to overcome this effect may have significant implication for the treatment of conditions characterized by neutrophil mediated vascular injury. Such syndromes would include ischemia-reperfusion injury (32, 82–85), systemic lupus erythematosus (86), and the Adult Respiratory Distress Syndrome, the last a condition in which treatment with inhaled NO has improved survival (52).

The complexity of the role of NO—whether it is acting to protect or injure—is perhaps well illustrated by its role in septic shock where elevated levels of serum nitrates have been described (50). In a preliminary study the NOS inhibitor, L-NMMA was used to successfully reverse hypotension in two septic patients; however, survival was not affected (51). In experimental sepsis, the inhibition of NOS causes increased liver and intestinal damage (82, 87). This is consistent with intravital microscopy studies by Kubes *et al.*, which demonstrated that NOS inhibitors increased neutrophil adherence, protein extravasation, and microvascular injury following the infusion of endotoxin (77). Indeed, the administration of NO donors in experimental endotoxemia prevents microthrombosis and oxygen radical-mediated damage (88, 89). Such observations have led to the appreciation of the protective effects of endothelial cell–derived nitric oxide with regard to defending against vascular injury. Successful strategies to treat pathological processes in which NO is clearly playing a pro-inflammatory role will require specific inhibition of nonendothelial NOS, in order not to interfere with the physiologically protective role of NO in the microvasculature (Fig. 2).

Effects of NO on Phagocyte Signaling. NO exerts a variety of effects on neutrophils which could account for its capacity to inhibit cellular functions. As noted above, these include inhibition of the NADPH oxidase, depletion of intracellular energy stores via the ADP-ribosylation of GADPH, and the inhibition of functions dependent upon actin polymerization, such as adhesion and migration. In addition, NO can also affect cellular signaling functions via the targeting of a variety of intracellular proteins. These include the capacity of NO to nitrosylate intracellular thiol-containing molecules, such as glutathione and protein kinase C, or to react with Fe-containing enzymes, such as guanylate cyclase and aconitase (17, 33, 90, 91). Our laboratory has demonstrated that neutrophils exposed to NO convert intracellular glutathione to a nitrosylated adduct, which may act as a stable intracellular intermediary of NO action (33). The NO-dependent depletion of reduced glutathione within the neutrophil provokes the prompt activation of the hexose monophosphate shunt (HMPS) (33) (Fig. 3). These findings are consistent with the work of Albina et al. (92) who demonstrated that treatment of elicited rat macrophages with L-NMA inhibited basal activity of HMPS. Similarly, Mauel et al. (93, 94) demonstrated in cytokine-activated macrophages that an increase in the production of nitrite was accompanied by activation of the HMPS. Taken together, these observations indicate that nitric oxide, in either an autocrine or paracrine fashion, reacts with and depletes intracellular glutathione and, at least in phagocytes, activates the HMPS (Fig. 3). This ability of NO to react with intracellular glutathione and to activate the HMPS has important implications for resistance to nitric oxide-dependent cytotoxicity. Firstly, nitric oxide, as a nitrosothiol, is less reactive with oxygen and superoxide anion reducing the likelihood of toxic peroxynitrite formation (17, 95). Secondly, the nitrosylation of glutathione could compete with the nitrosylation of mitochondrial Fe-containing proteins, as protecting critical respiratory enzymes (24–26, 96, 97). Finally, different capacities of various cell types to replenish reduced glutathione stores via the hexose monophosphate shunt could be an important individual determinant of susceptibility to the cytotoxic effects of nitric oxide. This hypothesis is supported by the observation that macrophages and neutrophils, which utilize nitric oxide for microbial killing, have high HMPS activity and are resistant to attack by nitric oxide (33, 92–94, 98).

