Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: Implications for immunity, autoimmune diseases, and apoptosis

Ebru Karpuzoglu, S. Ansar Ahmed *

Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Vet. Med., Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Received 17 October 2005; revised 2 March 2006
Available online 2 May 2006

Abstract

Nitric oxide plays a central role in the physiology and pathology of diverse tissues including the immune system. It is clear that the levels of nitric oxide must be carefully regulated to maintain homeostasis. Appropriate levels of nitric oxide derived from iNOS assist in mounting an effective defense against invading microbes. Conversely, inability to generate nitric oxide results in serious, even fatal, susceptibility to infections. Further, dysregulation or overproduction of nitric oxide has been implicated in the pathogenesis of many disorders, including atherosclerosis, neurodegenerative diseases, inflammatory autoimmune diseases, and cancer. Therefore, depending upon the levels of nitric oxide generated, the potential exists for nitric oxide to behave like a “double-edged” biological sword. Taking these issues into consideration, it is thus pivotal to understand the regulation of nitric oxide. Nitric oxide is regulated by many endogenous factors including hormones such as estrogens. While the effects of estrogen on the generation of nitric oxide in non-immune tissues are relatively well documented, the effect of estrogen on iNOS/nitric oxide in immune cells is only now becoming apparent. Our laboratory has recently shown that estrogen treatment of mice markedly upregulates the levels of iNOS mRNA, iNOS protein, and nitric oxide in activated splenocytes. This upregulation of nitric oxide is in part mediated through interferon-gamma (IFN-γ), a pro-inflammatory cytokine that is enhanced by estrogen. These findings are important considering that estrogens are not only involved in regulation of normal immune responses, but also are implicated in many autoimmune and inflammatory diseases. To date, there are no reviews on the effects of estrogen on immune tissue-derived nitric oxide and therefore this review will address this critical gap in the literature. Given the increasing importance of immune-tissue-derived iNOS in health and disease, studies on estrogen-induced regulation of iNOS may offer a better understanding of diseases and aid in devising new therapeutic interventions.

Keywords: Nitric oxide; iNOS; Estrogen; IFN-γ; Apoptosis; Splenocytes; Infection; Autoimmunity; Lymphocytes

Introduction

Role of nitric oxide in immunity, autoimmune diseases, and apoptosis

Intense research in many biological disciplines has revealed that nitric oxide has pleotropic effects on a wide-range of tissues, including the immune system [1]. Nitric oxide readily interacts with varied substances including aqueous oxygen, superoxide, transition metals, as well as iron–sulphur and zinc–sulphur clusters, to affect the functions of diverse types of cells. Therefore, it is not surprising that extensive research in many disciplines has revealed the central role of nitric oxide in the physiology and pathology of diverse tissues including the immune system. Nitric oxide is synthesized by three known forms of nitric oxide synthases (NOS) including (i) neuronal NOS (nNOS, NOS1), (ii) inducible NOS (iNOS, NOS2), and (iii) endothelial...
NOS (eNOS, NOS3), which catalyze the formation of nitric oxide and L-citrulline by oxidation of L-arginine in the presence of cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin-adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (BH4), and thiol donors (Fig. 1).

