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Review Article

Inducible Nitric Oxide Synthase and Inflammatory Diseases

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Introduction

Nitric oxide (NO) is a colorless gas at room temperature and one of the simplest molecules known, yet it has been implicated in a wide variety of regulatory mechanisms ranging from vasodilatation and blood pressure control to neurotransmission. It is also involved in nonspecific immunity and participates in the complex mechanism of tissue injury as a major mediator of inflammatory processes and apoptosis (1). This work focuses on the complex role of NO produced by the inducible form of nitric oxide synthase (iNOS) in inflammatory and autoimmune diseases. Although the earliest studies in the field suggested that NO is a strictly pro-inflammatory macrophage product, it is clear from the current literature that, in fact, NO is made by numerous cell types and is often anti-inflammatory. Much of this dichotomy can be explained by the particular responses of given cells involved in the inflammatory response, but another variable involves the complex chemistry in which NO can participate. As we outline below, various facets of the immune response can be examined from these perspectives.

Nitric Oxide and Inflammation

The physiological defense response of the body to any kind of injurious stimulus is called inflammation. There is no clear dividing line between acute and chronic inflammation, but the former generally refers to a response that has an abrupt onset and is of short duration. Acute inflammation may become chronic (in the temporal sense) if the injurious agent is persistent. On the other hand, chronic inflammation is characterized by a proliferation of fibroblasts and formation of blood vessels (angiogenesis), as well as an influx of chronic inflammatory cells, namely granulocytes (neutrophils, eosinophils, and basophils), lymphocytes, plasma cells and macrophages. [See (2) for a comprehensive work providing an up-to-date look at the basics of inflammatory processes.]

Nearly two decades ago, the production of nitrogen oxides was associated with inflammation. It was already known in 1981 that a marked increase in urinary nitrate excretion occurs in humans with diarrhea and fever (3). Nitrate formation was then believed to be a result of microbial metabolism, but these observations suggested that mammals also form nitrogen oxides and that a correlation between immunostimulation and nitrate synthesis may exist (4). In 1985, production of nitrite and nitrate-generating compounds by mammalian cells in vitro was first demonstrated in the mouse macrophage (5). Since that time, the production of NO has been considered of primary importance in the host's antimicrobial mechanisms.

The metabolic pathway known as the L-arginine:NO pathway is the main source for the production of NO in mammalian cells by a group of enzymes known as the nitric oxide synthases (NOS). Endothelial cells and neurons express isoforms of NOS (eNOS and nNOS, respectively), which produce NO at low levels under the physiological control of the Ca^{2+}/calmodulin system, will not be discussed

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extensively in this review. The enzyme primarily responsible for the roles of NO in inflammatory processes is the inducible NOS (iNOS; NOS2; or type II NOS), which is not typically expressed in resting cells and must first be induced by certain cytokines or microbial products. The molecular biology and regulation of NO synthases have been reviewed extensively (6,7). We would like to highlight that iNOS remains very stable at both the mRNA and protein levels, and generates large amounts of NO over a period of days (6,8,9). At least two general conclusions can be gleaned from these studies and numerous others. First, sustained production of NO at high levels will lead to the production of numerous reactive nitrogen oxide species (RNOS), which can mediate a broad spectrum of physiological and pathological effects (10). Second, due to the possibility of deleterious effects to the host as a consequence of prolonged exposure to such RNOS, iNOS must be regulated carefully (11). Finally, one can hypothesize that microorganisms may have developed means for suppressing the expression and/or activity of iNOS, perhaps by co-opting the host's own regulatory machinery. Viewed from this perspective, the balance between induction and suppression of iNOS may underlie much of the physiology and pathology of inflammation.

In recent years, NO has emerged as a major mediator of inflammation. As might be expected from such a pleiotropic molecule, there are contradictory reports in the literature concerning its role as an anti-inflammatory or pro-inflammatory agent. The inconsistencies reported probably are due to the multiple cellular actions of this molecule, the level and site of NO production, and the redox milieu into which it is released. Therefore, the type, concentration, and flux of RNOS. Nitric oxide itself activates soluble guanylyl cycles, which leads to synthesis of cGMP. This activation of soluble guanylyl cyclase constitutes a common pathway in many processes, including vascular smooth muscle cell relaxation, inhibition of platelet activity, inhibition of neutrophil chemotaxis, and signal transduction in the central and peripheral nervous systems (12). As stated above, under both physiological and pathological conditions the reaction of NO with ROS, for example superoxide, results in the formation of RNOS. These agents are directly involved in the activation or inhibition of key enzymes in various metabolic processes, such as mitochondrial respiration and DNA synthesis and repair, as well as in the modulation of various genes (for reviews see 9,10, 13–17). Many of the regulatory and physiological functions of NO (Table 1) can be considered as protective or "anti-inflammatory," and are mainly related to NO produced by the other isoforms of NOS. However, in the last years, data have accumulated about iNOS expression in an increasing number of human disorders.

Interactions of NO with the Chemical Mediators of Inflammation

Inflammation is controlled by the presence of a group of chemical mediators, each with a specific role at some definite stage of the inflammatory reaction. These mediators may be exogenous, arising from bacteria or chemical irritants, or endogenous in origin. The most important endogenous mediators identified include the vasoactive amines histamine and serotonin, the kinin system, the fibrinolytic system, the complement system, the arachidonic acid metabolites like prostaglandins and leukotrienes, platelet-activating factor (PAF), neuropeptides, reactive oxygen species, and inflammatory cytokines (2). We will discuss only those components of inflammation directly related to the actions of NO.

Inducible NOS and Inflammatory Cytokines

Cytokines are small-molecular weight proteins comprising regulatory factors of the immune system, hematopoiesis, tissue repair, cell proliferation and inflammation. It has been reported that in different cell types, in vitro, so-called pro- and anti-inflammatory cytokines can have both enhancing and suppressing effects on the expression of iNOS and NO production. The biological activities of cytokines *vis-à-vis* iNOS expression have been mainly investigated in vitro and in vivo by using purified or recombinant proteins and neutralizing antibodies, but the use of genetically modified animals gives better insights as to the roles of cytokines in experimental diseases. For example, disruption of the transforming growth factor-beta 1 (TGF-β1) gene in mice resulted in a severe wasting syndrome with multifocal inflammation and early death (18). In these TGF-β1 null mice, systemic NO production was greatly elevated over that of

Table 1. Regulatory and anti-inflammatory actions of NO

Tissue Organ	Physiological Action of NO Related to Inflammation	NOS Isoform	Refs.*
Vascular endothelium	— Maintains vasodilator tone	eNOS	(239)
	— Inhibits smooth muscle cell migration and proliferation	eNOS; iNOS eNOS	(240,241)
	— Inhibition of blood cell-vessel wall interactions and adhesion to endothelium		(242)
Blood cells	— Inhibition of platelet adhesion and aggregation, and inhibition of microvascular thrombosis	eNOS; iNOS eNOS	(243,244) (242) (245)
	— Prevents aggregation and adhesion of white cells	iNOS	(9,246)
	— Mediates cytostatic and cytotoxic activity of macrophages for antimicrobial and antitumor defense	iNOS	(247,248) (249)
	— Inhibition of mast cell degranulation		
Heart	— Maintains coronary perfusion and regulates cardiac contractility	eNOS	(250,251)
	— Inhibits cardiac contractility (pathology of myocarditis)	iNOS	(252–254)
Lung	— Maintains ventilation/perfusion ratio and regulates bronchociliar motility and mucus secretion	?	(255,256) (257)
Pancreas	— Modulates endocrine secretion	eNOS, iNOS	(258,259)
Intestinal system	— Modulates peristalsis and exocrine secretion	eNOS	(208,260)
	— Contributes to protection of mucosa		(261,262)

* The quoted references are mostly recent reviews of significant relevance. eNOS, endothelial isoform of nitric oxide synthase (NOS); iNOS, inducible form of NOS; NO, nitric oxide.

wild-type littermates, in association with aberrant iNOS expression in multiple organs (19).

The activated macrophage is one of the most important effector cells in the inflammatory response. In addition to NO (9), macrophages secrete pro-inflammatory cytokines including tumor necrosis factor (TNF-α) and interleuken-1β (IL-1β) and immunomodulatory cytokines, such as IL-2, IL-10, TGF-β1, and IL-6 (20,21). IL-4 (22), IL-6 (23), IL-10 (24) and TGF-β1 (8) have been reported to suppress the induction of NO from macrophages or to down-regulate the expression of iNOS in activated macrophages, but the list continues to grow. In a murine model of endotoxemia, human recombinant IL-11 attenuated the inflammatory response through down-regulation of pro-inflammatory cytokine release and NO production (21). Furthermore, IL-13 was recently found to suppress macrophage NO production in both mouse peritoneal macrophages and J774 macrophage cell line. Regulation of iNOS occurred at both the mRNA and translational levels, depending on the macrophage population (25). In the case of IL-13, its similarity to IL-4 in its spectrum of actions may suggest that IL-4 and IL-13 overlap with regard to suppression of iNOS, as well.

Since the release of cytokines constitutes a major event in inflammatory and immune responses, their opposing effects on the production of NO may partially explain why pro-inflammatory cytokines induce their detrimental effects, while anti-inflammatory cytokines may have beneficial effects in inflammation. A recent study showed that the levels of pro-inflammatory (TNF-α, IL-6, IL-8) and anti-inflammatory cytokines (IL-10, TNFsrI, TNFsrII) relate to serum nitrate levels in patients with severe sepsis. An excessive production of pro-inflammatory cytokines was related to an excessive production of NO in the acute phase of sepsis; whereas, during the secondary phase, the production of NO was reduced and the anti-inflammatory cytokines predominantly were present (26). Although administration of exogenous anti-inflammatory

cytokines, such as IL-10, to septic patients may possibly lead to diminished NO production, the efficacy of this treatment remains to be established due to the possible protective role of NO in sepsis. Our group demonstrated a decade ago that inhibition of systemic NO production with the nonselective NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) in endotoxemic mice was associated with increased liver damage (27). In a similar vein, Cobb et al. (28) found increased mortality following treatment of conscious endotoxemic dogs with another nonselective NOS inhibitor, N-omega-L-arginine. This paradox was illustrated further in a recent study of endotoxemia in TGF-β1 transgenic mice, in which mortality was higher in the transgenic animals, compared with controls, in conjunction with a greatly suppressed systemic NO production. Paradoxically, TGF-β1 transgenic animals also expressed very high circulating levels of TNF-α, which might explain this increased mortality (29). Indeed, sepsis and septic shock are complex, though their symptoms can be mimicked to a degree by administration of lipopolysaccharide (LPS) and/or TNF-α.

TGF-β1 negatively regulates iNOS expression both in vitro and in vivo (19), but endogenous and exogenous TGF-β1 can act differently to suppress NO production (30). Though overexpression of endogenous TGF-β1 was associated with the aforementioned increase in endotoxin-induced mortality, exogenous TGF-β1 has been reported to reduce the expression of iNOS, improve hemodynamic parameters, and decrease mortality of endotoxemic rats (31,32). Thus, the fact that a single cytokine may display opposite effects in different experimental models has to be considered when evaluating a possible therapeutic use of recombinant cytokines.

Nitric oxide may have an important regulatory role in the process of cytokine activation. Nitric oxide was recently reported to be a potent inhibitor of cysteine proteases, such as IL-1β-converting enzyme (33). NO suppressed IL-1β and interferon-γ (IFN-γ)-inducing factor (IGIF or IL-18) processing in activated RAW 264.7 mouse macrophages by inhibiting caspase-1 activity (34). Furthermore, stimulated peritoneal macrophages from wild-type mice released more IL-1β if exposed to the NOS inhibitor L-NMMA; whereas, macrophages from iNOS null mice did not (34). This indicates that regulation of pro-inflammatory cytokines release by iNOS may contribute to the pathogenesis of certain inflammatory processes. On the other hand, NO could lead to the indirect activation of TGF-β1, possibly through suppression of the capacity of latency-associated peptide to neutralize TGF-β1 (35). In this way, a negative feedback cycle may be established by which iNOS expression could be reduced in the presence of high levels of NO.

Inducible NOS and Arachidonic Acid Metabolites

It is known that prostaglandin E_2 (PGE_2) is a regulator of macrophage functions and displays a functional dualism in immunoinflammatory conditions (36). The expression of inducible NO synthase after stimulation by bacterial endotoxin and other cytokines is accompanied by the release of other mediators, such as PGE_2 and prostacyclin, via the cyclooxygenase (COX) pathway (37,38). This synergistic production has been the subject of several studies (39,40), which suggests a crucial link between the NO synthase and cyclooxygenase pathways in certain pathological conditions, such as nephrosis, sepsis or rheumatoid arthritis (38). Most studies have focused on the role of NO in the expression and/or activity of cyclo-oxygenase (38,41). NO has been reported to increase prostaglandin production via activation of both constitutive and inducible forms of COX in a number of cell types (38,42–44). Moreover, a recent study showed the existence of both NO-dependent and -independent pathways of prostaglandin synthesis after cytokine stimulation of rat osteoblasts in vitro (45). On the other hand, NOS inhibitors increased PGE_2 synthesis in Kupffer cells (46) and chondrocytes (47). More recently, two unrelated NO donors, namely GEA 3175 and S-nitroso-N-acetetyl-D,L-penicillamine (SNAP), were shown to inhibit prostacyclin production in human umbilical endothelial cells (48).

The effect of eicosanoids on the NO synthesis by the activation of the inducible NO synthase also has been studied (49–51). After stimulation of macrophages with bacterial endotoxin plus IFN-γ, induction of iNOS and NO production is accompanied by the release of prostaglandins via the cyclo-oxygenase pathway (37,38). Like many other laboratories, we showed that incubation with LPS plus IFN-γ led to a dose-dependent production of NO in murine J774 macrophage-like cells, an effect

prevented by the NOS inhibitor L-NMMA. Addition of the cyclo-oxygenase inhibitor, indomethacin, did not affect NO_2^- production significantly (44). These findings indicate that the products of the cyclo-oxygenase pathway do not play a major role in the regulation of iNOS and confirm previous studies, which demonstrate that the endogenous release of prostanoids from the RAW 264.7 and J774.2 murine macrophages is insufficient to affect the activity of iNOS (38,41). However, the effects of prostaglandins on iNOS activity are still controversial. Low concentrations of indomethacin have been reported to reduce NO formation significantly (51) and the amount of iNOS protein (52) in LPS-stimulated J774 macrophages. Also, in LPS plus IFN-γ-stimulated J774 macrophages, a significant reduction in NO production could only be found when indomethacin was used at very high concentrations (44). Similarly, anti-inflammatory drugs, such as aspirin and sodium salicylate, have been shown to inhibit induced NO production by immunostimulated RAW 264.7 cells at the high end of therapeutic concentrations. Moreover, this effect was not simply the result of inhibition of prostaglandin synthesis, because exogenous PGE_2 failed to overcome the effects of both drugs (53,54). In another study, high doses of aspirin inhibited IL-1β-induced iNOS protein expression in bovine vascular smooth muscle cells and decreased NF-κB translocation and TNF-α production. This study suggests new mechanisms of action for aspirin in the treatment of cytokine-induced inflammatory diseases (55).

In a recent study, inhibition of endogenous PGE_2 synthesis with indomethacin or ibuprofen had no effect on NO synthesis (56). Thus, the inhibitory effects of the high concentration of COX inhibitors like indomethacin have to be interpreted with caution. Interestingly, exogenous, but not endogenous, PGE_2 decreased the levels of iNOS mRNA and iNOS protein in LPS-stimulated RAW 264.7 cells. This inhibition of macrophage iNOS expression was shown to be dependent on the time and concentration of prostaglandin exposure (56).