In summary, while it is clear nitric oxide plays an important role in inflammatory states, the pathologic processes are complex. The pro- or anti-inflammatory properties of NO may vary according to the NO con-

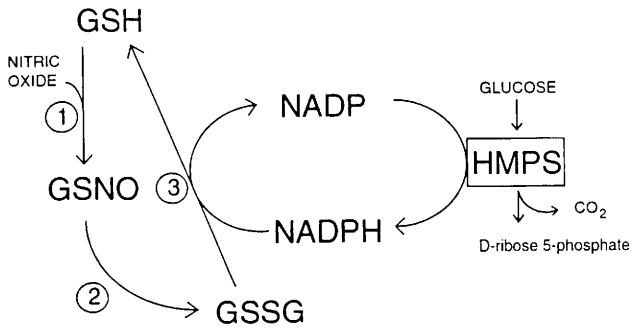

Figure 3. Model for nitric oxide reaction with intracellular glutathione and activation of the hexose monophosphate shunt in human neutrophils. There is a depletion of reduced glutathione when nitric oxide (*under aerobic conditions*) reacts with reduced glutathione (Reaction 1) which may require the involvement of glutathione transferase. S-nitrosoglutathione is nonenzymatically converted to oxidized glutathione (Reaction 2). In a reaction that requires NADPH, GSSG is converted to GSH by glutathione reductase (Reaction 3). NADPH is restored by the activity of the hexose monophosphate shunt pathway (HMPS).

centration, the potential for the formation of toxic derivatives such as peroxynitrites, the site of the pathological process, and the adaptive responses of the target cell. Successful strategies to inhibit the excessive and damaging production of NO in tissues in response to phlogistic cytokines must spare the physiologic protective function of NO produced by the vascular endothelium.

This work was supported by a Biomedical Science Grant from the National Arthritis Foundation to S.A.; and by a grant from the New York Chapter of the Arthritis Foundation to R.C.

1. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature **351:**714–718, 1991.
2. Xie QW, Cho HJ, Calaycay J, Mumford RA, Swiderek KM, Lee TD, Ding A, Troso T, Nathan C. Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science **256:**225–228, 1992.
3. Lamas S, Mardsden PA, Li GK, Tempst P, Michel T. Endothelial nitric oxide synthase: Molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci USA **89:**6348–6352, 1992.
4. Janssens SP, Shimouchi A, Quertermous T, Bloch DB, Bloch KD. Cloning and expression of a cDNA encoding human endothelium derived relaxing factor/nitric oxide synthase. J Biol Chem **267:**14519–14522, 1992.
5. Lyons CR, Orloff GJ, Cunningham JM. Molecular cloning and functional expression of an inducible nitric oxide synthase from a murine macrophage cell line. J Biol Chem **267:**6370–6374, 1992.
6. Nishida K, Harrison DG, Navas JP, Fisher AA, Dockery SP, Uematsu M, Nerem RM, Alexander RW, Murphy TJ. Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. J Clin Invest **90:**2092–2096, 1992.
7. Lowenstein CJ, Glatt CS, Bredt DS, Snyder SH. Cloned and expressed macrophage nitric oxide synthase contrasts with the brain enzyme. Proc Natl Acad Sci USA **89:**6711–6715, 1992.
8. Baek KJ, Thiel BA, Lucas S, Stuehr DJ. Macrophage Nitric oxide synthase subunits: Purification, characterization and role of prosthetic groups and substrate in regulating their association into a dimeric enzyme. J Biol Chem **268:**21120–21129, 1993.
9. Bredt DS, Glatt CE, Hwang PM, Fotuhi M, Dawson TM, Snyder SH. Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron **7:**615–624, 1991.
10. Denis M. Tumor necrosis factor and granulocyte macrophage colony stimulating factor stimulate human macrophages to restrict growth of virulent Mycobacterium avium and to kill avirulent *M. avium:* Killing effector mechanism depends on the generation of reactive nitrogen intermediates. J Leukocyte Bio **49:**380–387, 1991.
11. Munoz-Fernandez MA, Fernandez MA, Fresno M. Activation of human macrophages for the killing of intracellular Trypanosoma cruzi by TNF alpha and IFN gamma through a nitric oxide dependent mechanism. Immunol Lett **33:**35–40, 1992.
12. Hunt NCA, Goldin RD. Nitric oxide production by monocytes in alcoholic liver disease. J Hepatol **14:**146–150, 1992.
13. Marletta MA, Yoon PS, Iyengar R, Leaf CD, Wishnok JS. Macrophage oxidation of L-arginine to nitrite and nitrate: nitric oxide is an intermediate. Biochemistry **27:**8706–8711, 1988.
14. Iyengar R, Stuehr DJ, Marletta MA. Macrophage-synthesis of nitrite, nitrate, and N-nitrosamines: Precursors and role of the respiratory burst. Proc Natl Acad Sci USA **84:**6369–6373, 1978.