Key similarities and dissimilarities among the three NOS isoforms (nNOS, eNOS, and iNOS) are summarized in Table 1. The iNOS-derived nitric oxide is principally generated from cells of the macrophage–monocyte lineage such as monocytes, macrophages [2], peritoneal macrophages [3], microglia, and Kupffer cells. In addition, iNOS-derived nitric oxide has also been reported in certain T [4] and B cell lines [5], optic nerve astrocytes [6], hepatocytes, neutrophils, vascular smooth muscle cells, and endothelial cells [7]. Inducible NOS differs from nNOS and eNOS in that it is not constitutively present, rather it is induced upon activation (of mostly macrophages or monocytes) in response to inflammatory cytokines such as IFN-γ, IL-1β, and TNF-α [8]. A notable feature of iNOS is its ability to induce higher levels of nitric oxide compared to other two NOS isoforms. High levels of nitric oxide produced by iNOS exert potent anti-microbial effects to control infections and hence is critical in immune defense [8] (Table 1). Infected or activated macrophages produce high levels of iNOS-derived nitric oxide, which in turn results in reactive oxygen intermediates such as O2•−, H2O2, OH, and peroxynitrite (ONOO−). These reactive oxygen intermediates are highly effective in killing infectious agents such as viruses, bacteria, protozoa, and fungi, by decreasing glutathione, increasing double-stranded DNA breaks, and via oxidation of lipids and DNA [9]. Reactive oxygen intermediates are also known to further enhance nitric oxide synthesis by these macrophages [10]. Interestingly, nitric oxide is also involved in negative feedback regulation of cytokines. For example, IFN-γ is a potent inducer of high levels of nitric oxide. High levels of nitric oxide in turn downregulate IFN-γ [4]. The ability of nitric oxide to switch off IFN-γ may be an effective homeostasis mechanism in preventing the generation of dangerous levels of pro-inflammatory IFN-γ. While a “burst” of elevated levels of nitric oxide is beneficial in the elimination of intracellular pathogens, dysregulated or sustained enhanced levels of nitric oxide are believed to be detrimental to the host. Enhanced levels of nitric oxide have been implicated in tissue injury in several female-predominant inflammatory autoimmune diseases including...
rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and glomerulonephritis [8,11].

In addition to the central role of nitric oxide in immunity against infections, and autoimmune diseases nitric oxide regulates the viability or apoptosis of a broad range cell types including immune cells [12]. iNOS-derived nitric oxide has both pro-apoptotic and anti-apoptotic effects on the cells of the immune system. Appropriate regulation of apoptosis of immune cells is essential for survival. This includes apoptosis of dangerous autoreactive and defective lymphocytes during development, elimination of clonally expanded immune cells after effectively countering infection, and death of infected or normal cells by cytotoxic lymphocytes. Inappropriate survival of cells in the immune system may result in serious health consequences, including the development of autoimmunity and/or cancer [12]. To better understand how nitric oxide may influence apoptosis, it is pertinent to briefly discuss key molecular events involved in apoptosis. Apoptosis is often triggered by the activation of initiator caspases such as caspase-8 by a variety of apoptotic ligands (such as interactions of Fas-FasL, TNF, and TNFR). This eventually results in upregulation of pro-apoptotic Bax homodimers and/or downregulation of anti-apoptotic Bcl-2 proteins. This results in the release of cytochrome c from mitochondria, which binds to ATP-dependent activation of the death regulator apoptotic protease-activating factor-1 (APAF-1), and caspase-9 to form an ATP-forming complex called an apoptosome [13]. Activation of initiator caspases such as caspase-9 or -8 in turn stimulates downstream pro-effector caspases such as caspase-3, caspase-7, and caspase-6. [14]. This leads to the degradation of Inhibitor of caspase-activated DNase (ICAD) and poly-ADP-ribose polymerase (PARP) and release of CAD into the nucleus [15]. Cleavage of PARP by caspase-3 results in inhibition of homopolymers of ADP-ribose synthesis in response to DNA damage and therefore promotes DNA fragmentation (cleavage of chromatin DNA into nucleosomes of 180 base pairs) and eventually cell death.

Deliberate culturing of macrophages [16,17] or CD4⁺CD8⁺ thymocytes [18] with high levels of exogenous nitric oxide (higher than 100 up to 1000 μM) has been shown to result in increased release of cytochrome c into the cytosol accompanied by enhanced enzymatic activity of caspase-3 and eventually DNA fragmentation. In contrast to the pro-apoptotic effects of nitric oxide discussed above, nitric oxide has also been reported to inhibit apoptosis in LPS-stimulated mature B cells, eosinophils, murine and human lymphocytes, γδT cells, certain tumor cell lines, human endothelial cells, and rat cardiac muscle cells subjected to mechanical stress [19–25]. Exposure of certain cell types (e.g., macrophages or B cells) to lower levels of nitric oxide (1–10 μM) has been shown to increase anti-apoptotic Bcl-2 and/or Bcl-xL [20,26–28] but not pro-apoptotic Bax mRNA. Another mechanism by which iNOS-derived nitric oxide downregulates apoptosis is by S-nitrosylation of the thiol group in cysteine residues of key apoptotic executor proteins such as caspases, PARP, and ICAD (Fig. 2). Denitrosylation by the addition of dithiothreitol (DTT) has been shown to reverse the anti-apoptotic effects of nitric oxide in some cells [29]. DTT is able to de-S-nitrosylate directly the caspase catalytic cysteine residue (Cys163), a functionally essential amino acid conserved among ICE/CPP-32-like proteases, and reverse the nitric oxide inhibition of apoptosis in conditions where there are low concentrations of nitric oxide in the cellular environment [29,30].