Nitric Oxide in Acute Inflammation

Inducible NOS and the Vascular Response to Injury

Injury to an organ or tissue results in progressive changes in the damaged area. As the result of vascular alterations in the area, three main signs of vascular response appear: redness, heat, and swelling. The redness and heat result from an increase in blood flow, which is the result of local vasodilatation, first involving arterioles and then capillaries and venules. The production of NO by the eNOS in endothelial cells activates soluble guanylyl cyclase, leading to the synthesis of cyclic guanosine monophosphate (cGMP), which in turn leads to relaxation of vascular smooth muscle cells. This pathway has been investigated extensively and constitutes a common process in both human and many animal tissues (57). Swelling is the result of alterations in vascular permeability. The endothelial cells become leaky, leading to exudation of fluid, plasma proteins and white blood cells (inflammatory edema).

The carrageenan-induced edema model has been a useful experimental tool with which to assess the contribution of mediators involved with the vascular changes associated with acute inflammation and for screening efficacious anti-inflammatory drugs (58). The development of carrageenan-induced edema in the rat hindpaw is a biphasic event in which the early phase is related to the production of histamine, leukotrienes, PAF, bradykinin, and possibly cyclo-oxygenase products. The delayed phase is linked to local neutrophil infiltration and activation. The contribution to edema of NO, superoxide and peroxynitrite also has been demonstrated in this model (59). Both the nonselective NOS inhibitors N^G-nitro-L-arginine methyl ester (L-NAME) and L-NMMA (at the early phase), and the selective iNOS inhibitors N^G-iminoethyl-L-lysine (L-NIL) and mercaptoethylguanidine (MEG) (at the late phase) have a potent inhibitory effect, which strongly suggets a pro-inflammatory effect for both constitutive and induced NO production (58,60). However, the location and identity of the NOS isoforms responsible for NO synthesis at the site of inflammation remains to be determined. In this context, a recent study using the selective nNOS inhibitor 7-Nitroindazole (7-NI) suggests that NO synthesized by a nNOS isoform located in sensory nerves plays an important part in the early phase response to carrageenan in this model of inflammation. In addition, NO synthesized by an iNOS isoform located in inflammatory leukocytes contributes to the late phase response (61). Interestingly, injection of carrageenan into the pleural cavity of mice reduces the induction of iNOS protein

in both macrophages and airway epithelial cells in the lungs of both IL-6 null mice, as well as in wild-type mice pretreated with an antibody against IL-6. This finding suggests that endogenous IL-6 amplifies the induction of iNOS caused by carragenan in the lung (62).

Neutrophils are the first leukocytes to emerge from the vessels in significant numbers during acute inflammation. Although the carrageenan-induced paw edema is neutrophil-dependent and mediated by both the NOS and COX pathways (60), no evidence is found for the involvement of either cyclo-oxygenase products or neutrophils in mediating the iNOS inflammatory component in a model of dermal inflammation (63). In a recent study, the development of an inflammatory reaction induced by injection of specific agonists of proteinase-activated receptor-2 (PAR2)-activating peptides in the rat hindpaw was shown to be largely independent of the production of prostanoids and NO (64). However, as part of the zymosan-induced inflammatory response in the rat skin, NO contributed to edema formation by increasing blood flow. The sources of iNOS appeared to be cells other than neutrophils. It was suggested that other cell types, such as dermal fibroblasts and keratinocytes, that are also known to express iNOS, could be important sources of NO in the skin (65).

This process of leukocyte recruitment initiates with the adhesion of leukocytes to the endothelium, an event regulated by a series of adhesive interactions. Activated endothelium expresses surface adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin, which interact with peripheral blood leukocytes and facilitate their attachment to the endothelial cell surface. The inhibitory effects of endogenous NO in endothelial-leukocyte interaction were shown previously (66). Recently, a novel physiologic mechanism was identified by which macrophage-derived NO can inhibit endothelial VCAM-1 expression and modulates the activation of resident and nonresident vascular wall cells in an autocrine and paracrine manner (67,68). While administration of LPS to wild-type mice increased sequestration of neutrophils in the lung and their adhesion to the endothelium, these responses were markedly exaggerated in mice lacking iNOS (69). Although this suggests a beneficial effect for iNOS expression, in other experiments, the expression of iNOS had no impact on the mobilization of leukocytes into the peritoneal cavity induced by a number of inflammatory irritants such as thioglycollate broth or LPS plus IFN-γ (70). Similarly, in an oyster glycogen-induced model of acute peritonitis in rats, the iNOS-specific inhibitor, L-NIL, significantly inhibited NO production without altering polymorphonuclear neutrophils (PMN) recruitment, compared with vehicle-treated rats. The authors concluded that PMN-associated, iNOS-derived NO does not play an important role in modulating extravasion of these leukocytes in vivo in this model of acute inflammation (71).

The putative formation of peroxynitrite from NO during nitrosative stress may cause DNA single-strand breakage, which stimulates the activation of the nuclear enzyme poly(ADP-ribose) synthetase (PARS). Rapid activation of PARS depletes the intracellular concentration of its substrate, nicotinamide adenine dinucleotide (NAD^+), slowing the rate of glycolysis, electron transport and subsequent formation of ATP. This process can result in acute cell dysfunction and cell death (72,73). PARS also regulates the expression of a number of genes, including the genes for ICAM-1, collagenase, and iNOS. Inhibition of PARS protects against zymosan- or endotoxin-induced multiple organ failure, arthritis, allergic encephalomyelitis and diabetic islet cell destruction (72). Inhibition of PARS reduced neutrophil recruitment and reduced the extent of edema in zymosan- and carrageenan-triggered models of local inflammation (74). Furthermore, PARS null mice were more resistant against inflammation and organ injury than wild-type animals. Part of the anti-inflammatory effects of PARS inhibition were attributed to a reduced neutrophil recruitment, which may be related to maintained endothelial integrity (75).

Inducible NOS in Acute Inflammatory Responses

Sepsis and septic shock are caused by bacterial infection and represent an acute systemic inflammatory response. Septic shock is characterized by systemic hypotension, vascular smooth muscle hyporeactivity to adrenergic mimics, and myocardial depression (76,77). Cellular activation by cell wall components of Gram-negative or Gram-positive bacteria results in the production of a variety of inflammatory mediators that are essential for the development of

septic shock and its complications (26). Nitric oxide is crucial in the pathogenesis of septic shock (70,78–82).

Infusion of endotoxin or TNF-α into experimental animals is used to mimic septic shock and systemic NO production (83). Several studies showing an antihypotensive and protective effect for NOS inhibitors in rodent models of septic shock (31,84,85) suggest that the inhibition of NO production could be useful for the treatment of this condition. In contrast, in a murine model of chronic hepatic inflammation (administration of *Corynebacterium parvum* followed by LPS), our group has consistently shown the deleterious effect of NOS inhibition causing liver damage, intravascular thrombosis and oxygen radical-mediated hepatic injury (27, 86–88). The use of NOS inhibitors also has led to controversial results. In a murine model of endotoxemia, the nonselective NOS inhibitor, L-NAME, enhanced liver damage and tended to accelerate the time of death; whereas, the iNOS selective inhibitor, L-canavanine, significantly reduced mortality and had no deleterious effects in terms of organ damage (89). L-NAME also aggravated liver damage in a rat model of endotoxemia, while the iNOS selective inhibitor S-methylisothiourea did not increase LPS-induced damage (90). Inhibition of iNOS with L-NMMA also resulted in reduction of Cu/Zn SOD expression levels in rat glomerular mesangial cells treated with LPS. This finding suggests that up-regulation of Cu/Zn SOD by endogenous NO may serve as a protective mechanism against formation of peroxynitrite or other potentially damaging RNOS, such as nitroxyl anion (91), in conditions associated with iNOS induction during endotoxic shock (92).

Recently, mice lacking the iNOS gene were reported to be protected against LPS-induced mortality (70,93). However, another study demonstrated that mice lacking iNOS were not resistant to LPS-induced death (94). In two models of endotoxic shock in iNOS and respiratory burst oxidase deficient mice, major reductions in the ability to form NO or superoxide also failed to improve survival (95). Recently, Cobb et al. (96) used iNOS null mice to examine the effect of inducible NO production in a clinically relevant model of polymicrobial abdominal sepsis treated with antibiotics. The survival study showed that iNOS gene deficiency increased the mortality of sepsis in mice, suggesting a beneficial role for iNOS gene function in septic mice.

These contradictory effects of septic insults in iNOS null mice may be explained by modulation of NO production in the liver. The liver is a major site of the response to endotoxin (97). The dual effects of NO, both as hepatoprotective and hepatotoxic agent (98), and its role as a bifunctional regulator of apoptosis (99) were reviewed recently.

Nonspecific inhibition of iNOS also has been reported to be detrimental, rather than beneficial (27,83,100,101). Recent results showing that iNOS-deficient mice have enhanced leukocyte-endothelium interactions in endotoxemia raised the possibility that induction of iNOS is a homeostatic regulator for leukocyte recruitment (69). Although iNOS expression can protect the liver in acute hepatic inflammation, it may account for hepatic necrosis in ischemia/reperfusion and hemorrhagic shock (98). In a murine model of hemorrhagic shock, it was found that the expression of iNOS and NO production caused an increase in PMN influx, activation of the transcriptional factor NF-κB, and upregulation of IL-6 and granulocyte colony stimulating factor (G-CSF) mRNA levels. These changes were associated with marked lung and liver injury [(102), see also a recent review on the novel roles of NO in the pathogenesis of hemorrhagic shock and resuscitation (103)]. Thus, factors, such as the cellular redox status, the production of reactive oxygen species and pro-inflammatory cytokines, the type of insult, and the isoform selectivity of different NOS inhibitors, will determine the possible therapeutic use of NOS inhibitors in the future.

A critical role for the transcription factor NF-κB has been demonstrated in the transcriptional regulation of the murine and human iNOS gene induced by LPS and cytokines in cultured cells (104–107). More recently, it was reported that LPS activated NF-κB in vivo, which, in turn, induced transcription of the iNOS gene and expression of the iNOS protein in a rat model of septic shock. The authors suggested that targeting NF-κB might be a more effective strategy for the treatment of septic shock, because inhibition of NF-κB activation selectively prevented the increase in iNOS activity and iNOS-mediated NO production (108). Of course, this hypothesis is based on the putative detrimental role for NO in sepsis. In a recent study, treating poly(ADP-ribose)

polymerase-1 (PARP-1)-deficient mice with LPS did not result in the rapid activation of NF-κB seen in macrophages from wild-type mice. The PARP-1 null mice were extremely resistant to LPS-induced endotoxic shock, which was explained by the almost complete abrogation of NF-κB-dependent accumulation of TNF-α in the serum, as well as the down-regulation of iNOS (109). This study suggests PARP-1 may be a possible target for therapeutic interventions.

Nitric Oxide in Immunity and Chronic Inflammation

Inducible NOS and the Specific Immune Response

Release of NO has been reported in inflammatory responses initiated by microbial products or autoimmune reactions. Although the role of NO in nonspecific immunity is well-established in animal models (9), it still awaits definitive confirmation in humans. The effects of NO on specific immunity, however, need extensive investigation. In the generation of an inflammatory response, the defensive machinery of the immune system is based mainly on the activity of effector cells, such as T lymphocytes, macrophages and neutrophils. The activation of these cells results in the production of immune modulators, including cytokines, chemokines, and reactive oxygen and nitrogen species that form a complex regulatory network that determine the intensity and duration of inflammation. Based on the cytokine secretion pattern of $CD4^+$ helper T lymphocytes, two main subsets of T helper cells are defined (110): T helper type I (Th1) and T helper type II (Th2). The former are mainly implicated in cell-mediated immune reactions, macrophage activation and the production of opzonizing antibodies. At least in mice, this subset of T cells secrete IL-2, IFN-γ, and TNF-β. Th2 cells, on the other hand, secrete IL-4, IL-5, IL-6, IL-10 and IL-13, and are key players in humoral immunity and activate mast cells and eosinophils (110,111).

Several relevant diseases that are positive for iNOS also exhibit pro-inflammatory, Th1-type cytokine expression or cytokine response profiles (112,113). As is the case with sepsis, the elevated expression of iNOS in affected tissues suggests that iNOS is involved in the pathogenesis of certain immune diseases, but a number of controversial reports in the literature again suggest a possible dual role for NO. Although NO generation from L-arginine was required for DNA synthesis in human peripheral blood lymphocytes in one study (114), another study showed that human lymphocytes did not produce the appreciable amounts of NO needed to affect lymphocyte mitogenesis (115). Furthermore, the inhibitory effects of two NO donors (sodium nitroprusside and nitroglycerin) on lymphocyte function were shown to be nonspecific and unrelated to NO production (115). Recently, allogeneic (mixed leukocyte cultures), mitogenic and superantigenic stimulation of bovine blood mononuclear cells induced NO production at a low level and without having any effect on cellular activation and proliferation (116).

In fact, most of the existing data suggest that NO suppresses, rather than enhances, lymphocyte activation and proliferation. Antigen-stimulated mouse Th1 cells produce high levels of NO that result in a concomitant reduction of IL-2 secretion and lymphocyte proliferation. This is reversed by addition of recombinant IL-2 (117). Moreover, there is evidence that NO exerts different effects on discrete subpopulations of T cells, for example by inhibiting secretion of IL-2 by murine Th1 cells and increasing secretion of IL-4 by Th2 cells (118). This preferential effect, however, was not observed in activated human T cells and human T-cell clones in vitro, where the Th1- and Th2-associated cytokine production was equally impaired by the NO donors SIN-1 and SNAP (119). The question of whether NO differentially affects T lymphocyte function merits special attention, because the outcome of numerous diseases appears to depend critically on the Th1/Th2 balance in accompanying immune responses (111,113). Nitric oxide may amplify inflammation by altering this balance. Furthermore, the increased proliferation of Th2 lymphocytes may, in turn, produce a cytokine profile that was associated with exacerbation of asthma. These observations, however, have not been extended yet to humans, where the Th1/Th2 paradigm is less well-defined (120).

Both cGMP-independent and -dependent pathways have been described to explain the antiproliferative effects of NO. In human lymphocytes activated by lectin mitogen concanavalin (ConA), two oxotriazole derivatives (GEA 3162 and 3175) and the nitrosothiol SNAP caused inhibition of cell proliferation and enhanced cGMP production. While a guanylyl cyclase inhibitor inhibited the NO donor-induced cGMP production, the antipro-

liferative action remained unaltered (121). In contrast, another study showed that T cells activated in the presence of alveolar macrophages were unable to proliferate, despite the expression of IL-2 receptor and secretion of IL-2. The NO-mediated T cell suppression was reversible by the guanylyl cyclase inhibitors, methylene blue and LY-83583, and was reproduced by a cell-permeable analogue of cGMP. In addition, this effect could be reproduced by the addition of SNAP and inhibited by the NOS inhibitor L-NAME (122).

The involvement of NO production by iNOS in important autoimmune diseases, such as immunologically induced diabetes, inflammatory arthritis and graft versus host disease (GVHD), appears to be unquestionable. However, because of differences in the experimental animals and disease-induction methods, it is unclear whether NO is beneficial or detrimental. In several studies, administration of selective iNOS inhibitors to rodents with autoimmune diseases, led to conflicting results. One possible explanation for these often contradictory results is that iNOS inhibition is detrimental to the host during priming of pathogenic T-cell responses in the periphery, but largely protective at the site of disease (123). Early studies in a rodent model of arthritis suggested that selective inhibition of iNOS was beneficial in this disease state (124). In a different study of rats with adjuvant arthritis, however, administration of the NOS inhibitor, L-NIL, was without effect (125). Moreover, the protective role for NO in the host response to infections with *Staphylococcus aureus* strongly cautioned against the clinical use of selective NOS inhibitor therapy in diseases such as septic arthritis (126).