15. Ford PC, Wink DA, Stanbury DM. Autooxidation kinetics of aqueous nitric oxide. FEBS Lett **326:**1–3, 1993.
16. Wink DA, Darbyshire JF, Nims RW, Saavedra JE, Ford PC. Reactions of the bioregulatory agent nitric oxide in oxygenated aqueous media: Determination of the kinetics for oxidation and nitrosation by intermediates generated in the NO/O_2 reaction. Chem Res Toxicol **6:**23–27, 1993.
17. Stamler JS, Singel DJ, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. Science **258:**1898–1902, 1992.
18. Pryor WA, Squadrito GL. The chemistry of peroxynitrite: A product from the reaction of nitric oxide with superoxide. Am J Physiol **268**(5):L699–L722, 1995.
19. Ignarro LJ, Degnan JN, Baricos WH, Kadowitz PJ, Wolin MS. Activation of purified guanylate cyclase by nitric oxide requires heme: comparison of heme-deficient, heme reconstituted, and heme-containing forms of soluble enzyme from bovine lung. Biochem Biophys Acta **718:**49–55, 1982.
20. Waldman SA, Murad F. Biochemical mechanisms underlying vascular smooth muscle relaxation: The guanylate cyclase-cyclic GMP system. J Cardiovasc Pharmacol 12(Suppl 5): S115–S118, 1988.
21. Wink DA, Osawa Y, Darbyshire JF, Jones CR, Eshenaur SC, Nims RW. Inhibition of cytochromes P450 by nitric oxide and a nitric oxide releasing agent. Arch Biochem Biophys **220:**115–123, 1993.
22. Hibbs JB, Taintor RR, Vavrin Z, Rachlin EM. Nitric oxide: A cytotoxic activated macophage effector molecule. Biochem Biophys Res Commun **157:**87–94, 1988.
23. Hibbs JB, Taintor RR, Vavrin Z. Iron depletion: Possible cause of tumor cell cytotoxicity induced by activated macrophages. Biochem Biophys Res Commun **123:**716, 1984.
24. Drapier JC, Hibbs JB. Differentiation of murine macrophages to express nonspecific cytotoxicity for tumor cells results in L-arginine dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophage effector cells. J Clin Invest **78:**790–797, 1986.
25. Stadler J, Billiar TR, Curran RD, Stuehr DJ, Ochoa JB, Simmons RL. Effect of exogenous and endogenous nitric oxide on mitochondrial respiration of rat hepatocytes. Am J Physiol **260:**C910, 1991.
26. Henry Y, Ducrocq C, Drapier J-C, Servent D, Pellat C, Guissani A. Nitric oxide, a biological effector: Electron paramagnetic resonance detection of nitrosyl-iron-protein complexes in whole cells. Eur Biophys J **20:**1–15, 1991.
27. Wharton M, Granger DL, Durack DT. Mitochondrial iron loss from leukemia cells injured by macrophages. J Immunol **141:**1311–1317, 1988.
28. Clancy RM, Abramson SB. Novel synthesis of S-nitrosoglutathione and degradation by human neutrophils. Anal Biochem **204:**365–371, 1992.
29. Myers PR, Minor RL Jr, Guerra R Jr, Bates JN, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. Nature **345:**161–163, 1990.
30. Wink DA, Nims RW, Darbyshire JF, Christodoulou D, Hanbuer I, Cox GW, Laval R, et al. Reaction kinetics for nitrosation of cysteine and glutathione in aerobic nitric oxide solutions at neutral pH. Insights into the fate and physiological effects of intermediates generated in the NO/O_2 reaction. Chem Res Toxicol **7**(4):519–525, 1994.
31. Stamler JS, Jaraki O, Osborne J, Simon DI, Keaney J, Vita J, Singel D, Valeri CR, Loscalzo J. Nitric oxide circulates in mam-

malian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci USA **89:**7674–7677, 1992.
32. Palmer RMJ, Ferrige G, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxant factor. Nature **327:**524, 1987.
33. Clancy RM, Levartovsky D, Leszczynska-Piziak J, Yegudin J, Abramson SB. Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: Evidence for S-nitrosoglutathione as a bioactive intermediary. Proc Natl Acad Sci USA **91:**3680–3684, 1994.
34. Brune B, Lapetina EG. Activation of a cytosolic ADP-ribosyltransferase by nitric oxide-generating agents. J Biol Chem **264:**8455–8458, 1989.