Although the precise reasons underlying these divergent effects of nitric oxide on cell viability or death are not known, many recent studies have suggested that induction or prevention of apoptosis depends upon several factors. These include: 1. Cell type: nitric oxide has been shown to induce apoptosis in macrophages, thymocytes, and neurons, while it prevented apoptosis in B cells, splenocytes, hepatocytes, and ovarian follicles. Submillimolar concentrations of nitric oxide rapidly induce apoptosis of macrophages but not hepatocytes [31,32]. 2. Nitric oxide level and duration: higher sustained levels of nitric oxide are believed to induce apoptosis, while lower levels may inhibit apoptosis. 3. Redox status and availability of transition metals: cell specific sensitivity to nitric oxide-mediated apoptosis may be associated with the redox state and transition metal complexes within the cell [32]. It is plausible that the ability to S-nitrosylate and the threshold of nitric oxide level that triggers apoptosis varies from one cell type to another [33]. S-nitrosylation by nitric oxide requires donation of an electron, which is accepted by transitional metals such as iron, a biochemical reaction that can affect apoptosis [31]. For example, MCF-7 and RAW264.7 macrophage cells are susceptible to nitric oxide-mediated apoptosis and preloading these cells with iron renders them resistant to apoptosis [31]. 4. Induction of anti-apoptotic and pro-apoptotic proteins and cytoprotective proteins: it is conceivable that various cell types (subjected to different activation signals) may have differential ability to trigger the induction of either anti-apoptotic or pro-apoptotic proteins [32]. Altering the balance of pro-apoptotic (e.g., Bax) and anti-apoptotic (e.g., Bcl-2) proteins by nitric oxide may determine the fate of cells. Further, other proteins such as heat shock proteins can influence apoptosis. For example, exposure of some cell types (such as hepatocytes) to nitric oxide has been shown to induce Hsp70, the levels of which correlate with the prevention of apoptosis [34].

iNOS, nitric oxide, and estrogen

Given that nitric oxide plays a central role in immunity against infectious agents, autoimmune diseases, and apoptosis, it becomes pivotal to understand factors that regulate nitric oxide. Recent studies have shown that nitric oxide is regulated by many endogenous factors including hormones such as estrogen. Therefore, this section will focus on the role of estrogen on nitric oxide. The effects of estrogen on iNOS and nitric oxide have been investigated in non-immune tissues such as aortic cells [35,36] and cardiac
myocytes [37]. Estrogen has a suppressive effect on iNOS expression from rat isolated aortic endothelial cells [15,35,36], microglial cells [38], and vascular endothelial cells [39], while it increases the level of iNOS and/or nitric oxide in other tissues such as rat uterus (in vivo estrogen) [40], ovine coronary artery cells (single dose of estrogen in vivo) [41], and rat cardiac myocytes (in vitro estrogen) [37]. Divergent effects of estrogen on nitric oxide could be due to intrinsic differences in cell types, the dose of estrogen, levels and types of estrogen receptors, as well as differences in the time of administration and culture. While the effects of estrogen on the generation of nitric oxide in non-immune tissues are reported, there are only a limited number of studies that have demonstrated the effect of estrogen on iNOS/nitric oxide by immune cells. The regulation of nitric oxide by estrogen is clearly important considering that estrogen is a potent immunomodulator, including modulating the activity of macrophages, which are important producers of iNOS-derived nitric oxide. Estrogen treatment activates phagocytic activity [42] and enhances Fc-γR expression on splenic-macrophage [43]. In vitro exposure of peritoneal macrophages [44] or macrophage-

![Diagram](image)