Experimental allergic encephalomyelitis (EAE) is a well-studied animal model of organ-specific autoiimmunity that mimics human multiple sclerosis. Treatment with aminoguanidine ameliorated EAE in both mice and rats (127–129), but it led to aggravation and prolongation of disease in myelin and T cell-mediated EAE (130). Similarly, L-NIL administration caused a marked worsening in disease expression in myelin basic protein (MBP)-immunized Lewis rats, but ameliorated the severity of disease following adoptive transfer of MBP-reactive T cells into L-NIL-treated recipients. Also in Lewis rats, aminoguanidine was shown to ameliorate experimental autoimmune myocarditis (131). Detrimental effects for iNOS inhibition also were reported in a model of autoimmune interstitial nephritis (132) and experimental autoimmune uveoretinitis (EAU) (133). More recently, it was reported that IL-12 protected mice from Th1-mediated EAU through a mechanism involving IFN-γ-induced NO production and bcl-2-regulated apoptotic deletion of the antigen-specific T cells (134).

To address the limitations of the administration of NOS inhibitors, iNOS null mice and antisense nucleotides were used to identify the functional roles of iNOS in different pathologies. In a model of EAE, mice deficient in or with reduced iNOS activity showed more disease and less remission than wild-type mice (135). In contrast, intraventricular administration of antisense oligodeoxynucleotide complementary to iNOS to SJL/S mice significantly reduced the clinical score of EAE and blocked the iNOS mRNA, the protein synthesis and the iNOS activity within the CNS (136). More recently, inhibition of allergic airway inflammation was observed in mice lacking iNOS (137).

The consequences of iNOS disruption were studied in MRL-lpr/lpr mice. These mice produce an excess of NO and develop a systemic autoimmune disease associated with a number of inflammatory manifestations, like glomerulonephritis, arthritis and vasculitis. iNOS-disrupted and wild-type mice displayed equivalent degrees of nephritis and arthritis, but the former showed markedly reduced vasculitis, suggesting heterogeneity in mechanisms of inflammation in MRL-lpr/lpr mice (138). Similar results showing that, in iNOS null mice, glomerulonephritis did not differ from that in mice with an intact iNOS gene, suggest that iNOS does not play an essential role in this autoimmune disease in the mouse (139).

Nitric oxide may also affect inflammation that occurs due to organ transplantation. Acute rejection is an immunoinflammatory process characterized by an intense inflammatory cell infiltrate and progressive destruction of the grafted organ. Although it has become apparent that NO contributes to allograft rejection, GVHD and tissue damage in alloimmune responses (140,141), the complex regulatory and effector mechanisms that underlie the rejection process are not completely understood. Production of NO was shown to partially account for the destruction of both lymphoid and erythroid host tissue, as well as the reduced lym-

phoproliferative responses associated with the acute phase of GVHD in mice (142). Also in mice with acute GVHD, induction of the NOS pathway by TNF-α was reported to suppress B-cell proliferation (143). A recent study showed that NO production by non-T, non-B, L-leucine methyl ester-sensitive cells mediated the graft versus host reaction-associated, IFN-γ-dependent immunosuppression of T-cell proliferation and of antibody synthesis by CD5(+) B cells (144). Administration of NOS inhibitors in models of GVHD was reported to prolong graft survival in mice undergoing GVHD (145), or to have no effect at all in mice receiving allogeneic heterotopic heart transplants (146). Administration of the nonselective NOS inhibitor, L-NMMA, produced only a small increase in graft survival in a model of heterotopic cardiac transplantation in the rat (147). Generation of NO also was observed in acute rejection of rat hepatic allografts (148) and of pancreas allografts in hyperglycemic rats, where electron spin resonance measurement of NO was suggested as a useful marker for the diagnosis of acute rejection in pancreas transplantation (149).

In humans, expression of iNOS was localized in lung transplant recipients with obliterative bronchiolitis (150) and in the coronary arteries of transplanted human hearts with accelerated graft arteriosclerosis. The role of NO in the pathogenesis, however, remains unknown (151). In transplanted rat aortic allografts, the inhibition of NO production significantly increased the intimal thickening, suggesting that NO suppresses the development of allograft arteriosclerosis (152). In the same study, transduction with iNOS using an adenoviral vector completely suppressed the development of allograft arteriosclerosis, indicating that iNOS may be important for the suppression of transplant vasculopathy in chronic rejection associated with cardiac transplantation (152). It was recently reported that cardiac myocyte apoptosis was closely associated with expression of iNOS in macrophages and myocytes and with nitration of myocyte proteins by peroxynitrite during human cardiac allograft rejection (153). Moreover, it was suggested that NO plays a role in modulating the localized bone resorption that accompanies the aseptic loosening of prosthetic joints (154). The identification of iNOS in the setting of organ transplantation will certainly open novel possibilities for the use of selective inhibitors and pharmacological intervention in the treatment of these alloimmune conditions. Other relevant immunologic effects attributed to NO are the inhibition of lymphokine-activated killer-cell induction by inducing apoptosis of cytolytic lymphocyte precursors (155); inhibition of major histocompatibility class II expression on mouse peritoneal macrophages and antigen presentation by lung dendritic cells; tumor-induced immunosuppression; and the reduced immunological response resulting from administration of morphine (13).

The demonstration of the involvement of NO in a number of autoimmune and inflammatory diseases does not necessarily imply that NO itself is the effector molecule. Whether these pathologies are directly mediated by NO or by RNOS, such as peroxynitrite or nitroxyl anion, requires further investigation. Inflammatory mediators that enhance the cellular production of NO also increase cellular superoxide production from various cellular sources, including NOS itself (156). Nitration of tyrosine, thought to occur due to the production of peroxynitrite and assessed by the degree of formation of nitrotyrosine, is used as a footprint of peroxynitrite activity (157–160). The putative role of peroxynitrite as an inflammatory mediator has been suggested after detection of nitrotyrosine in animal models of endotoxemia (161–163), lung injury (164,165), ileitis (166,167), experimental autoimmune encephalomyelitis (168, 169), myocardial ischemia-reperfusion injury (170,171), myocardial dysfunction (172), and glomerulonephritis (173), as well as in human atherosclerotic plaques (174,175), adult respiratory distress syndrome (176), airways of asthmatic patients (177), multiple sclerosis (178, 179), and human sepsis and myocarditis (180). However, it is still not clear whether the apparent requirement for the simultaneous presence of equimolar concentrations of NO and superoxide to form peroxynitrite (181) and, subsequently, form nitrotyrosine residues on proteins (182) can be fulfilled under pathologically relevant circumstances. Furthermore, recent evidence suggests that both myeloperoxidase (183,184) and eosinophil peroxidase (185) can oxidize NO_2^- to form nitrotyrosine, which suggests pathways of nitrotyrosine formation independent of peroxynitrite may exist.

Inducible NOS in Chronic Inflammatory Diseases

Chronic inflammation is characterized by a proliferation of fibroblasts and small blood vessels, as well as an influx of chronic in-

flammatory cells (lymphocytes, plasma cells, macrophages). In certain immunologic conditions, chronic inflammation is primary and not preceded by an acute inflammatory response. It also differs from acute inflammation in that it is orchestrated almost entirely by cells of the immune system (2). Although more tightly regulated than the rodent iNOS gene, expression of human iNOS has been found in chronic inflammatory diseases of the airways, the vessels, the bowels, the kidney, the heart, the skin and the apex of teeth (112), strongly indicating that NO plays an important role in the pathogenesis of chronic inflammation. Table 2 summarizes the most relevant chronic inflammatory conditions related to NO in humans. Below, we discuss some of the relevant studies both in humans and in animal models.

The involvement of NO in chronic localized inflammatory diseases has been demonstrated in a number of experimental animal models. Nitric oxide stimulates TNF-α production by synoviocytes and its catabolic effects on chondrocyte function promote the degradation of articular cartilage implicated in certain rheumatic diseases (16,186). Most studies indicate that NO is at least partly responsible for IL-1-induced suppression of glycosaminoglycan and collagen synthesis (187). In human chondrocytes, IL-18 has been identified as a cytokine that regulates chondrocyte responses and contributes to cartilage destruction through stimulation of the expression of several genes, including iNOS, inducible COX, IL-6, and stromelysin (188). The beneficial effects of inhibition of NOS and scavenging of NO have been shown in murine systemic lupus erythematosus (SLE) (16,189), suppression of rat adjuvant arthritis by N^G-iminoethyl-L-ornithine (L-NIO) (190) or L-NMMA (191), attenuation of streptococcal cell wall-induced arthritis in rats by L-NMMA (124), reduction of inflammation and injury in synovial tissue from joints with inflammatory arthritis by hemoglobin (192), prevention of chondrocyte death and cartilage erosion by local IL-4 application in the knee joint of mice with collagen-induced arthritis (193), and the suppression by diphenylene diodonium chloride of potassium peroxocromate-induced arthritis in mice (194). Cyclic tensile stress, which mimics the tensile stress experienced by chondrocytes on the surface of cartilage during movement, was also found to suppress pathologic effects of IL-1β through inhibition of inducible NO production in primary rabbit chondrocytes in vitro (195). Furthermore, in a model of osteoarthritis (OA) in dogs, inhibition of NOS reduced the progression of cartilage lesions and the production of metalloproteinases and IL-1 (16). In syn-

Table 2. Nitric oxide in human autoimmune and chronic inflammatory diseases

Disease	Reference
Rheumatic diseases	
Systemic lupus erythematosus	(263–265)
Vasculitis	(266,267)
Rheumatoid arthritis	(264,268–271)
Osteoarthritis	(186,268, 272–274)
Inflammatory airway disease	
Asthma	(177,230, 275–277)
Respiratory tract infections	(278–282)
Idiopathic pulmonary fibrosis	(283–285)
Bronchiectasis	(286–288)
Gastrointestinal system	
Inflammatory bowel disease	(289)
Ulcerative colitis	(290–293)
Crohn's disease	(290–292,294)
Diverticulitis	(290,295)
Necrotizing enterocolitis	(296–298)
Celiac disease	(299–302)
Helicobacter pylori-associated chronic gastritis	(303–306)
Kidney	
Glomerulonephritis	(307–310)
Lupus nephritis	(310–312)
Pancreas	
Diabetes	(313–316)
Pancreatitis	(317)
Liver	
Chronic hepatitis	(318,319)
Bladder	
Infectious and noninfectious cystitis	(320–323)
Central and peripheral nervous system	
Parkinson's disease	(324–326)
Multiple sclerosis	(327–330)
Severe AIDS dementia	(331–333)
Vasculitic and optic neuropathy	(334,335)
Skin	(336)
Psoriasis	(337–340)
Cutaneous lupus erythematosus	(341)
Systemic sclerosis	(342–344)
Dermatitis	(345–347)
Atherosclerosis	(219–221, 348–350)
Periapical periodontitis	(351–353)
Sjögren's syndrome	(354–356)

oviocytes from OA patients, two NO donors (SNAP and sodium nitroprusside) markedly increased p53 protein expression and DNA fragmentation in vitro (196). This suggests that iNOS-derived NO may be a major inducer of synoviocyte apoptosis in OA in vivo. Recently, iNOS-deficient mice were used to investigate the role of NO and IL-1 in joint inflammation and cartilage destruction in a nonimmunologic model of inflammation, the zymosan-induced gonarthritis (197). In this study, IL-1 and NO played only a minor role in edema and neutrophil influx, but a major role in cartilage destruction. Moreover, the results obtained from anti-IL-1 treatment of wild-type mice were comparable to those found in iNOS null mice, which suggests that most IL-1-related effects in arthritis were mediated by NO (197).

Although most experimental findings suggest that the actions of NO in the cartilage are detrimental, there is also evidence for protective functions of NO. In a recent study, intravenous inoculation with *S. aureus* induced significantly increased clinical severity of septic arthritis, with attendant septicemia in iNOS-deficient mice, compared with similarly infected heterozygous or wild-type mice. This was associated with enhanced production of IFN-γ and TNF-α in vivo and in vitro, which indicated a shift towards increased production of Th1-type cytokines (126). Apart from antimicrobial activity, other beneficial effects of NO include stimulation of proteoglycan synthesis during certain conditions, participation in wound healing, and stimulation of collagen production (187).

The NO-mediated destruction of both rat and mouse islets of Langerhans and its effects on insulin secretion provide strong evidence for the involvement of NO in human diabetes (112). Administration of a natural IL-12 antagonist, which suppressed the progression of islet inflammation and concomitant upregulation of iNOS (198), and overexpression of the anti-apoptotic gene A20, which abrogated cytokine-induced NO production and protected both human and rat islet cells against apoptosis (199), suggest possible strategies for therapeutic intervention against NO-mediated toxicity in islet inflammation. However, it is not known whether the inhibition of human iNOS will reduce the destruction of >90% of the pancreatic islets found in type-1 diabetes.

NO also contributes to mucosal damage in inflammatory bowel disease (200,201) and the beneficial effects of NOS inhibitors for reducing intestinal inflammation is shown in various models of colitis (202–204). Furthermore, NO also is reported to promote mucosal integrity (205). The isoform nonselective NOS inhibitor L-NAME worsens acute edematous and necrotizing pancreatitis; whereas, NO donors reduces pancreatic injury (206). Indeed, there is increasing evidence that iNOS is beneficial, rather than detrimental, for resolving intestinal inflammation (207). Evidence for the dual roles of inducible NO in modulating gastrointestinal mucosal defense and injury is presented in a recent review (208).

The relationship between inflammation and atherosclerosis is well established, but the biologic events that trigger the local inflammatory response within plaque are not fully understood. The development of atherosclerosis and hyperlipaemia per se is accompanied by impairment of endothelium-dependent vasodilation. Atherosclerosis is associated with marked changes in the activity of NOS isoforms in the artery wall, including increased expression of the iNOS in complex human lesions, as well as in the neointima of experimental animal models. Defective NO production by eNOS, together with inducible NO and superoxide anions generated by inflammatory cells, are detrimental events that may cause apoptosis and injury to both the endothelium and myocytes, and possibly lead to plaque rupture. In this way, the balance between the possible protective effects of NO and the deleterious effects of RNOS may be disturbed (10). In endothelial cells, NOS prevents apoptosis (209,210); whereas, it induces apoptosis in smooth muscle cells (211–215).

The presence of iNOS in atherosclerotic plaques suggests a role for NO in atherosclerosis (216–218), but its exact role is still unknown. Inducible NOS and nitrotyrosine is detected within the atheroma (219), and COX-2 and iNOS/nitrotyrosine co-localize predominantly in macrophages/foam cells in both native and transplanted human coronary arteries (220). These findings may suggest an ongoing production of both prostanoids and RNOS, such as peroxynitrite, both of which may have proatherosclerotic effects. Interestingly, high expression levels of the anti-inflammatory cytokine, IL-10, are associated with significant decreases, in iNOS expression and cell death in a recent study of advanced human atheroscle-

rotic plaques (221). Additionally, TGF-β1 and its signaling system are perturbed in atherosclerosis (222–226). These findings suggest that a balance between iNOS-inducing and iNOS-suppressing mediators might modulate the expression of iNOS in atherosclerosis.