35. DeMatteis MA, DiGirolamo M, Colanzi A, Pallas M, DiTullio G, McDonald LJ, Moss J, Santini G, Bannykh S, Corda D, Luini A. Stimulation of endogenous ADP-ribosylation by brefeldin A. Proc Natl Acad Sci USA **91:**1114–1118, 1994.
36. Molina-Vedia L, McDonald B, Reep B, Brune B, DiSilvio M, Billiar TR, Lapetina EG. Nitric oxide-induced S-nitrosylation of glyceraldehyde-3-phosphate dehydrogenase inhibits enzymatic activity and increases endogenous ADP-ribosylation. J Biol Chem **267:**24929–24932, 1992.
37. Clancy RM, Leszczynska-Piziak J, Abramson SB. Nitric oxide stimulates the ADP-ribosylation of actin in human neutrophils. Biochem Biophys Res Comm **191:**847–852, 1993.
38. Clancy RM, Leszczynska-Piziak J, Amin A, Levartovsky D, Abramson SB. Nitric oxide ADP-ribosylates actin in association with the inhibition of cytoskeletal assembly in activated neutrophils. J Leukocyte Biol **58:**196–202, 1995.
39. Beasley D, Schaneartz JH, Brenner BM. Interleukin-1 induces prolonged L-arginine-dependent cyclic guanosine monophosphate and nitrite production in rat vascular smooth muscle cells. J Clin Invest **87:**602–608, 1991.
40. Stadler J, Stefanovic-Racic M, Billiar TR, Curran RD, McIntyre L, Georgescu HI, Simmons RL, Evans CH. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J Immunol **147:**3915–3920, 1991.
41. LePoivre M, Chenai B, Yapo A, Lemair G, Thelander L, Tenu JP. Alteration of ribonucleotide reductase activity following induction of nitrite-generating pathway in adenocarcinoma cells. J Biol Chem **265:**14143–14149, 1990.
42. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol **3**(1):65–70, 1991.
43. Merryman PF, Clancy RM, He XH, Abramson SB. Modulation of human T cell responses by nitric oxide and its derivative, S-nitrosoglutathione. Arthritis Rheum **36:**1414–1422, 1993.
44. Laval F, Wink DA. Inhibition by nitric oxide of the repair protein, O6-methylguanine-DNA-methyltransferase. Carcinogen **15:**443–447, 1994.
45. Kubes P. Nitric oxide modulates epithelial permeability in the feline small intestine. Am J Physiol **262:**G1138–G1142, 1992.
46. Kubes P, Granger DN. Nitric oxide modulates microvascular permeability. Am J Physiol **262:**H611–H615, 1992.
47. Mulligan MS, Hevel JM, Marletta MA, Ward PA. Tissue injury caused by deposition of immune complexes is L-arginine dependent. Proc Natl Acad Sci USA **88:**6338–6342, 1991.
48. Kolb H, Kolb-Bachofer V. Nitric oxide: a pathogenic factor in autoimmunity. Immunol Today **13:**157–160, 1992.
49. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med **329:**2002–2112, 1993.
50. Ochoa JB, Udekwu AO, Billiar TR, Curran RD, Cerra FB, Simmons RL, Peitzman AB. Nitrogen oxide levels in patients after trauma and sepsis. Ann Surg **214:**621–626, 1991.
51. Petros A, Bennett D, Vallance P. Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. Lancet **338:**1557–1558, 1991.
52. Roissaint R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM. Inhaled oxide for the adult respiratory distress syndrome. N Engl J Med **328:**399–405, 1993.
53. Middleton SJ, Shorthouse M, Hunter JO. Increased nitric oxide synthesis in ulcerative colitis. Lancet **341:**465–466, 1993.
54. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharmacol Exp Ther **264:**11–16, 1993.
55. Kolb-Bachofen V, Fehsel K, Michel G, Ruzicka T. Epiderman keratinocyte expression of inducible nitric oxide synthase in skin lesions of psoriasis vulgaris. Lancet **344:**139, 1994.
56. Farrell AJ, Blake D, Palmer RMJ, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis **51:**1219–1222, 1992.
57. Stefanovic-Racic M, Stadler J, Evans CH. Nitric oxide and arthritis. Arthritis Rheum **36:**1036–1044, 1993.
58. McCartney-Francis N, Allen JB, Mizel DE, Albina JE, Xie QW, Nathan CF, Wahl SM. Suppression of arthritis by an inhibitor of nitric oxide synthase. J Exp Med **178:**749–754, 1993.