Fig. 2. S-nitrosylation by nitric oxide and apoptosis. Apoptotic signals such as Fas-FasL or TNF-TNFR could induce apoptotic pathway via upregulation the activation of initiator caspases (such as caspase-8). The activation of caspase-8 results in release of cytochrome c from mitochondria to cytosol, binding of APAF-1 to caspase-9 disruption of the expression of pro (e.g., Bax) vs anti-apoptotic (e.g., Bcl-2) proteins as well as activation of effector caspases (caspase-3). This leads to the degradation of inhibitor of caspase-activated DNase (ICAD) and poly-ADP-ribose polymerase (PARP) and release of CAD into nucleus. This molecular event together with degradation of PARP results in DNA fragmentation and apoptosis. In the presence of high levels of iNOS-derived nitric oxide, initiator and effector caspases are S-nitrosylated, which blocks the apoptotic molecular pathways and thus saving cells from apoptotic cell death.
monocyte-like cell lines (RAW 264.7) [45] to 17-β estradiol has been shown to increase iNOS expression and release nitric oxide.

Since there were no studies on in vivo effects of estrogen on iNOS expression and nitric oxide release by splenocytes, we addressed this gap in our recent studies. Our laboratory was the first to show that in vivo estrogen treatment promotes that the generation of pro-inflammatory nitric oxide and iNOS gene upregulation in splenocytes activated with T cell stimulants. We showed that Con-A-activated splenic lymphocytes from outbred CD-1 mice exposed to short-term, relatively low doses of subcutaneous injections of estrogen (2 and 4 μg/100g body weight) increased nitric oxide in supernatants [46]. This increase is especially apparent with higher doses of 17-β estradiol (4 μg/100g body weight). Further, in our recent studies we have shown that splenocytes from orchietomized inbred C57BL/6 male mice given estrogen implants (1–3 months) when activated in vitro with T cell mitogens [Concanavalin-A (Con-A) or anti-CD3 antibodies] released copious amounts of nitric oxide in supernatants [47]. This increase in nitric oxide in splenocytes from estrogen-treated (but not placebo-treated) orchietomized male mice was in part due to increased transcription of iNOS mRNA as determined by the Real Time PCR and increased levels of iNOS protein as determined with Western blot assay [47]. To further determine whether estrogen also enhances iNOS/nitric oxide in female mice, we next prepubertally orchietomized inbred C57BL/6 mice and gave either placebo and estrogen implants. After 1–3 months of treatment, splenocytes from these female mice were cultured in the presence or absence of Con-A. Similar to our findings in gonadectomized male mice given estrogen, we found that Con-A-stimulated splenocytes from orchietomized estrogen-treated C57BL/6 female mice demonstrated an apparent increase in the expression of relative levels of iNOS mRNA (data not shown) when compared to placebo-treated mice (control). In addition, we find that level of nitric oxide was significantly increased in the supernatants from Con-A-activated splenocytes from estrogen-treated orchietomized female C57BL/6 mice when compared to similar cultures from placebo-treated mice (Fig. 3; p < 0.001). It is important to note that high levels of nitric oxide was detectable only in splenocytes from estrogen-treated mice that were activated with a T cell mitogen (e.g., Con-A) and not in unstimulated (media only) cells. This implies that estrogen is not directly stimulating splenocytes to induce iNOS, rather estrogen alters the response (i.e., upregulates iNOS) of splenocytes upon activation. In our recent elaborate studies, we find that estrogen-induced upregulation of iNOS/nitric oxide in activated splenocytes is likely to be mediated through IFN-γ [47]. This is based on the following findings: (1) estrogen treatment of IFN-γ-knockout orchietomized male mice did not induce iNOS or nitric oxide. (2) Estrogen treatment of interferon regulatory factor-1 (IRF-1) knockout orchietomized male mice (in collaboration with Dr. G. Senaldi), which also have low levels of IFN-γ, did not induce detectable levels of nitric oxide. (3) Direct addition of recombinant IFN-γ to splenocytes (that were briefly activated with Con-A) from estrogen-treated but not placebo-treated mice resulted in upregulation of iNOS protein. (4) Blocking the interactions of co-stimulatory molecule, CD28 on T cells with B7 molecules on antigen presenting cells with a specific CTLA-4Ig fusion protein markedly diminishes IFN-γ levels as well as nitric oxide [47]. In our previous studies, we have shown that estrogen upregulates iNOS gene and protein expression as well as nitric oxide in orchietomized male mice [46,47]. The level of nitric oxide was significantly increased in supernatants from Con-A-activated splenocytes from orchietomized estrogen-treated mice, and the increase in nitric oxide level in Con-A-activated splenocytes from estrogen-treated orchietomized female mice could be abrogated by blocking CD28 and B7 interactions implying the importance of CD28-B7 pathway in regulating nitric oxide levels (Fig. 4). Together these studies suggest that estrogen upregulation of iNOS is mediated in part by IFN-γ. These findings are consistent with studies from others who have shown that iNOS expression is activated by selected immunological stimuli especially cytokines such as IFN-γ. IFN-γ, secreted by T cells, is required for the production of nitric oxide and the expression of iNOS [48].
Macrophages are notable target cells for IFN-\(\gamma\). This cytokine has been shown to activate macrophages, increase iNOS activity [48], and confer immunity against intracellular pathogens [49]. IFN-\(\gamma\) binds to specific IFN-\(\gamma\) receptors [50] and activates Janus kinase (JAK) and signal transducers and activators of transcription-1 (STAT-1) pathway [51]. Activated STAT-1 dimerizes and binds to specific DNA response elements to regulate STAT-1-controlled genes including iNOS gene, which can culminate in the induction of the enzyme iNOS protein (Fig. 4). In addition to estrogen-induced IFN-\(\gamma\) and subsequent IFN-\(\gamma\)-mediated upregulation of iNOS, estrogen has been shown to upregulate other cytokines such as TNF-\(\alpha\) [52,53], that induce iNOS-derived nitric oxide production. Although several cytokines such as IL-1, IL-6, and IL-17 are known to modulate iNOS expression and nitric oxide production [48,54], there is no conclusive data as yet to demonstrate that estrogen upregulation of iNOS/nitric oxide is through these cytokines in lymphocyte cultures.