In situ hybridization and immunohistochemical techniques localize iNOS to human lung in certain disease states. The expression of iNOS is described in a murine model of allergic asthma (227), foreign body-induced granulomatous lung inflammation (228), as well as in radiation pneumonitis and fibrosis in rats (229). Exhaled NO levels are increased in patients with asthmatic flares, bronchiectasis, and active tuberculosis, and are considered as a marker of inflammatory injury; however, the precise role of NO in lung inflammation is still under debate (120,230). Given the increasing evidence that viruses are a major cause of acute exacerbation of asthma, the cytototoxic and potent antiviral properties of inducible NO may be beneficial (120). The interaction of NO with the transcription factor NF-κB, which is activated by diverse inflammatory stimuli, is causally linked to respiratory cell inflammation and pulmonary disease, but has not been demonstrated unambiguously (231). It should be noted that high concentrations of NO are capable of killing *Mycobacterium tuberculosis* and this may be significant for the control of infection in the lung (232).

Cerebrospinal fluid concentrations of the stable NO metabolite nitrite are elevated both in animal models of bacterial meningitis (BM) and in patients with the disease (233). In a model of BM, production of nitrite in the cerebrospinal fluid of rats correlated with elevated blood-brain barrier permeability, which suggests that NO contributes to the pathophysiology of BM (233). However, NO produced by iNOS may be beneficial as well. Recently, inhibition of iNOS, primarily localized to the cerebral vasculature and inflammatory cells in the subarachnoid and ventricular space, increased cortical hypoperfusion and ischemic neuronal injury in an infant rat model of meningitis caused by group B streptococci (234).

One of the primary functions of the inflammatory response is to heal wounded tissue. Healing commences soon after injury, while acute inflammation in still in full swing. Aseptic wounding induces iNOS, which may modulate wound healing (235); however, definitive proof of this concept requires wound-healing studies in iNOS null mice. Our group showed a delay in closure of excisional wounds in iNOS-deficient mice, compared with wild-type mice. This defect in healing of excisional wounds could be quantitatively corrected by a single topical administration of an adenoviral vector expressing iNOS cDNA (236). Interestingly, the cytokine most associated with wound healing, TGF-β1, may be the most potent suppressor of iNOS (237). The recent finding that exposure to NO of cells, which express latent TGF-β1, could lead to the activation of this cytokine (35). This raises the intriguing suggestion that one of the roles of iNOS in wound healing is to modulate TGF-β1. Most importantly, these findings suggest caution with the use of iNOS inhibitors in settings that require appropriate wound healing.

Conclusions

It is now clear that NO cannot be rigidly catalogued as either an anti-inflammatory or a pro-inflammatory molecule, but it can be considered a true inflammatory mediator. Although all three NOS isoforms are involved to a greater or lesser extent in the course of inflammation, the role of iNOS appears to be dominant. Inducible, high-level NO production mediates a number of inflammatory and infectious diseases by acting both as a direct effector and as a regulator of other effector pathways. The dichotomous role of NO in inflammation, often referred to as the NO paradox, is based mainly on the conflicting data showing the effects of NOS inhibitors of varying selectivity in different animal models. In addition, the use of iNOS null animals for exploring the role of NO further reinforces this view and clearly demonstrates that caution should be taken when extrapolating experimental results to possible therapeutic benefits. For example, the basal upregulation of heat shock protein 72 in iNOS null mice, rather than the inhibition of inducible NO production, is implicated in their protection from renal ischemia-reperfusion injury (238). Finally, the spectrum of activity of NO itself versus that of the reaction products of NO present under physiological and pathological conditions (10) may help account for this seeming paradox.

The expression of iNOS is reported in a variety of diseases. The level of iNOS expression and high output NO formation trigger short- and long-term effects that may be either bene-

ficial or deleterious, and ultimately depend on five factors:

(1) the existence of additional metabolic pathways that provide the iNOS with substrate and cofactors;
(2) the effect of other pathways that influence or affect the enzyme induction and activity;
(3) the molecular targets with which NO and NO-derived species interact;
(4) local factors, such as the cellular redox state; and
(5) the presence and concentration of endogenous defense and antioxidant/scavenging mechanisms.

Administration of NO donors (in hypertension, angina, atherosclerosis, and gastrointestinal and genitourinary disorders), inhalation of NO gas (in chronic pulmonary hypertension or adult respiratory distress syndrome), or increased intake of L-arginine (in atherosclerosis) may be suitable therapies for disease states in which impaired NO production appears to exacerbate inflammation. The biggest challenge, therefore, is to develop strategies that target the cytotoxic and damaging actions of NO/RNOS without interfering with essential protective functions. Besides selectively inhibiting iNOS, a number of other therapeutic strategies are conceivable in order to alleviate the deleterious effects of excessive NO formation. These alternative therapies involve scavenging of NO/RNOS, and/or inhibition of metabolic pathways triggered by these molecules. The advantage of preserving the beneficial effects of iNOS also needs to be considered when implementing any therapeutic approach. Counteracting vascular hyper-responsiveness to endogenous vasoconstrictor agonists in septic shock, or inducing cardiac protection against ischemia-reperfusion injury are examples of such beneficial effects of iNOS. Only the identification of the roles of NO and of the cells that produce it, as well as the more complete elucidation of the mechanisms that regulate its cellular production in inflammation, will help in the development of therapeutic applications for both acute and chronic inflammatory diseases.

References

1. Moncada S. (1999) Nitric oxide: discovery and impact on clinical medicine. *J. R. Soc. Med.* **92:** 164–169.
2. Trowbridge HO, Emling RC. (1997) In: *Inflammation: a review of the process, 5th Ed.* Quintessence Pub. Co., Chicago
3. Wagner DA, Young VR, Tannenbaum SR. (1983) Mammalian nitrate biosynthesis: Incorporation of $^{15}NH_3$ into nitrate is enhanced by endotoxin treatment. *Proc. Natl. Acad. Sci. U.S.A.* **80:** 4518–4521.
4. Moncada S, Palmer RMJ, Higgs EA. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* **43:** 109–142.
5. Stuehr DJ, Marletta MA. (1985) Mammalian nitrate biosynthesis: mouse macrophages produce nitrite and nitrate in response to Escherichia coli lipopolysaccharide. *Proc. Natl. Acad. Sci. U.S.A.* **82:** 7738–7742.
6. Geller DA, Billiar TR. (1998) Molecular biology of nitric oxide synthases. *Cancer Metastasis Rev.* **17:** 7–23.
7. Stuehr DJ. (1999) Mammalian nitric oxide synthases. *Biochim. Biophys. Acta* **1411:** 217–230.
8. Vodovotz Y, Bogdan C, Paik J, et al. (1993) Mechanisms of suppression of macrophage nitric oxide release by transforming growth factor β. *J. Exp. Med.* **178:** 605–613.
9. Macmicking JD, Xie Qw, Nathan C. (1997) Nitric oxide and macrophage function. *Annu. Rev. Immunol.* **15:** 323–350.
10. Wink DA, Feelisch M, Vodovotz Y, et al. (1999) The chemical biology of nitric oxide. In: Colton CA, Gilbert DL (eds.) *Reactive Oxygen Species in Biological Systems: An Interdisciplinary approach.* Kluwer Academic/Plenum Publishing, New York, pp. 245–291.
11. Nathan C, Xie QW. (1994) Regulation of biosynthesis of nitric oxide. *J. Biol. Chem.* **269:** 13725–13728.
12. Denninger JW, Marletta MA. (1999) Guanylate cyclase and the .NO/cGMP signaling pathway. *Biochim. Biophys. Acta* **1411:** 334–350.
13. Evans CH. (1995) Nitric oxide: what role does it play in inflammation and tissue destruction? *Agents Actions Suppl.* **47:** 107–116.
14. Lyons CR. (1995) The role of nitric oxide in inflammation. *Adv. Immunol.* **60:** 323–371.
15. Nathan C. (1997) Inducible nitric oxide synthase: what difference does it make? *J. Clin. Invest.* **100:** 2417–2423.
16. Clancy RM, Amin AR, Abramson SB. (1998) The role of nitric oxide in inflammation and immunity. *Arthritis Rheum.* **41:** 1141–1151.
17. Zamora R, Billiar TR. (2000) Nitric oxide: A true inflammatory mediator. In: Mayer B (ed.) *Handbook in Experimental Pharmacology.* Springer-Verlag, Berlin.
18. Ryffel B. (1997) Impact of knockout mice in toxicology. *Crit. Rev. Toxicol.* **27:** 135–154.
19. Vodovotz Y, Geiser AG, Chesler L, et al. (1996) Spontaneously increased production of nitric oxide and aberrant expression of the inducible

nitric oxide synthase in vivo in the transforming growth factor β 1 null mouse. *J. Exp. Med.* **183:** 2337–2342.
20. Nathan C, Xie Qw. (1994) Nitric oxide synthases: roles, tolls, and controls. *Cell* **78:** 915–918.
21. Trepicchio WL, Bozza M, Pedneault G, et al. (1996) Recombinant human IL-11 attenuates the inflammatory response through down-regulation of proinflammatory cytokine release and nitric oxide production. *J. Immunol.* **157:** 3627–3634.
22. Bogdan C, Vodovotz Y, Paik J, et al. (1994) Mechanism of suppression of nitric oxide synthase expression by interleukin-4 in primary mouse macrophages. *J. Leukoc. Biol.* **55:** 227–233.
23. Barton BE, Jackson JV. (1993) Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model. *Infect. Immun.* **61:** 1496–1499.
24. Howard M, Muchamuel T, Andrade S, et al. (1993) Interleukin 10 protects mice from lethal endotoxemia. *J. Exp. Med.* **177:** 1205–1208.
25. Bogdan C, Thuring H, Dlaska M, et al. (1997) Mechanism of suppression of macrophage nitric oxide release by IL-13: influence of the macrophage population. *J. Immunol.* **159:** 4506–4513.
26. Groeneveld PH, Kwappenberg KM, Langermans JA, et al. (1997) Relation between pro- and anti-inflammatory cytokines and the production of nitric oxide (NO) in severe sepsis. *Cytokine.* **9:** 138–142.
27. Billiar TR, Curran RD, Harbrecht BG, et al. (1990) Modulation of nitrogen oxide synthesis in vivo: N^G-monomethyl-L-arginine inhibits endotoxin-induced nitrate/nitrate biosynthesis while promoting hepatic damage. *J. Leukoc. Biol.* **48:** 565–569.
28. Cobb JP, Natanson C, Hoffman WD, et al. (1992) N omega-amino-L-arginine, an inhibitor of nitric oxide synthase, raises vascular resistance but increases mortality rates in awake canines challenged with endotoxin. *J. Exp. Med.* **176:** 1175–1182.
29. Vodovotz Y, Kopp JB, Takeguchi H, et al. (1998) Increased mortality, blunted production of nitric oxide, and increased production of TNF-α in endotoxemic TGF-β1 transgenic mice. *J. Leukoc. Biol.* **63:** 31–39.
30. Vodovotz Y, Letterio JJ, Geiser AG, et al. (1996) Control of nitric oxide production by endogenous TGF-β1 and systemic nitric oxide in retinal pigment epithelial cells and peritoneal macrophages. *J. Leukoc. Biol.* **60:** 261–270.
31. Perrella MA, Hsieh CM, Lee WS, et al. (1996) Arrest of endotoxin-induced hypotension by transforming growth factor β1. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 2054–2059.
32. Pender BS, Chen H, Ashton S, et al. (1996) Transforming growth factor β 1 alters rat peritoneal macrophage mediator production and improves survival during endotoxic shock. *Eur. Cytokine Netw.* **7:** 137–142.
33. Dimmeler S, Haendeler J, Nehls M, et al. (1997) Suppression of apoptosis by nitric oxide via inhibition of interleukin- 1β-converting enzyme (ICE)-like and cysteine protease protein (CPP)- 32-like proteases. *J. Exp. Med.* **185:** 601–607.
34. Kim YM, Talanian RV, Li J, et al. (1998) Nitric oxide prevents IL-1β and IFN-γ-inducing factor (IL-18) release from macrophages by inhibiting caspase-1 (IL-1β-converting enzyme). *J. Immunol.* **161:** 4122–4128.
35. Vodovotz Y, Chesler L, Chong H, et al. (1999) Regulation of transforming growth factor β1 by nitric oxide. *Cancer Res.* **59:** 2142–2149.
36. Bonta IL, Ben-Efraim S. (1993) Involvement of inflammatory mediators in macrophage antitumor activity. *J. Leukoc. Biol.* **54:** 613–626.
37. Nathan CF. (1987) Secretory products of macrophages. *J. Clin. Invest.* **79:** 319–326.
38. Salvemini D, Misko TP, Masferrer JL, et al. (1993) Nitric oxide activates cyclooxygenase enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 7240–7244.
39. Salvemini D, Seibert K, Marino MH. (1996) PG release, as a consequence of NO-driven COX activation, contributes to the proinflammatory effects of NO. *Drugs News Perspect.* **4:** 204–219.
40. Di Rosa M, Ialenti A, Ianaro A, et al. (1996) Interaction between nitric oxide and cyclooxygenase pathways. *Prostagl. Leukotr. Essential Fatty Acids* **54:** 229–238.
41. Swierkosz TA, Mitchell JA, Warner TD, et al. (1995) Co-induction of nitric oxide synthase and cyclo-oxygenase: interactions between nitric oxide and prostanoids. *Br. J. Pharmacol.* **114:** 1335–1342.
42. Corbett JA, Kwon G, Turk J, et al. (1993) IL-1 β induces the coexpression of both nitric oxide synthase and cyclooxygenase by islets of Langerhans: activation of cyclooxygenase by nitric oxide. *Biochemistry* **32:** 13767–13770.
43. Inoue T, Fukuo K, Morimoto S, et al. (1993) Nitric oxide mediates interleukin-1-induced prostaglandin E2 production by vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* **194:** 420–424.
44. Zamora R, Bult H, Herman AG. (1998) The role of prostaglandin E2 and nitric oxide in cell death in J774 murine macrophages. *Eur. J. Pharmacol.* **349:** 307–315.
45. Hughes FJ, Buttery LK, Hukkanen MJ, et al. (1999) Cytokine-induced prostaglandin E2 synthesis and cyclooxygenase-2 activity are regulated both by a nitric oxide-dependent