59. Ialenti A, Moncada S, DiRosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol **110:**701–706, 1993.
60. Stefanovic-Racic M, Meyers K, Meschter C, Coffey JW, Hoffman RA, Evans CH. N-monomethylarginine, an inhibitor of nitric oxide synthase, suppresses the development of adjuvant arthritis in rats. Arthritis Rheum **37:**1062–1069, 1994.
61. Amin AR, DiCesare PE, Vyas P, Attur M, Abramson SB. The expression and regulation of inducible nitric oxide synthase in human osteoarthritic chondrocytes. J Exp Med (in press).
62. Buyon JP, Seligman S, Clancy R, Belmont HM, Young B, Abramson SB. Increased serum nitrite levels in lupus pregnancies. Arthritis Rheum **37**(9):S324, 1994.
63. Weinberg JB, Granger DL, Pisetsky DS, Seldin MF, Misukonis MA, Mason SN, Pippen AM, Ruiz P, Wood ER, Gilkeson GS. The role of nitric oxide in the pathogenesis of spontaneous murine autoimmune disease: Increased nitric oxide production and nitric oxide synthase expression in MRL-lpr/lpr Mice, and reduction of spontaneous glomerulonephritis and arthritis by orally administered NG-monomethyl-l-arginine. J Exp Med **179:**651–660, 1994.
64. Hickory MS, Palmer RMJ, Charles IG, Moncada S, Bayliss MT. The role of nitric oxide in IL-1 and TNF-alpha induced inhibition of proteoglycan synthesis in human articular cartilage. Trans Orthop Res Soc **19:**77–80, 1994.
65. Taskiran D, Stefanovic-Racid M, Georgescu H, Evans C. Nitric oxide mediates supression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun **200:**142–148, 1994.
66. Abramson SB, Clancy RM, Koehne C, Rediske J. Nitric oxide mediates cytokine dependent susceptibility to oxidant injury in articular chondrocytes. Clin Res (in press).
67. Ohlstein EH, Horhonich S. Selective inhibition of endothelium-dependent relaxation by gold-containing compounds. Pharmacology **38:**93–100, 1989.
68. DiRosa M, Randomski M, Carnuccio R, Moncada S. Glucocorticoids inhibit the induction of nitric oxide synthase in macrophages. Biochem Biophys Res Commun **172:**1246–1252, 1990.
69. Gross SS, Levi R. Tetrahydrobiopterin synthesis. An absolute requirement for cytokine-induced nitric oxide generation by vascular smooth muscle. J Biol Chem **267:**25722–25729, 1992.
70. Inoue T, Fukuo K, Morimoto S, Koh E, Ogihara T. Nitric oxide mediates interleukin-1 induced prostaglandin E2 production by vascular smooth muscle cells. Biochem Biophys Res Commun **194:**420–424, 1993.

71. Mitchell JA, Kohlhaas KL, Sorentino R, Warner TD, Murad F, Vane JR. Induction by endotoxin of nitric oxide synthase in the rat mesentery: Lack of effect on action of vasoconstrictors. Br J Pharmacol **109**:265–270, 1993.
72. Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P. Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci USA **90**:7240–7244, 1993.
73. Tetsuka T, Daphna-Iken D, Srivastava SK, Baier LD, DuMaine J, Morrison AR. Cross-talk between cyclooxygenase and nitric oxide pathways: Prostaglandin E2 negatively modulates induction of nitric oxide synthase by interleukin 1. Proc Natl Acad Sci USA **91**:12168–12172, 1994.
74. Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet **2**(8567):1057–1058, 1987.
75. Maragos CM, Morley D, Wink DA, Dunams TM, Saavedra JE, Hoffman A, Bove AA, Isaac L, Hrabie JA, Keefer LK. Complexes of NO with nucleophiles as agents for the controlled biological release of nitric oxide. Vasorelaxant effects. J Med Chem **34**:3242–3247, 1991.
76. Clancy RM, Leszczynska-Piziak J, Abramson SB. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPH oxidase. J Clin Invest **90**:1116–1121, 1992.