Overall, these studies demonstrate that estrogen regulates iNOS expression and/or release of nitric oxide in many non-immune and immune (splenocytes) cells. The precise effects of estrogen on nitric oxide are dependent upon several factors including different cell types, the dose of estrogen, the duration of cell culture period, and the response to IFN-\(\gamma\).

**Nitric oxide, COX-2, and estrogen: Implications for inflammation**

iNOS-derived nitric oxide can induce several genes and their products in various cell types. One of these iNOS-inducible genes is cyclooxygenase-2 (COX-2). COX-2, a member of cyclooxygenases family, is also known as prostaglandin H synthase. It catalyzes arachidonic acid for the formation of prostaglandin in tissues, PGE\(_2\) [55]. COX-2 is expressed in activated cells and as a result of pathologic stimuli. COX-2 is also upregulated in several pathological conditions including cancer and autoimmune diseases. COX-2 mRNA and protein are induced in a time and dose-dependent manner in inflammatory cells, such as human macrophages and polymorphonuclear leukocytes in response to pro-inflammatory cytokines (IL-1\(\beta\), IL-8, TNF-\(\alpha\), and IFN-\(\gamma\)) [56,57], and murine and rat macrophages.
that are stimulated with LPS and IFN-γ [58,59]. Studies show that iNOS and COX-2 are frequently expressed together in tumor cells [60] and in various cells such as keratinocytes, macrophage cell lines, and endothelial cells [58,61]. Prolonged COX-2 mRNA expression in IFN-γ and LPS-activated murine macrophages is maintained by endogenous nitric oxide [62]. In addition to the increased COX-2 enzyme activity, due to iNOS-derived nitric oxide in macrophages, elevated COX-2 expression is observed in murine cholangiocytes [63] and colon cancer cell lines [60] in relation to iNOS-derived nitric oxide. The relationship between IFN-γ, iNOS, and COX-2 can be deciphered in studies using iNOS knockout and IFN-γ knockout mice. Peritoneal macrophages from iNOS knockout mice that are stimulated with IFN-γ and LPS have no nitric oxide production [64]. Moreover, IFN-γ knockout mice have not only decreased nitric oxide levels but also the formation of the COX-2 product PGE2 (prostaglandin E2) is significantly decreased (80%) [64]. Interestingly, although COX-2 product PGE2 is decreased in IFN-γ knockout mice, the levels of COX-2 itself are not different compared to control mice [64]. These results indicate that the absence of iNOS-derived nitric oxide affects the formation of PGE2 from the COX-2 enzyme, rather than protein expression of COX-2 [65]. Other studies have shown that the suppression of LPS and IFN-γ-mediated nitric oxide and iNOS via capsaicin treatment of the murine macrophage cell line (RAW264.7) decrease COX-2 mRNA expression and PGE2 secretion [65].