and independent mechanism in rat osteoblasts in vitro. *J. Biol. Chem.* **274:** 1776–1782.
46. Stadler J, Harbrecht BG, Di Silvio M, et al. (1993) Endogenous nitric oxide inhibits the synthesis of cyclooxygenase products and interleukin-6 by rat Kupffer cells. *J. Leukoc. Biol.* **53:** 165–172.
47. Stadler J, Stefanovic-Racic M, Billiar TR, et al. (1991) Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. *J. Immunol.* **147:** 3915– 3920.
48. Kosonen O, Kankaanranta H, Malo-Ranta U, et al. (1998) Inhibition by nitric oxide-releasing compounds of prostacyclin production in human endothelial cells. *Br. J. Pharmacol.* **125:** 247–254.
49. Marotta P, Sautebin L, Di Rosa M. (1992) Modulation of the induction of nitric oxide synthase by eicosanoids in the murine macrophage cell line J774. *Br. J. Pharmacol.* **107:** 640–641.
50. Bulut V, Severn A, Liew FY. (1993) Nitric oxide production by murine macrophages is inhibited by prolonged elevation of cyclic AMP. *Biochem. Biophys. Res. Commun.* **195:** 1134–1138.
51. Milano S, Arcoleo F, Dieli M, et al. (1995) Prostaglandin E2 regulates inducible nitric oxide synthase in the murine macrophage cell line J774. *Prostaglandins* **49:** 105–115.
52. Pang L, Hoult JRS. (1996) Induction of cyclooxygenase and nitric oxide synthase in endotoxin-activated J774 macrophages is differentially regulated by indomethacin: Enhanced cyclooxygenase-2 protein expression but reduction of inducible nitric oxde synthase. *Eur. J. Pharmacol.* **317:** 151–155.
53. Brouet I, Ohshima H. (1995) Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochem. Biophys. Res. Commun.* **206:** 533–540.
54. Kepka-Lenhart D, Chen L-C, Morris SM. (1996) Novel actions of aspirin and sodium salicylate: discordant effects on nitric oxide synthesis and induction of nitric oxide synthase mRNA in a murine macrophage cell line. *J. Leukoc. Biol.* **59:** 840–846.
55. Sanchez dM, de Frutos T, Gonzalez-Fernandez F, et al. (1999) Aspirin inhibits inducible nitric oxide synthase expression and tumour necrosis factor-alpha release by cultured smooth muscle cells. *Eur. J. Clin. Invest* **29:** 93–99.
56. Harbrecht BG, Kim YM, Wirant EA, et al. (1997) Timing of prostaglandin exposure is critical for the inhibition of LPS- or IFN-γ-induced macrophage NO synthesis by PGE_2. *J. Leukoc. Biol.* **61:** 712–720.
57. Moncada S. (1997) Nitric oxide in the vasculature: physiology and pathophysiology. *Ann. N. Y. Acad. Sci.* **811:** 60–67.
58. Cuzzocrea S, Zingarelli B, Hake P, et al. (1998) Antiinflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. *Free Radic. Biol. Med.* **24:** 450–459.
59. Salvemini D, Wang ZQ, Bourdon DM, et al. (1996) Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. *Eur. J. Pharmacol.* **303:** 217–220.
60. Salvemini D, Wang ZQ, Wyatt PS, et al. (1996) Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *Br. J. Pharmacol.* **118:** 829–838.
61. Handy RL, Moore PK. (1998) A comparison of the effects of L-NAME, 7-NI and L-NIL on carrageenan-induced hindpaw oedema and NOS activity. *Br. J. Pharmacol.* **123:** 1119–1126.
62. Cuzzocrea S, Sautebin L, De Sarro G, et al. (1999) Role of IL-6 in the pleurisy and lung injury caused by carrageenan. *J. Immunol.* **163:** 5094–5104.
63. Ridger VC, Pettipher ER, Bryant CE, et al. (1997) Effect of the inducible nitric oxide synthase inhibitors aminoguanidine and L-N6-(1-iminoethyl)lysine on zymosan-induced plasma extravasation in rat skin. *J. Immunol.* **159:** 383–390.
64. Vergnolle N, Hollenberg MD, Sharkey KA, et al. (1999) Characterization of the inflammatory response to proteinase-activated receptor-2 (PAR2)-activating peptides in the rat paw. *Br. J. Pharmacol.* **127:** 1083–1090.
65. Wang R, Ghahary A, Shen YJ, et al. (1996) Human dermal fibroblasts produce nitric oxide and express both constitutive and inducible nitric oxide synthase isoforms. *J. Invest. Dermatol.* **106:** 419–427.
66. Kubes P, Suzuki M, Granger DN. (1991) Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc. Natl. Acad. Sci. U.S.A.* **88:** 4651–4655.
67. Spiecker M, Peng HB, Liao JK. (1997) Inhibition of endothelial vascular cell adhesion molecule-1 expression by nitric oxide involves the induction and nuclear translocation of IκBα. *J. Biol. Chem.* **272:** 30969–30974.
68. Peng HB, Spiecker M, Liao JK. (1998) Inducible nitric oxide: an autoregulatory feedback inhibitor of vascular inflammation. *J. Immunol.* **161:** 1970–1976.
69. Hickey MJ, Sharkey KA, Sihota EG, et al. (1997) Inducible nitric oxide synthase-deficient mice have enhanced leukocyte-endothelium interactions in endotoxemia. *FASEB J.* **11:** 955–964.
70. Macmicking JD, Nathan C, Hom G, et al. (1995) Altered responses to bacterial infection and endotoxic shock in mice lacking inducible nitric oxide synthase. *Cell* **81:** 641–650.

71. Cockrell A, Laroux FS, Jourd'heuil D, et al. (1999) Role of inducible nitric oxide synthase in leukocyte extravasation in vivo. *Biochem. Biophys. Res. Commun.* **257:** 684–686.
72. Szabo C. (1998) Role of poly(ADP-ribose)-synthetase in inflammation. *Eur. J. Pharmacol.* **350:** 1–19.
73. Szabo C, Dawson VL. (1998) Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. *Trends. Pharmacol. Sci.* **19:** 287–298.
74. Cuzzocrea S, Costantino G, Zingarelli B, et al. (1999) Protective effects of poly (ADP-ribose) synthase inhibitors in zymosan- activated plasma induced paw edema. *Life Sci.* **65:** 957–964.
75. Szabo C, Lim LH, Cuzzocrea S, et al. (1997) Inhibition of poly (ADP-ribose) synthetase attenuates neutrophil recruitment and exerts antiinflammatory effects. *J. Exp. Med.* **186:** 1041–1049.
76. Glauser MP, Zanetti G, Baumgartner JD, et al. (1991) Septic shock: pathogenesis. *Lancet* **338:** 732–736.
77. Parrillo JE. (1993) Pathogenetic mechanisms of septic shock. *N. Engl. J. Med.* **328:** 1471–1477.
78. Kilbourn RG, Gross SS, Jubran A, et al. (1990) NG-methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc. Natl. Acad. Sci. U.S.A.* **87:** 3629–3632.
79. Petros A, Bennett D, Vallance P. (1991) Effect of nitric oxide synthase inhibitors on hypotension in patients with septic shock. *Lancet* **338:** 1557–1558.
80. Liu S, Adcock IM, Old RW, et al. (1993) Lipopolysaccharide treatment in vivo induces widespread tissue expression of inducible nitric oxide synthase mRNA. *Biochem. Biophys. Res. Commun.* **196:** 1208–1213.
81. Symeonides S, Balk RA. (1999) Nitric oxide in the pathogenesis of sepsis. *Infect. Dis. Clin. North Am.* **13:** 449–463.
82. Wolkow PP. (1998) Involvement and dual effects of nitric oxide in septic shock. *Inflamm. Res.* **47:** 152–166.
83. Nussler AK, Billiar TR. (1993) Inflammation, immunoregulation, and inducible nitric oxide synthase. *J. Leukoc. Biol.* **54:** 171–178.
84. Szabo C, Southan GJ, Thiemermann C. (1994) Beneficial effects and improved survival in rodent models of septic shock with S-methylisothiourea sulfate, a potent and selective inhibitor of inducible nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.* **91:** 12472–12476.
85. Wu CC, Chen SJ, Szabo C, et al. (1995) Aminoguanidine attenuates the delayed circulatory failure and improves survival in rodent models of endotoxic shock. *Br. J. Pharmacol.* **114:** 1666–1672.
86. Harbrecht BG, Billiar TR, Stadler J, et al. (1992) Inhibition of nitric oxide synthesis during endotoxemia promotes intrahepatic thrombosis and an oxygen radical-mediated hepatic injury. *J. Leukoc. Biol.* **52:** 390–394.
87. Harbrecht BG, Stadler J, Demetris AJ, et al. (1994) Nitric oxide and prostaglandins interact to prevent hepatic damage during murine endotoxemia. *Am. J. Physiol.* **266:** G1004–G1010.
88. Ou J, Carlos TM, Watkins SC, et al. (1997) Differential effects of nonselective nitric oxide synthase (NOS) and selective inducible NOS inhibition on hepatic necrosis, apoptosis, ICAM- 1 expression, and neutrophil accumulation during endotoxemia. *Nitric. Oxide.* **1:** 404–416.
89. Liaudet L, Rosselet A, Schaller MD, et al. (1998) Nonselective versus selective inhibition of inducible nitric oxide synthase in experimental endotoxic shock. *J. Infect. Dis.* **177:** 127–132.
90. Vos TA, Gouw AS, Klok PA, et al. (1997) Differential effects of nitric oxide synthase inhibitors on endotoxin-induced liver damage in rats. *Gastroenterology* **113:** 1323–1333.
91. Wink DA, Feelisch M, Fukuto J, et al. (1998) The cytotoxicity of nitroxyl: possible implications for the pathophysiological role of NO. *Arch. Biochem. Biophys.* **351:** 66–74.
92. Frank S, Zacharowski K, Wray GM, et al. (1999) Identification of copper/zinc superoxide dismutase as a novel nitric oxide-regulated gene in rat glomerular mesangial cells and kidneys of endotoxemic rats. *FASEB J.* **13:** 869–882.
93. Wei XQ, Charles IG, Smith A, et al. (1995) Altered immune responses in mice lacking inducible nitric oxide synthase. *Nature* **375:** 408–411.
94. Laubach VE, Shesely EG, Smithies O, et al. (1995) Mice lacking inducible nitric oxide synthase are not resistant to lipopolysaccharide-induced death. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 10688–10692.
95. Nicholson SC, Grobmyer SR, Shiloh MU, et al. (1999) Lethality of endotoxin in mice genetically deficient in the respiratory burst oxidase, inducible nitric oxide synthase, or both. *Shock* **11:** 253–258.
96. Cobb JP, Hotchkiss RS, Swanson PE, et al. (1999) Inducible nitric oxide synthase gene deficiency increases the mortality of sepsis in mice. *Surgery* **126:** 438–442.
97. Sriskandan S, Cohen J. (1995) The pathogenesis of septic shock. *J. Infect.* **30:** 201–206.
98. Li J, Billiar TR. (1999) Determinants of Nitric Oxide Protection and Toxicity in Liver. *Am. J. Physiol.* **276:** G1069–G1073.
99. Kim YM, Bombeck CA, Billiar TR. (1999) Nitric oxide as a bifunctional regulator of apoptosis. *Circ. Res.* **84:** 253–256.

100. Fukatsu K, Saito H, Fukushima R, et al. (1995) Detrimental effects of a nitric oxide synthase inhibitor (N-omega-nitro-L-arginine-methyl-ester) in a murine sepsis model. *Arch. Surg.* **130:** 410–414.
101. Park JH, Chang SH, Lee KM, et al. (1996) Protective effect of nitric oxide in an endotoxin-induced septic shock. *Am. J. Surg.* **171:** 340–345.
102. Hierholzer C, Harbrecht B, Menezes JM, et al. (1998) Essential role of induced nitric oxide in the initiation of the inflammatory response after hemorrhagic shock. *J. Exp. Med.* **187:** 917–928.
103. Szabo C, Billiar TR. (1999) Novel roles of nitric oxide in hemorrhagic shock. *Shock* **12:** 1–9.
104. Xie Qw, Kashiwabara Y, Nathan C. (1994) Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. *J. Biol. Chem.* **269:** 4705–4708.
105. Spink J, Cohen J, Evans TJ. (1995) The cytokine responsive vascular smooth muscle cell enhancer of inducible nitric oxide synthase. Activation by nuclear factor-kappa B. *J. Biol. Chem.* **270:** 29541–29547.
106. Wong HR, Finder JD, Wasserloos K, et al. (1996) Transcriptional regulation of iNOS by IL-1 β in cultured rat pulmonary artery smooth muscle cells. *Am. J. Physiol.* **271:** L166–L171.
107. Taylor BS, de Vera ME, Ganster RW, et al. (1998) Multiple NF-kappa B enhancer elements regulate cytokine induction of the human inducible nitric oxide synthase gene. *J. Biol. Chem.* **273:** 15148–15156.
108. Liu SF, Ye X, Malik AB. (1997) In vivo inhibition of nuclear factor-kappa B activation prevents inducible nitric oxide synthase expression and systemic hypotension in a rat model of septic shock. *J. Immunol.* **159:** 3976–3983.
109. Oliver FJ, Menissier-de Murcia J, Nacci C, et al. (1999) Resistance to endotoxic shock as a consequence of defective NF-kappa B activation in poly (ADP-ribose) polymerase-1 deficient mice. *EMBO J.* **18:** 4446–4454.
110. Abbas AK, Murphy KM, Sher A. (1996) Functional diversity of helper T lymphocytes. *Nature* **383:** 787–793.
111. Mosmann TR, Sad S. (1996) The expanding universe of T-cell subsets: Th1, Th2 and more [see comments]. *Immunol. Today* **17:** 138–146.
112. Kroncke KD, Fehsel K, Kolb-Bachofen V. (1998) Inducible nitric oxide synthase in human diseases. *Clin. Exp. Immunol.* **113:** 147–156.
113. Kolb H, Kolb-Bachofen V. (1998) Nitric oxide in autoimmune disease: cytotoxic or regulatory mediator? *Immunol. Today* **19:** 556–561.
114. Efron DT, Kirk SJ, Regan MC, et al. (1991) Nitric oxide generation from L-arginine is required for optimal human peripheral blood lymphocyte DNA synthesis. *Surgery* **110:** 327–334.
115. Shoker AS, Yang H, Murabit MA, et al. (1997) Analysis of the in vitro effect of exogenous nitric oxide on human lymphocytes. *Mol. Cell Biochem.* **171:** 75–83.
116. Schuberth HJ, Hendricks A, Leibold W. (1998) There is no regulatory role for induced nitric oxide in the regulation of the in vitro proliferative response of bovine mononuclear cells to mitogens, alloantigens or superantigens. *Immunobiology* **198:** 439–450.
117. Taylor-Robinson AW. (1997) Counter-regulation of T helper 1 cell proliferation by nitric oxide and interleukin-2. *Biochem. Biophys. Res. Commun.* **233:** 14–19.
118. Chang RH, Feng MH, Liu WH, et al. (1997) Nitric oxide increased interleukin-4 expression in T lymphocytes. *Immunology* **90:** 364–369.
119. Bauer H, Jung T, Tsikas D, et al. (1997) Nitric oxide inhibits the secretion of T-helper 1- and T-helper 2-associated cytokines in activated human T cells. *Immunology* **90:** 205–211.
120. Sanders SP. (1999) Nitric oxide in asthma. Pathogenic, therapeutic, or diagnostic? *Am. J. Respir. Cell Mol. Biol.* **21:** 147–149.
121. Kosonen O, Kankaanranta H, Lahde M, et al. (1998) Nitric oxide-releasing oxatriazole derivatives inhibit human lymphocyte proliferation by a cyclic GMP-independent mechanism. *J. Pharmacol. Exp. Ther.* **286:** 215–220.
122. Bingisser RM, Tilbrook PA, Holt PG, et al. (1998) Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. *J. Immunol.* **160:** 5729–5734.
123. Gold DP, Schroder K, Powell HC, et al. (1997) Nitric oxide and the immunomodulation of experimental allergic encephalomyelitis. *Eur. J. Immunol.* **27:** 2863–2869.
124. McCartney-Francis N, Allen JB, Mizel DE, et al. (1993) Suppression of arthritis by an inhibitor of nitric oxide synthase. *J. Exp. Med.* **178:** 749–754.
125. Fletcher DS, Widmer WR, Luell S, et al. (1998) Therapeutic administration of a selective inhibitor of nitric oxide synthase does not ameliorate the chronic inflammation and tissue damage associated with adjuvant-induced arthritis in rats. *J. Pharmacol. Exp. Ther.* **284:** 714–721.
126. McInnes IB, Leung B, Wei XQ, et al. (1998) Septic arthritis following *Staphylococcus aureus* infection in mice lacking inducible nitric oxide synthase. *J. Immunol.* **160:** 308–315.
127. Cross AH, Misko TP, Lin RF, et al. (1994) Aminoguanidine, an inhibitor of inducible nitric oxide synthase, ameliorates experimental autoimmune encephalomyelitis in SJL mice. *J. Clin. Invest.* **93:** 2684–2690.
128. Zhao W, Tilton RG, Corbett JA, et al. (1996) Experimental allergic encephalomyelitis in the