77. Kubes PM, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA **88**:4651–4655, 1991.
78. Kubes P, Kurose I, Granger DN. NO donors prevent integrin-induced leukocyte adhesion but not P-selectin-dependent rolling in postischemic venules. Am J Physiol **267**:H931–H937, 1994.
79. Oliver JA. Endothelium-derived relaxing factor contributes to the regulation of endothelial permeability. J Cell Physiol **151**:506–511, 1992.
80. Pavalko FM, LaRoche SM. Activation of human neutrophils induces an interaction between the integrin $beta_2$-subunit (CD18) and the actin binding protein alpha-actinin. J Immunol **151**:3795–3897, 1993.
81. Cooke JP, Tsao PS. Cytoprotective effects of nitric oxide. Circulation **88**:2451–2455, 1993.
82. Seekamp A, Mulligan MS, Till GO, Ward PA. Requirements for neutrophil products and L-arginine in ischemia-reperfusion injury. Am J Pathol **142**:1217–1226, 1993.
83. Buckberg GD, Matheis G, Sherman MP, Haybron DM, Ignarro LJ. Myocardial reoxygenation injury after ischaemia is mediated by the L-arginine:nitric oxide pathway. In: Moncada S, Marletta MA, Hibbs JB Jr., Higgs EA, Eds. The Biology of Nitric Oxide. London: Portland Press, pp 52–54, 1992.
84. Lefer AM. Cytoprotective actions of nitric oxide donors in ischaemia-reperfusion injury. In: Moncada S, Marletta MA, Hibbs JB Jr., Higgs EA, Eds. The biology of nitric oxide. London: Portland Press, pp 55–56, 1992.
85. Zimmerman BJ, Granger DN. Reperfusion-induced leukocyte infiltration: Role of elastase. Am J Physiol **259**(2 Pt 2): H390–H394, 1990.
86. Abramson SB, Weissmann G. Complement split products and the pathogenesis of systemic lupus erythematosus. Hospital Practice **23**(12):45–56, 1988.
87. Hutcheson AR, Whittle BJR, Boughton-Smith NK. Role of nitric oxide in maintaining vascular integrity in endotoxin-induced acute intestinal damage in the rat. Br J Pharmacol **101**:815–820, 1990.
88. Wink DA, Hanbauer I, Krishna MC, DeGraff W, Gamson J, Mitchell JB. Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. Proc Natl Acad Sci USA **90**:9813–9817, 1993.
89. Harbrecht BG, Billiar TR, Stadler J, Demetris AJ, Ochoa J, Curran RD, Simmons RL. Inhibition of nitric oxide synthesis during endotoxemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. J Leukocyte Bio **52**:390–394, 1992.
90. Gopalakrishna R, Gundimeda U. Nitric oxide and nitric oxide-generating agents induce a reversible inactivation of protein kinase C activity and phorbol ester binding. J Biol Chem **268**:27180–27185, 1993.
91. Kaplan SS, Billiar T, Curran RD, Zdziarski UE, Simmons RL, Basford RE. Inhibition of chemotaxis with N-monomethyl-L-arginine: A role for cyclic GMP. Blood **74**:1885–1887, 1989.
92. Albina JE, Mastrofrancesco B. Modulation of glucose metabolism in macrophages by products of nitric oxide synthase. Am J Physiol **363**:c1594–c1599, 1993.
93. Mauel J, Corradin SB. Phagocytosis of Leishmania enhances macrophage activation by IFN-gamma and lipopolysaccharide. J Immunol **146**:279–285, 1991.
94. Buchmuller-Rouiller Y, Mauel J. Macrophage activation for intracellular killing as induced by calcium ionophore. J Immunol **146**:217–223, 1993.
95. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxy radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA **87**:1620–1624, 1990.
96. Stuehr DJ, Gross SS, Sakuma I, Levin R, Nathan CF. Activated murine macrophages secrete a metabolite of arginine with the bioactivity of endothelium-derived relaxing factor and the chemical reactivity of nitric oxide. J Exp Med **169**:1011–1020, 1989.
97. Drapier JC, Pellat C, Henry Y. Generation of EPR-detectable nitrosyl iron complexes in tumor target cells cocultured with activated macrophages. J Biol Chem **266**:10162–10167, 1991.
98. Malawista SE, Montgomery RR, Van Blarican G. Evidence for reactive nitrogen intermediates in killing of staphylococci by human neutrophil cytoplasts. J Clin Invest **90**:631, 1992.