The effect of estrogen on COX-2 in various tissues is demonstrated by several studies. Estrogen treatment of vascular endothelial cells [66], as well as human umbilical vein endothelial cells [67] increases COX-2 expression. Egan et al. [68] have shown that estrogen upregulates COX-2 activity and PGE2 production via estrogen receptor subunit alpha (ERα) providing atheroprotection. Although it was shown that macrophage PGE2 production was increased in burn-traumatized proestrus female mice compared to traumatized males or control proestrus female mice [69,70], there is no relevant data that demonstrate the effect of estrogen on COX-2 expression in non-autoimmune and non-traumatized immune system. We have previously shown that estrogen upregulates IFN-γ, IFN-γ-inducible iNOS, and its end-product nitric oxide from splenocytes compared to controls. Therefore, we evaluated the effect of estrogen treatment on iNOS-inducible COX-2 expression in the immune system. Our studies demonstrated that estrogen treatment increased iNOS-inducible COX-2 protein expression in Con-A-stimulated splenocytes [47]. These data imply that estrogen upregulates iNOS and nitric oxide which may have a vital impact in inflammatory disorders.

iNOS, nitric oxide, estrogen, and apoptosis

Several studies in various cell types have shown that estrogen (17-β estradiol) can promote the escape from apoptosis [71]. It has been shown that at physiological levels, in vivo estrogen treatment rescues several cell types from apoptosis, including B lymphocytes [71,72], bone marrow mesenchymal stem cells [73], brain, endothelium, testes, uterus, and aortic endothelial cells [74,75], neuronal cells [76,77], osteocytes [78], as well as B cells [71,79], and breast cancer cells [80] cultured with estrogen in vitro. On the other hand, estrogen levels above physiological concentrations can result in the induction of apoptosis of lymphocytes from gonadectomized female C57BL/6 mice [81].

One of the several proposed mechanisms by which estrogen can modulate apoptosis is by altering the expression of iNOS and/or response to iNOS-derived nitric oxide. To date, the data on the effect of estrogen on apoptosis in relation to iNOS/nitric oxide are inconclusive. For example, anterior pituitary cells from ovariectomized rats cultured in the presence of estrogen (10−9 M) with TNF-α and the nitric oxide inhibitor, NAME (0.5 mM), have increased apoptosis and enhanced pro-apoptotic action of TNF-α in the absence of nitric oxide [52]. This particular study does not specify which NOS (nNOS, eNOS, or iNOS) is responsible for this action. Estrogen exposure upregulates FasL expression as well as increases the levels of superoxide through a nitric oxide-dependent mechanism in rabbit endothelial cells [82] and spermatogenic cells [83]. On the other hand, subcutaneous one-time estrogen treatment (100 μg/ml) of ovariectomized female rats with ischemic damage demonstrates a protective effect on neuronal cells from apoptosis [84]. In this case, estrogen treatment results in diminished NF-κB, IκB, and iNOS expression, which are rapidly activated during ischemia/reperfusion. In addition, in vitro estrogen treatment of the rat PC12 neural cell line activates the Akt/NOS pathway, which resulted in survival from apoptosis [85]. In addition to its effect on iNOS expression, estrogen treatment decreases apoptosis in nerves, smooth muscle, vascular endothelium, and epithelium of the rat vagina by upregulating vaginal eNOS and nNOS expression [86]. In summary, the discrepancy between these studies may be due to the effect of estrogen and/or the nature of nitric oxide (either cytotoxic or cytoprotective) on different cell types or experimental conditions, a subject that requires further investigation. We are currently investigating the effects of estrogen on apoptosis of splenocytes in relation to iNOS and nitric oxide.