rat is inhibited by aminoguanidine, an inhibitor of nitric oxide synthase. *J. Neuroimmunol.* **64**: 123–133.
129. Brenner T, Brocke S, Szafer F, et al. (1997) Inhibition of nitric oxide synthase for treatment of experimental autoimmune encephalomyelitis. *J. Immunol.* **158**: 2940–2946.
130. Zielasek J, Jung S, Gold R, et al. (1995) Administration of nitric oxide synthase inhibitors in experimental autoimmune neuritis and experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* **58**: 81–88.
131. Shin T, Tanuma N, Kim S, et al. (1998) An inhibitor of inducible nitric oxide synthase ameliorates experimental autoimmune myocarditis in Lewis rats. *J. Neuroimmunol.* **92**: 133–138.
132. Gabbai FB, Boggiano C, Peter T, et al. (1997) Inhibition of inducible nitric oxide synthase intensifies injury and functional deterioration in autoimmune interstitial nephritis. *J. Immunol.* **159**: 6266–6275.
133. Hoey S, Grabowski PS, Ralston SH, et al. (1997) Nitric oxide accelerates the onset and increases the severity of experimental autoimmune uveoretinitis through an IFN-gamma-dependent mechanism. *J. Immunol.* **159**: 5132–5142.
134. Tarrant TK, Silver PB, Wahlsten JL, et al. (1999) Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. *J. Exp. Med.* **189**: 219–230.
135. Fenyk-Melody JE, Garrison AE, Brunnert SR, et al. (1998) Experimental autoimmune encephalomyelitis is exacerbated in mice lacking the NOS2 gene. *J. Immunol.* **160**: 2940–2946.
136. Ding M, Zhang M, Wong JL, et al. (1998) Antisense knockdown of inducible nitric oxide synthase inhibits induction of experimental autoimmune encephalomyelitis in SJL/J mice. *J. Immunol.* **160**: 2560–2564.
137. Xiong Y, Karupiah G, Hogan SP, et al. (1999) Inhibition of allergic airway inflammation in mice lacking nitric oxide synthase 2. *J. Immunol.* **162**: 445–452.
138. Gilkeson GS, Mudgett JS, Seldin MF, et al. (1997) Clinical and serologic manifestations of autoimmune disease in MRL-lpr/lpr mice lacking nitric oxide synthase type 2. *J. Exp. Med.* **186**: 365–373.
139. Cattell V, Cook HT, Ebrahim H, et al. (1998) Anti-GBM glomerulonephritis in mice lacking nitric oxide synthase type 2. *Kidney Int.* **53**: 932–936.
140. Langrehr JM, Hoffman RA, Lancaster JRJ, et al. (1993) Nitric oxide – a new endogenous immunomodulator. *Transplantation* **55**: 1205–1212.
141. Billiar TR. (1995) Nitric oxide. Novel biology with clinical relevance. *Ann. Surg.* **221**: 339–349.
142. Hoffman RA, Langrehr JM, Berry LM, et al. (1996) Bystander injury of host lymphoid tissue during murine graft-versus-host disease is mediated by nitric oxide. *Transplantation* **61**: 610–618.
143. Falzarano G, Krenger W, Snyder KM, et al. (1996) Suppression of B-cell proliferation to lipopolysaccharide is mediated through induction of the nitric oxide pathway by tumor necrosis factor-α in mice with acute graft-versus-host disease. *Blood* **87**: 2853–2860.
144. Bobe P, Benihoud K, Grandjon D, et al. (1999) Nitric oxide mediation of active immunosuppression associated with graft-versus-host reaction. *Blood* **94**: 1028–1037.
145. Hoffman RA, Nussler NC, Gleixner SL, et al. (1997) Attenuation of lethal graft-versus-host disease by inhibition of nitric oxide synthase. *Transplantation* **63**: 94–100.
146. Bastian NR, Xu S, Shao XL, et al. (1994) N-omega-monomethyl-L-arginine inhibits nitric oxide production in murine cardiac allografts but does not affect graft rejection. *Biochim. Biophys. Acta* **1226**: 225–231.
147. Winlaw DS, Schyvens CG, Smythe GA, et al. (1995) Selective inhibition of nitric oxide production during cardiac allograft rejection causes a small increase in graft survival. *Transplantation* **60**: 77–82.
148. Goto M, Yamaguchi Y, Ichiguchi O, et al. (1997) Phenotype and localization of macrophages expressing inducible nitric oxide synthase in rat hepatic allograft rejection. *Transplantation* **64**: 303–310.
149. Tanaka S, Kamiike W, Ito T, et al. (1995) Generation of nitric oxide as a rejection marker in rat pancreas transplantation. *Transplantation* **60**: 713–717.
150. McDermott CD, Gavita SM, Shennib H, et al. (1997) Immunohistochemical localization of nitric oxide synthase and the oxidant peroxynitrite in lung transplant recipients with obliterative bronchiolitis. *Transplantation* **64**: 270–274.
151. Lafond-Walker A, Chen CL, Augustine S, et al. (1997) Inducible nitric oxide synthase expression in coronary arteries of transplanted human hearts with accelerated graft arteriosclerosis. *Am. J. Pathol.* **151**: 919–925.
152. Shears LL, Kawaharada N, Tzeng E, et al. (1997) Inducible nitric oxide synthase suppresses the development of allograft arteriosclerosis. *J. Clin. Invest.* **100**: 2035–2042.
153. Szabolcs MJ, Ravalli S, Minanov O, et al. (1998) Apoptosis and increased expression of inducible nitric oxide synthase in human allograft rejection. *Transplantation* **65**: 804–812.
154. Watkins SC, Macaulay W, Turner D, et al. (1997) Identification of inducible nitric oxide synthase in human macrophages surrounding

loosened hip prostheses. *Am. J. Pathol.* **150:** 1199–1206.
155. Samlowski WE, Yim CY, McGregor JR. (1998) Nitric oxide exposure inhibits induction of lymphokine-activated killer cells by inducing precursor apoptosis. *Nitric. Oxide.* **2:** 45–56.
156. Pou S, Pou WS, Bredt DS, et al. (1992) Generation of superoxide by purified brain nitric oxide synthase. *J. Biol. Chem.* **267:** 24173–24176.
157. Wink DA, Hanbauer I, Grisham MB, et al. (1996) Chemical biology of nitric oxide: regulation and protective and toxic mechanisms. *Curr. Top. Cell Regul.* **34:** 159–187.
158. Beckman JS, Koppenol WH. (1996) Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am. J. Physiol.* **271:** C1424–C1437.
159. Mayer B, Hemmens B. (1997) Biosynthesis and action of nitric oxide in mammalian cells [published erratum appears in *Trends Biochem. Sci.* 1998, 23:87]. *Trends. Biochem. Sci.* **22:** 477–481.
160. Halliwell B. (1997) What nitrates tyrosine? Is nitrotyrosine specific as a biomarker of peroxynitrite formation in vivo? *FEBS Lett.* **411:** 157–160.
161. Wizemann TM, Gardner CR, Laskin JD, et al. (1994) Production of nitric oxide and peroxynitrite in the lung during acute endotoxemia. *J. Leukoc. Biol.* **56:** 759–768.
162. Szabo C, Salzman AL, Ischiropoulos H. (1995) Endotoxin triggers the expression of an inducible isoform of nitric oxide synthase and the formation of peroxynitrite in the rat aorta in vivo. *FEBS Lett.* **363:** 235–238.
163. Kamisaki Y, Wada K, Ataka M, et al. (1997) Lipopolysaccharide-induced increase in plasma nitrotyrosine concentrations in rats. *Biochim. Biophys. Acta* **1362:** 24–28.
164. Numata M, Suzuki S, Miyazawa N, et al. (1998) Inhibition of inducible nitric oxide synthase prevents LPS-induced acute lung injury in dogs. *J. Immunol.* **160:** 3031–3037.
165. Cuzzocrea S, Zingarelli B, Costantino G, et al. (1999) Beneficial effects of Mn(III)tetrakis (4-benzoic acid) porphyrin (MnTBAP), a superoxide dismutase mimetic, in carrageenan-induced pleurisy. *Free Radic. Biol. Med.* **26:** 25–33.
166. Miller MJ, Thompson JH, Zhang XJ, et al. (1995) Role of inducible nitric oxide synthase expression and peroxynitrite formation in guinea pig ileitis. *Gastroenterology* **109:** 1475–1483.
167. Sadowska-Krowicka H, Mannick EE, Oliver PD, et al. (1998) Genistein and gut inflammation: role of nitric oxide. *Proc. Soc. Exp. Biol. Med.* **217:** 351–357.
168. van der Veen RC, Hinton DR, Incardonna F, et al. (1997) Extensive peroxynitrite activity during progressive stages of central nervous system inflammation. *J. Neuroimmunol.* **77:** 1–7.
169. Cross AH, Manning PT, Stern MK, et al. (1997) Evidence for the production of peroxynitrite in inflammatory CNS demyelination. *J. Neuroimmunol.* **80:** 121–130.
170. Liu P, Hock CE, Nagele R, et al. (1997) Formation of nitric oxide, superoxide, and peroxynitrite in myocardial ischemia-reperfusion injury in rats. *Am. J. Physiol.* **272:** H2327–H2336.
171. Liu P, Yin K, Nagele R, et al. (1998) Inhibition of nitric oxide synthase attenuates peroxynitrite generation, but augments neutrophil accumulation in hepatic ischemia-reperfusion in rats. *J. Pharmacol. Exp. Ther.* **284:** 1139–1146.
172. Oyama J, Shimokawa H, Momii H, et al. (1998) Role of nitric oxide and peroxynitrite in the cytokine-induced sustained myocardial dysfunction in dogs in vivo. *J. Clin. Invest.* **101:** 2207–2214.
173. Heeringa P, van Goor H, Moshage H, et al. (1998) Expression of iNOS, eNOS, and peroxynitrite-modified proteins in experimental anti-myeloperoxidase associated crescentic glomerulonephritis. *Kidney Int.* **53:** 382–393.
174. Beckmann JS, Ye YZ, Anderson PG, et al. (1994) Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. *Biol. Chem. Hoppe Seyler* **375:** 81–88.
175. Luoma JS, Stralin P, Marklund SL, et al. (1998) Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic lesions: colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins. *Arterioscler. Thromb. Vasc. Biol.* **18:** 157–167.
176. Kooy NW, Royall JA, Ye YZ, et al. (1995) Evidence for in vivo peroxynitrite production in human acute lung injury. *Am. J. Respir. Crit. Care Med.* **151:** 1250–1254.
177. Saleh D, Ernst P, Lim S, et al. (1998) Increased formation of the potent oxidant peroxynitrite in the airways of asthmatic patients is associated with induction of nitric oxide synthase: effect of inhaled glucocorticoid. *FASEB J.* **12:** 929–937.
178. Bagasra O, Michaels FH, Zheng YM, et al. (1995) Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 12041–12045.
179. Cross AH, Manning PT, Keeling RM, et al. (1998) Peroxynitrite formation within the central nervous system in active multiple sclerosis. *J. Neuroimmunol.* **88:** 45–56.
180. Kooy NW, Lewis SJ, Royall JA, et al. (1997) Extensive tyrosine nitration in human myocardial inflammation: evidence for the presence of peroxynitrite. *Crit. Care Med.* **25:** 812–819.
181. Wink DA, Cook JA, Kim SY, et al. (1997) Superoxide modulates the oxidation and nitrosation of thiols by nitric oxide-derived reactive

intermediates. Chemical aspects involved in the balance between oxidative and nitrosative stress. *J. Biol. Chem.* **272:** 11147–11151.
182. Beckman JS. (1996) Oxidative damage and tyrosine nitration from peroxynitrite. *Chem. Res. Toxicol.* **9:** 836–844.
183. van d, V, Eiserich JP, Halliwell B, et al. (1997) Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *J. Biol. Chem.* **272:** 7617–7625.
184. Hazen SL, Zhang R, Shen Z, et al. (1999) Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes: pathways for monocyte-mediated protein nitration and lipid peroxidation In vivo. *Circ. Res.* **85:** 950–958.
185. Wu W, Chen Y, Hazen SL. (1999) Eosinophil peroxidase nitrates protein tyrosyl residues. Implications for oxidative damage by nitrating intermediates in eosinophilic inflammatory disorders. *J. Biol. Chem.* **274:** 25933–25944.
186. Studer R, Jaffurs D, Stefanovic-Racic M et al. (1999) Nitric oxide in osteoarthritis. *Osteoarthritis Cartilage.* **7:** 377–379.
187. Lotz M. (1999) The role of nitric oxide in articular cartilage damage. *Rheum. Dis. Clin. North Am.* **25:** 269–282.
188. Olee T, Hashimoto S, Quach J, et al. (1999) IL-18 is produced by articular chondrocytes and induces proinflammatory and catabolic responses. *J. Immunol.* **162:** 1096–1100.
189. Oates JC, Ruiz P, Alexander A, et al. (1997) Effect of late modulation of nitric oxide production on murine lupus. *Clin. Immunol. Immunopathol.* **83:** 86–92.
190. Santos LL, Morand EF, Yang Y, et al. (1997) Suppression of adjuvant arthritis and synovial macrophage inducible nitric oxide by N-iminoethyl-L-ornithine, a nitric oxide synthase inhibitor. *Inflammation* **21:** 299–311.
191. Stefanovic-Racic M, Meyers K, Meschter C, et al. (1994) N-monomethyl arginine, an inhibitor of nitric oxide synthase, suppresses the development of adjuvant arthritis in rats. *Arthritis Rheum.* **37:** 1062–1069.
192. McCartney-Francis NL, Song XY, Mizel DE, et al. (1999) Hemoglobin protects from streptococcal cell wall-induced arthritis. *Arthritis Rheum.* **42:** 1119–1127.
193. Lubberts E, Joosten LA, van Den BL, et al. (1999) Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. *J. Immunol.* **163:** 4546–4556.
194. Miesel R, Kurpisz M, Kroger H. (1996) Suppression of inflammatory arthritis by simultaneous inhibition of nitric oxide synthase and NADPH oxidase. *Free Radic. Biol. Med.* **20:** 75–81.
195. Gassner R, Buckley MJ, Georgescu H, et al. (1999) Cyclic tensile stress exerts antiinflammatory actions on chondrocytes by inhibiting inducible nitric oxide synthase. *J. Immunol.* **163:** 2187–2192.
196. Borderie D, Hilliquin P, Hernvann A, et al. (1999) Apoptosis induced by nitric oxide is associated with nuclear p53 protein expression in cultured osteoarthritic synoviocytes. *Osteoarthritis. Cartilage.* **7:** 203–213.
197. van de Loo FA, Arntz OJ, van Enckevort FH, et al. (1998) Reduced cartilage proteoglycan loss during zymosan-induced gonarthritis in NOS2-deficient mice and in anti-interleukin-1-treated wild-type mice with unabated joint inflammation. *Arthritis Rheum.* **41:** 634–646.
198. Rothe H, Kolb H. (1999) Strategies of protection from nitric oxide toxicity in islet inflammation. *J. Mol. Med.* **77:** 40–44.
199. Grey ST, Arvelo MB, Hasenkamp W, et al. (1999) A20 inhibits cytokine-induced apoptosis and nuclear factor kappa B-dependent gene activation in islets. *J. Exp. Med.* **190:** 1135–1146.
200. Whittle BJ. (1997) Nitric oxide–a mediator of inflammation or mucosal defence. *Eur. J. Gastroenterol. Hepatol.* **9:** 1026–1032.
201. Guslandi M. (1998) Nitric oxide and inflammatory bowel diseases. *Eur. J. Clin. Invest.* **28:** 904–907.
202. Mourelle M, Vilaseca J, Guarner F, et al. (1996) Toxic dilatation of colon in a rat model of colitis is linked to an inducible form of nitric oxide synthase. *Am. J. Physiol.* **270:** G425–G430.
203. Kiss J, Lamarque D, Delchier JC, et al. (1997) Time-dependent actions of nitric oxide synthase inhibition on colonic inflammation induced by trinitrobenzene sulphonic acid in rats. *Eur. J. Pharmacol.* **336:** 219–224.
204. Zingarelli B, Szabo C, Salzman AL. (1999) Blockade of poly(ADP-ribose) synthetase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis. *Gastroenterology* **116:** 335–345.
205. Perner A, Rask-Madsen J. (1999) Review article: The potential role of nitric oxide in chronic inflammatory bowel disorders. *Aliment. Pharmacol. Ther.* **13:** 135–144.
206. Werner J, Rivera J, Fernandez-del CC, et al. (1997) Differing roles of nitric oxide in the pathogenesis of acute edematous versus necrotizing pancreatitis. *Surgery* **121:** 23–30.
207. McCafferty DM, Mudgett JS, Swain MG, et al. (1997) Inducible nitric oxide synthase plays a critical role in resolving intestinal inflammation. *Gastroenterology* **112:** 1022–1027.
208. Elliott SN, Wallace JL. (1998) Nitric oxide: A regulator of mucosal defense and injury. *J. Gastroenterol.* **33:** 792–803.
209. Dimmeler S, Haendeler J, Nehls M, et al. (1997) Suppression of apoptosis by nitric oxide via inhibition of interleukin-1β-converting enzyme (ICE)-like and cysteine protease protein