Concluding remarks

The production of iNOS-derived nitric oxide is critical to the homeostasis of the immune system, inflammation, and immunity. As reviewed above, the effects of iNOS-derived nitric oxide in relation to apoptosis are complex due to various factors such as the levels of nitric oxide produced, cell or tissue types exposed to nitric oxide, types of stimulants, the duration of exposure, as well as the setting of the experiments (in vivo versus in vitro exposure). In general, moderately high levels of nitric oxide can be cytoprotective for the immune system such as being anti-bacterial, anti-parasitic, anti-viral, and anti-apoptotic for
immune cells, whereas in the presence of prolonged or severe pathological conditions, very high levels of iNOS-derived nitric oxide can demonstrate a destructive character leading to apoptosis or even necrosis of immune cells. There is also evidence that high concentrations of nitric oxide can suppress IL-2 and IFN-γ secretion and macrophage activity resulting in the upregulation of IL-4 and driving T cell differentiation into a Th2 profile [11]. This double nature of iNOS-derived nitric oxide helps to maintain the homestasis of the immune system as well as other cell systems. Therefore, it is critical to take into consideration these factors before defining the role of iNOS and nitric oxide in the treatment of disorders related with high levels of nitric oxide and in designing of new drugs against these disorders.

In addition to the factors explained above, the profound effects of estrogen on the immune systems of normal and autoimmune individuals should also have been taken into consideration when evaluating the upregulation of iNOS-derived nitric oxide. In addition to natural estrogen, multiple sources of exposure to estrogenic compounds are recognized. These include, natural endogenous estrogens, intentional exposure to pharmaceutical estrogen (such as oral contraceptives and estrogen replacement therapy), inadvertent human exposure to estrogenic compounds including environmental estrogens [87], and through consumption of phytoestrogens that are found in soybeans, cabbage, and other edible plants [87]. The precise impact of estrogenic compounds on health is therefore of significant concern. One mechanism by which estrogens could modulate the immune system is through upregulating the level and/or response to cytokines such as IFN-γ, which in turn can induce iNOS and eventually increased release of nitric oxide. Our studies show that estrogen upregulates IFN-γ-inducible nitric oxide synthase (iNOS) and nitric oxide levels in Con-A-stimulated splenic lymphocytes from outbred CD-1 mice that were treated with relatively low doses of 17-β estradiol for short durations [46] as well as activated splenocytes from estrogen-treated C57BL/6 gonadectomized male [47] and orchietomized female (this study) mice.

These results are significant due to the fact that increased IFN-γ and IFN-γ-induced nitric oxide have been associated with autoimmune diseases such as systemic lupus erythematosus [88,89], and rheumatoid arthritis [90,91]. Further these autoimmune diseases are more prevalent in women compared to men, and estrogens have been shown to regulate autoimmune parameters [92,93]. Since iNOS and iNOS-derived nitric oxide are both key molecules in physiological processes such as immunity against intracellular infections and pathological conditions such as autoimmune diseases and cancer, it is important to understand the impact of nitric oxide on the immune system and the effects of estrogen in relation to the link between IFN-γ, iNOS, and nitric oxide in splenic lymphocytes. The latter aspect adds new dimension to our understanding of estrogen regulation of immunity. Therefore, mechanistic studies that decipher the molecular and cellular pathways of estrogen-induced IFN-γ, iNOS, and the cellular responses to exposure to these molecules, are critically important in understanding how estrogens influence healthy individuals and the induction or progression of disease. It is conceivable that new therapeutic approaches can be designed based on this information to disrupt or regulate the sequence of events involved in such diseases.

References


M. S. Gregory, L. A. Du

D.J. Perkins, D.A. Kniss, Blockade of nitric oxide formation down-}


C. Amant, P. Holm, S.H. Xu Sh, N. Tritman, M. Kearney, D.W. Los-


Y.I. Alexaki, I. Charalampopoulos, M. Kampa, H. Vassalou, P. Theo-
doropoulos, E.N. Stathopoulos, A. Hatzoglou, A. Gravanis, E. Ca-
tanas, Estrogen exerts neuroprotective effects via membrane estrogen receptors and rapid Akt/NOS activation, FASEB J. 18 (2004) 1594–1596.


S. Ansar Ahmed, The immune system as a potential target for envi-


J.B. Weinberg, Nitric oxide as an inflammatory mediator in autoim-


J.W. Choi, Nitric oxide production is increased in patients with rheu-