(CPP)- 32-like proteases. *J. Exp. Med.* **185:** 601–607.
210. Tzeng E, Kim YM, Pitt BR, et al. (1997) Adenoviral transfer of the inducible nitric oxide synthase gene blocks endothelial cell apoptosis. *Surgery* **122:** 255–263.
211. Iwashina M, Shichiri M, Marumo F, et al. (1998) Transfection of inducible nitric oxide synthase gene causes apoptosis in vascular smooth muscle cells. *Circulation* **98:** 1212–1218.
212. Smith JD, McLean SD, Nakayama DK. (1998) Nitric oxide causes apoptosis in pulmonary vascular smooth muscle cells. *J. Surg. Res.* **79:** 121–127.
213. Chiche JD, Schlutsmeyer SM, Bloch DB, et al. (1998) Adenovirus-mediated gene transfer of cGMP-dependent protein kinase increases the sensitivity of cultured vascular smooth muscle cells to the antiproliferative and pro-apoptotic effects of nitric oxide/cGMP. *J. Biol. Chem.* **273:** 34263–34271.
214. Best PJ, Hasdai D, Sangiorgi G, et al. (1999) Apoptosis. Basic concepts and implications in coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **19:** 14–22.
215. Zhang HY, Phan SH. (1999) Inhibition of myofibroblast apoptosis by transforming growth factor beta(1). *Am. J. Respir. Cell Mol. Biol.* **21:** 658–665.
216. Bult H. (1996) Nitric oxide and atherosclerosis: possible implications for therapy. *Mol. Med. Today* **2:** 510–518.
217. Maxwell AJ, Cooke JP. (1999) The role of nitric oxide in atherosclerosis. *Coron. Artery Dis.* **10:** 277–286.
218. Ross R. (1999) Atherosclerosis — an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
219. Hunter GC, Henderson AM, Westerband A, et al. (1999) The contribution of inducible nitric oxide and cytomegalovirus to the stability of complex carotid plaque. *J. Vasc. Surg.* **30:** 36–49.
220. Baker CS, Hall RJ, Evans TJ, et al. (1999) Cyclooxygenase-2 is widely expressed in atherosclerotic lesions affecting native and transplanted human coronary arteries and colocalizes with inducible nitric oxide synthase and nitrotyrosine particularly in macrophages. *Arterioscler. Thromb. Vasc. Biol.* **19:** 646–655.
221. Mallat Z, Heymes C, Ohan J, et al. (1999) Expression of interleukin-10 in advanced human atherosclerotic plaques: relation to inducible nitric oxide synthase expression and cell death. *Arterioscler. Thromb. Vasc. Biol.* **19:** 611–616.
222. Grainger DJ, Kemp PR, Metcalfe JC, et al. (1995) The serum concentration of active transforming growth factor-β is severely depressed in advanced atherosclerosis. *Nat. Med.* **1:** 74–79.
223. McCaffrey TA, Consigli S, Du B, et al. (1995) Decreased type II/type I TGF-β receptor ratio in cells derived from human atherosclerotic lesions. Conversion from an antiproliferative to profibrotic response to TGF-β1. *J. Clin. Invest* **96:** 2667–2675.
224. Blann AD, Wang JM, Wilson PB, et al. (1996) Serum levels of the TGF-β receptor are increased in atherosclerosis. *Atherosclerosis* **120:** 221–226.
225. McCaffrey TA, Du B, Consigli S, et al. (1997) Genomic instability in the type II TGF-β1 receptor gene in atherosclerotic and restenotic vascular cells [see comments]. *J. Clin. Invest* **100:** 2182–2188.
226. Scott L, Kerr A, Haydock D, et al. (1997) Subendothelial proteoglycan synthesis and transforming growth factor β distribution correlate with susceptibility to atherosclerosis. *J. Vasc. Res.* **34:** 365–377.
227. De Sanctis GT, MacLean JA, Hamada K, et al. (1999) Contribution of nitric oxide synthases 1, 2, and 3 to airway hyperresponsiveness and inflammation in a murine model of asthma. *J. Exp. Med.* **189:** 1621–1630.
228. Tsuji M, Dimov VB, Yoshida T. (1995) In vivo expression of monokine and inducible nitric oxide synthase in experimentally induced pulmonary granulomatous inflammation. Evidence for sequential production of interleukin–1, inducible nitric oxide synthase, and tumor necrosis factor. *Am. J. Pathol.* **147:** 1001–1015.
229. Nozaki Y, Hasegawa Y, Takeuchi A, et al. (1997) Nitric oxide as an inflammatory mediator of radiation pneumonitis in rats. *Am. J. Physiol.* **272:** L651–L658.
230. Frieri M. (1998) Nitric oxide in allergic rhin it is and asthma. *Allergy Asthma Proc.* **19:** 349–351.
231. Marshall HE, Stamler JS. (1999) Exhaled nitric oxide (NO), NO synthase activity, and regulation of nuclear factor (NF)-kappa B. *Am. J. Respir. Cell Mol. Biol.* **21:** 296–297.
232. Long R, Light B, Talbot JA. (1999) Mycobacteriocidal action of exogenous nitric oxide. *Antimicrob. Agents Chemother.* **43:** 403–405.
233. Buster BL, Weintrob AC, Townsend GC, et al. (1995) Potential role of nitric oxide in the pathophysiology of experimental bacterial meningitis in rats. *Infect. Immun.* **63:** 3835–3839.
234. Leib SL, Kim YS, Black SM, et al. (1998) Inducible nitric oxide synthase and the effect of aminoguanidine in experimental neonatal meningitis. *J. Infect. Dis.* **177:** 692–700.
235. Schaffer MR, Tantry U, Gross SS, et al. (1996) Nitric oxide regulates wound healing. *J. Surg. Res.* **63:** 237–240.
236. Yamasaki K, Edington HD, McClosky C, et al. (1998) Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-

mediated iNOS gene transfer. *J. Clin. Invest.* **101:** 967–971.
237. Vodovotz Y. (1997) Control of nitric oxide production by transforming growth factor-beta1: mechanistic insights and potential relevance to human disease. *Nitric. Oxide.* **1:** 3–17.
238. Ling H, Edelstein C, Gengaro P, et al. (1999) Attenuation of renal ischemia-reperfusion injury in inducible nitric oxide synthase knockout mice. *Am. J. Physiol* **277:** F383–F390.
239. Moncada S. (1994) Nitric oxide. *J. Hypertens. Suppl.* **12:** S35–S39.
240. Cooke JP, Dzau VJ. (1997) Nitric oxide synthase: role in the genesis of vascular disease. *Annu. Rev. Med.* **48:** 489–509.
241. Sarkar R, Webb RC. (1998) Does nitric oxide regulate smooth muscle cell proliferation? A critical appraisal. *J. Vasc. Res.* **35:** 135–142.
242. Ruschitzka F, Corti R, Noll G, et al. (1999) A rationale for treatment of endothelial dysfunction in hypertension. *J. Hypertens. Suppl* **17:** S25–S35.
243. Radomski MW, Zakar T, Salas E. (1996) Nitric oxide in platelets. *Methods Enzymol.* **269:** 88–107.
244. Cines DB, Pollak ES, Buck CA, et al. (1998) Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* **91:** 3527–3561.
245. Luscher TF, Noll G. (1995) The pathogenesis of cardiovascular disease: role of the endothelium as a target and mediator. *Atherosclerosis* **118 (Suppl):** S81–S90.
246. Liew FY, Xu D, Chan WL. (1999) Immune effector mechanism in parasitic infections. *Immunol. Lett.* **65:** 101–104.
247. Albina JE, Reichner JS. (1998) Role of nitric oxide in mediation of macrophage cytotoxicity and apoptosis. *Cancer Metastasis Rev.* **17:** 39–53.
248. Wink DA, Vodovotz Y, Cook JA, et al. (1998) The role of nitric oxide chemistry in cancer treatment. *Biochemistry (Mosc.)* **63:** 802–809.
249. Kubes P, Granger DN. (1996) Leukocyte-endothelial cell interactions evoked by mast cells. *Cardiovasc. Res.* **32:** 699–708.
250. Kojda G, Kottenberg K. (1999) Regulation of basal myocardial function by NO. *Cardiovasc. Res.* **41:** 514–523.
251. Drexler H, Hornig B. (1999) Endothelial dysfunction in human disease. *J. Mol. Cell Cardiol.* **31:** 51–60.
252. Schulz R, Nava E, Moncada S. (1992) Induction and potential biological relevance of a Ca(2+)–independent nitric oxide synthase in the myocardium. *Br. J. Pharmacol.* **105:** 575–580.
253. Roberts AB, Vodovotz Y, Roche NS, et al. (1992) Role of nitric oxide in antagonistic effects of transforming growth factor-beta and interleukin-1 beta on the beating rate of cultured cardiac myocytes. *Mol. Endocrinol.* **6:** 1921–1930.
254. Zaragoza C, Ocampo C, Saura M, et al. (1998) The role of inducible nitric oxide synthase in the host response to *Coxsackievirus myocarditis. Proc. Natl. Acad. Sci. U.S.A.* **95:** 2469–2474.
255. Singh S, Evans TW. (1997) Nitric oxide, the biological mediator of the decade: fact or fiction? *Eur. Respir. J.* **10:** 699–707.
256. Hart CM. (1999) Nitric oxide in adult lung disease. *Chest* **115:** 1407–1417.
257. Mashimo H, Goyal RK. (1999) Lessons from genetically engineered animal models. IV. Nitric oxide synthase gene knockout mice. *Am. J. Physiol.* **277:** G745–G750.
258. Sjoholm A. (1998) Aspects of the involvement of interleukin-1 and nitric oxide in the pathogenesis of insulin-dependent diabetes mellitus. *Cell Death. Differ.* **5:** 461–468.
259. Rabinovitch A, Suarez-Pinzon WL. (1998) Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. *Biochem. Pharmacol.* **55:** 1139–1149.
260. Muscara MN, Wallace JL. (1999) Nitric oxide. V. Therapeutic potential of nitric oxide donors and inhibitors. *Am. J. Physiol* **276:** G1313–G1316.
261. Holzer P. (1998) Neural emergency system in the stomach. *Gastroenterology* **114:** 823–839.
262. Lefer AM, Lefer DJ. (1999) Nitric oxide. II. Nitric oxide protects in intestinal inflammation. *Am. J. Physiol* **276:** G572–G575.
263. Belmont HM, Levartovsky D, Goel A, et al. (1997) Increased nitric oxide production accompanied by the up-regulation of inducible nitric oxide synthase in vascular endothelium from patients with systemic lupus erythematosus. *Arthritis Rheum.* **40:** 1810–1816.
264. Wigand R, Meyer J, Busse R, et al. (1997) Increased serum NG-hydroxy-L-arginine in patients with rheumatoid arthritis and systemic lupus erythematosus as an index of an increased nitric oxide synthase activity. *Ann. Rheum. Dis.* **56:** 330–332.
265. Gilkeson G, Cannon C, Oates J, et al. (1999) Correlation of serum measures of nitric oxide production with lupus disease activity. *J. Rheumatol.* **26:** 318–324.
266. Kausalya S, Nath J. (1998) Interactive role of nitric oxide and superoxide anion in neutrophil- mediated endothelial cell injury. *J. Leukoc. Biol.* **64:** 185–191.
267. Harper L, Savage CO. (1999) Mechanisms of endothelial injury in systemic vasculitis. *Adv. Nephrol. Necker Hosp.* **29:** 1–15.
268. Grabowski PS, Wright PK, Van 't H, et al. (1997) Immunolocalization of inducible nitric oxide synthase in synovium and cartilage in rheumatoid arthritis and osteoarthritis. *Br. J. Rheumatol.* **36:** 651–655.

269. Borderie D, Hilliquin P, Hernvann A, et al. (1999) Nitric oxide synthase is expressed in the lymphomononuclear cells of synovial fluid in patients with rheumatoid arthritis. *J. Rheumatol.* **26:** 2083–2088.
270. Amin AR, Attur M, Abramson SB. (1999) Nitric oxide synthase and cyclooxygenases: distribution, regulation, and intervention in arthritis. *Curr. Opin. Rheumatol.* **11:** 202–209.
271. Kim HA, Song YW. (1999) Apoptotic chondrocyte death in rheumatoid arthritis. *Arthritis Rheum.* **42:** 1528–1537.
272. McInnes IB, Leung BP, Field M, et al. (1996) Production of nitric oxide in the synovial membrane of rheumatoid and osteoarthritis patients. *J. Exp. Med.* **184:** 1519–1524.
273. Martel-Pelletier J, Mineau F, Jovanovic D, et al. (1999) Mitogen-activated protein kinase and nuclear factor kappa B together regulate interleukin-17-induced nitric oxide production in human osteoarthritic chondrocytes: possible role of transactivating factor mitogen-activated protein kinase-activated proten kinase (MAPKAPK). *Arthritis Rheum.* **42:** 2399–2409.
274. Salvatierra J, Escames G, Hernandez P, et al. (1999) Cartilage and serum levels of nitric oxide in patients with hip osteoarthritis. *J. Rheumatol.* **26:** 2015–2017.
275. Hamid Q, Springall DR, Riveros-Moreno V, et al. (1993) Induction of nitric oxide synthase in asthma. *Lancet* **342:** 1510–1513.
276. Barnes PJ. (1996) Pathophysiology of asthma. *Br. J. Clin. Pharmacol.* **42:** 3–10.
277. Wang CH, Hsieh WY, Shih LY, et al. (1999) Increased progenitor cell proliferation in the peripheral blood of patients with bronchial asthma: the role of nitric oxide. *J. Allergy Clin. Immunol.* **104:** 803–810.
278. Kharitonov SA, Yates D, Barnes PJ. (1995) Increased nitric oxide in exhaled air of normal human subjects with upper respiratory tract infections. *Eur. Respir. J.* **8:** 295–297.
279. Nicholson S, Bonecini–Almeida Md, Silva JR, et al. (1996) Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. *J. Exp. Med.* **183:** 2293–2302.
280. Flak TA, Goldman WE. (1996) Autotoxicity of nitric oxide in airway disease. *Am. J. Respir. Crit Care Med.* **154:** S202–S206.
281. Kwon OJ, Kim JH, Kim HC, et al. (1998) Nitric oxide expression in airway epithelial cells in response to tubercle bacilli stimulation. *Respirology.* **3:** 119–124.
282. Wang CH, Liu CY, Lin HC, et al. (1998) Increased exhaled nitric oxide in active pulmonary tuberculosis due to inducible NO synthase upregulation in alveolar macrophages. *Eur. Respir. J.* **11:** 809–815.
283. Saleh D, Barnes PJ, Giaid A. (1997) Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* **155:** 1763–1769.
284. Rogers DF, Laurent GJ. (1998) New ideas on the pathophysiology and treatment of lung disease. *Thorax* **53:** 200–203.
285. Paredi P, Kharitonov SA, Loukides S, et al. (1999) Exhaled nitric oxide is increased in active fibrosing alveolitis. *Chest* **115:** 1352–1356.
286. Tracey WR, Xue C, Klinghofer V, et al. (1994) Immunochemical detection of inducible NO synthase in human lung. *Am. J. Physiol.* **266:** L722–L727.
287. Barnes PJ. (1995) Nitric oxide and airway disease. *Ann. Med.* **27:** 389–393.
288. Kharitonov SA, Wells AU, O'Connor BJ, et al. (1995) Elevated levels of exhaled nitric oxide in bronchiectasis. *Am. J. Respir. Crit Care Med.* **151:** 1889–1893.
289. Leonard N, Bishop AE, Polak JM, et al. (1998) Expression of nitric oxide synthase in inflammatory bowel disease is not affected by corticosteroid treatment. *J. Clin. Pathol.* **51:** 750–753.
290. Singer II, Kawka DW, Scott S, et al. (1996) Expression of inducible nitric oxide synthase and nitrotyrosine in colonic epithelium in inflammatory bowel disease. *Gastroenterology* **111:** 871–885.
291. Gupta SK, Fitzgerald JF, Chong SK, et al. (1998) Expression of inducible nitric oxide synthase (iNOS) mRNA in inflamed esophageal and colonic mucosa in a pediatric population. *Am. J. Gastroenterol.* **93:** 795–798.
292. Herulf M, Ljung T, Hellstrom PM, et al. (1998) Increased luminal nitric oxide in inflammatory bowel disease as shown with a novel minimally invasive method. *Scand. J. Gastroenterol.* **33:** 164–169.
293. Iwashita E, Iwai A, Sawazaki Y, et al. (1998) Activation of microvascular endothelial cells in active ulcerative colitis and detection of inducible nitric oxide synthase. *J. Clin. Gastroenterol.* **27** (Suppl 1)**:** S74–S79.
294. Ina K, Itoh J, Fukushima K ,et al. (1999) Resistance of Crohn's disease T cells to multiple apoptotic signals is associated with a Bcl-2/Bax mucosal imbalance. *J. Immunol.* **163:** 1081–1090.
295. Izzo AA, Mascolo N, Capasso F. (1998) Nitric oxide as a modulator of intestinal water and electrolyte transport. *Dig. Dis. Sci.* **43:** 1605–1620.
296. Ford H, Watkins S, Reblock K, et al. (1997) The role of inflammatory cytokines and nitric oxide in the pathogenesis of necrotizing enterocolitis. *J. Pediatr. Surg.* **32:** 275–282.
297. Zamora SA, Amin HJ, McMillan DD, et al. (1997) Plasma L-arginine concentrations in premature infants with necrotizing enterocolitis. *J. Pediatr.* **131:** 226–232.

298. Sigge W, Wedel T, Kuhnel W, et al. (1998) Morphologic alterations of the enteric nervous system and deficiency of non-adrenergic non-cholinergic inhibitory innervation in neonatal necrotizing enterocolitis. *Eur. J. Pediatr. Surg.* **8**: 87–94.
299. ter Steege J, Buurman W, Arends JW, et al. (1997) Presence of inducible nitric oxide synthase, nitrotyrosine, CD68, and CD14 in the small intestine in celiac disease. *Lab. Invest.* **77**: 29–36.
300. Beckett CG, Dell'Olio D, Ellis HJ, et al. (1998) The detection and localization of inducible nitric oxide synthase production in the small intestine of patients with coeliac disease. *Eur. J. Gastroenterol. Hepatol.* **10**: 641–647.
301. ter Steege JC, Koster-Kamphuis L, van Straaten EA, et al. (1998) Nitrotyrosine in plasma of celiac disease patients as detected by a new sandwich ELISA. *Free Radic. Biol. Med.* **25**: 953–963.
302. Everts B, Stotzer P, Olsson M, et al. (1999) Increased luminal nitric oxide concentrations in the small intestine of patients with coeliac disease. *Eur. J. Clin. Invest* **29**: 692–696.
303. Mannick EE, Bravo LE, Zarama G, et al. (1996) Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in Helicobacter pylori gastritis: effect of antibiotics and antioxidants. *Cancer Res.* **56**: 3238–3243.
304. Hahm KB, Lee KJ, Choi SY, et al. (1997) Possibility of chemoprevention by the eradication of Helicobacter pylori: Oxidative DNA damage and apoptosis in *H. pylori infection. Am. J. Gastroenterol.* **92**: 1853–1857.
305. Fu S, Ramanujam KS, Wong A, et al. (1999) Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in *Helicobacter pylori* gastritis. *Gastroenterology* **116**: 1319–1329.
306. Sakaguchi AA, Miura S, Takeuchi T, et al. (1999) Increased expression of inducible nitric oxide synthase and peroxynitrite in *Helicobacter pylori* gastric ulcer. *Free Radic. Biol. Med.* **27**: 781–789.
307. Cattell V, Cook T. (1995) The nitric oxide pathway in glomerulonephritis. *Curr. Opin. Nephrol. Hypertens.* **4**: 359–364.
308. Kashem A, Endoh M, Yano N, et al. (1996) Expression of inducible-NOS in human glomerulonephritis: The possible source is infiltrating monocytes/macrophages. *Kidney Int.* **50**: 392–399.
309. Lianos EA. (1998) Activation and potential interactions between the arachidonic acid and L-arginine:nitric oxide pathways in glomerulonephritis. *Kidney Int.* **53**: 540–547.
310. Cattell V. (1999) Nitric oxide and glomerulonephritis. *Semin. Nephrol.* **19**: 277–287.
311. Wang JS, Tseng HH, Shih DF, et al. (1997) Expression of inducible nitric oxide synthase and apoptosis in human lupus nephritis. *Nephron* **77**: 404–411.
312. Furusu A, Miyazaki M, Abe K, et al. (1998) Expression of endothelial and inducible nitric oxide synthase in human glomerulonephritis. *Kidney Int.* **53**: 1760–1768.
313. Corbett JA, Sweetland MA, Wang JL, et al. (1993) Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. *Proc. Natl. Acad. Sci. U.S.A.* **90**: 1731–1735.
314. Vara E, Arias-Diaz J, Garcia C, et al. (1995) Production of TNF alpha, IL-1, IL-6 and nitric oxide by isolated human islets. *Transplant. Proc.* **27**: 3367–3371.
315. Arnush M, Heitmeier MR, Scarim AL, et al. (1998) IL-1 produced and released endogenously within human islets inhibits beta cell function. *J. Clin. Invest* **102**: 516–526.
316. Pavlovic D, Chen MC, Bouwens L, et al. (1999) Contribution of ductal cells to cytokine responses by human pancreatic islets. *Diabetes* **48**: 29–33.
317. Cortesi R, Ascenzi P, Colasanti M, et al. (1998) Cross-enzyme inhibition by gabexate mesylate: formulation and reactivity study. *J. Pharm. Sci.* **87**: 1335–1340.
318. Sharara AI, Perkins DJ, Misukonis MA, et al. (1997) Interferon (IFN)-alpha activation of human blood mononuclear cells in vitro and in vivo for nitric oxide synthase (NOS) type 2 mRNA and protein expression: Possible relationship of induced NOS2 to the anti-hepatitis C effects of IFN-α in vivo. *J. Exp. Med.* **186**: 1495–1502.
319. Cuzzocrea S, Zingarelli B, Villari D, et al. (1998) Evidence for in vivo peroxynitrite production in human chronic hepatitis. *Life Sci.* **63**: L25–L30.
320. Lundberg JO, Ehren I, Jansson O, et al. (1996) Elevated nitric oxide in the urinary bladder in infectious and noninfectious cystitis. *Urology* **48**: 700–702.
321. Lundberg JO, Lundberg JM, Alving K, et al. (1997) Nitric oxide and inflammation: the answer is blowing in the wind. *Nat. Med.* **3**: 30–31.
322. Wein AJ. (1997) Nitric oxide and interstitial cystitis. *J. Urol.* **158**: 993.
323. Ehren I, Hosseini A, Lundberg JO, et al. (1999) Nitric oxide: a useful gas in the detection of lower urinary tract inflammation. *J. Urol.* **162**: 327–329.
324. Snyder SH. (1996) No NO prevents parkinsonism [comment]. *Nat. Med.* **2**: 965–966.
325. Hirsch EC, Hunot S, Damier P, et al. (1998) Glial cells and inflammation in Parkinson's disease: a role in neurodegeneration? *Ann. Neurol.* **44**: S115–S120.
326. Gerlach M, Blum-Degen D, Lan J, et al. (1999)

Nitric oxide in the pathogenesis of Parkinson's disease. *Adv. Neurol.* **80:** 239–245.
327. Bagasra O, Michaels FH, Zheng YM, et al. (1995) Activation of the inducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **92:** 12041–12045.
328. Parkinson JF, Mitrovic B, Merrill JE. (1997) The role of nitric oxide in multiple sclerosis. *J. Mol. Med.* **75:** 174–186.
329. Hooper DC, Bagasra O, Marini JC, et al. (1997) Prevention of experimental allergic encephalomyelitis by targeting nitric oxide and peroxynitrite: implications for the treatment of multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **94:** 2528–2533.
330. Rouzaut A, Subira ML, de Miguel C, et al. (1999) Co-expression of inducible nitric oxide synthase and arginases in different human monocyte subsets. Apoptosis regulated by endogenous NO. *Biochim. Biophys. Acta* **1451:** 319–333.
331. Adamson DC, Wildemann B, Sasaki M, et al. (1996) Immunologic NO synthase: Elevation in severe AIDS dementia and induction by HIV-1 gp41. *Science* **274:** 1917–1921.
332. Rostasy K, Monti L, Yiannoutsos C, et al. (1999) Human immunodeficiency virus infection, inducible nitric oxide synthase expression, and microglial activation: Pathogenetic relationship to the acquired immunodeficiency syndrome dementia complex. *Ann. Neurol.* **46:** 207–216.
333. Adamson DC, McArthur JC, Dawson TM, et al. (1999) Rate and severity of HIV-associated dementia (HAD): correlations with Gp41 and iNOS. *Mol. Med.* **5:** 98–109.
334. Satoi H, Oka N, Kawasaki T, et al. (1998) Mechanisms of tissue injury in vasculitic neuropathies. *Neurology* **50:** 492–496.
335. Neufeld AH, Sawada A, Becker B. (1999) Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 9944–9948.
336. Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V. (1998) Nitric oxide in human skin: current status and future prospects. *J. Invest Dermatol.* **110:** 1–7.
337. Bruch-Gerharz D, Fehsel K, Suschek C, et al. (1996) A proinflammatory activity of interleukin 8 in human skin: expression of the inducible nitric oxide synthase in psoriatic lesions and cultured keratinocytes. *J. Exp. Med.* **184:** 2007–2012.
338. Morhenn VB. (1997) Langerhans cells may trigger the psoriatic disease process via production of nitric oxide [see comments]. *Immunol. Today* **18:** 433–436.
339. McKenzie RC, Weller R. (1998) Langerhans cells, keratinocytes, nitric oxide and psoriasis [letter; comment]. *Immunol. Today* **19:** 427–428.
340. Kolb-Bachofen V, Bruch-Gerharz D. (1999) Langerhans cells, nitric oxide, keratinocytes and psoriasis [letter]. *Immunol. Today* **20:** 289.
341. Kuhn A, Fehsel K, Lehmann P, et al. (1998) Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus. *J. Invest. Dermatol.* **111:** 149–153.
342. Yamamoto T, Katayama I, Nishioka K. (1998) Nitric oxide production and inducible nitric oxide synthase expression in systemic sclerosis. *J. Rheumatol.* **25:** 314–317.
343. Yamamoto T, Sawada Y, Katayama I, et al. (1998) Increased production of nitric oxide stimulated by interleukin-1beta in peripheral blood mononuclear cells in patients with systemic sclerosis. *Br. J. Rheumatol.* **37:** 1123–1125.
344. Cotton SA, Herrick AL, Jayson MI, et al. (1999) Endothelial expression of nitric oxide synthases and nitrotyrosine in systemic sclerosis skin. *J. Pathol.* **189:** 273–278.
345. Rowe A, Farrell AM, Bunker CB. (1997) Constitutive endothelial and inducible nitric oxide synthase in inflammatory dermatoses. *Br. J. Dermatol.* **136:** 18–23.
346. Bruch-Gerharz D, Ruzicka T, Kolb-Bachofen V. (1998) Nitric oxide and its implications in skin homeostasis and dise. *Arch. Dermatol. Res.* **290:** 643–651.
347. Clough GF. (1999) Role of nitric oxide in the regulation of microvascular perfusion in human skin in vivo. *J. Physiol (Lond)* **516:** 549–557.
348. Buttery LD, Springall DR, Chester AH, et al. (1996) Inducible nitric oxide synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. *Lab. Invest.* **75:** 77–85.
349. Lafond-Walker A, Chen CL, Augustine S, et al. (1997) Inducible nitric oxide synthase expression in coronary arteries of transplanted human hearts with accelerated graft arteriosclerosis. *Am. J. Pathol.* **151:** 919–925.
350. Kinscherf R, Wagner M, Kamencic H, et al. (1999) Characterization of apoptotic macrophages in atheromatous tissue of humans and heritable hyperlipidemic rabbits. *Atherosclerosis* **144:** 33–39.
351. Takeichi O, Saito I, Hayashi M, et al. (1998) Production of human-inducible nitric oxide synthase in radicular cysts. *J. Endod.* **24:** 157–160.
352. Takeichi O, Saito I, Okamoto Y, et al. (1998) Cytokine regulation on the synthesis of nitric oxide in vivo by chronically infected human polymorphonuclear leucocytes. *Immunology* **93:** 275–280.

353. Takeichi O, Hayashi M, Tsurumachi T et al. (1999) Inducible nitric oxide synthase activity by interferon-gamma-producing cells in human radicular cysts. *Int. Endod. J.* **32:** 124–130.
354. Konttinen YT, Platts LA, Tuominen S, et al. (1997) Role of nitric oxide in Sjogren's syndrome. *Arthritis Rheum.* **40:** 875–883.
355. Bacman SR, Berra A, Sterin-Borda L et, al. (1998) Human primary Sjogren's syndrome autoantibodies as mediators of nitric oxide release coupled to lacrimal gland muscarinic acetylcholine receptors. *Curr. Eye Res.* **17:** 1135–1142.
356. Ludviksdottir D, Janson C, Hogman M, et al. (1999) Increased nitric oxide in expired air in patients with Sjogren's syndrome. BHR study group. Bronchial hyperresponsiveness. *Eur. Respir. J.* **13:** 739–743.