Cytokines and Nitric Oxide in Islet Inflammation and Diabetes (43950D)

MICHAEL L. MCDANIEL,[1] GUIM KWON, JEANETTE R. HILL, CONNIE A. MARSHALL, AND JOHN A. CORBETT
Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110-1010

Abstract. **Cytokines released by both T lymphocytes and activated macrophages, in particular interleukin-1 (IL-1), have been implicated as immunological effector molecules that both inhibit insulin secretion from the pancreatic β cell and induce β-cell destruction. Recent findings have demonstrated that production of the free radical nitric oxide (NO), resulting from the expression of the cytokine-inducible isoform of NO synthase (iNOS), mediates these deleterious effects. The cellular mechanism responsible for inhibition of β-cell function and destruction by NO involves, in part, inactivation of enzymes specifically localized to the β-cell mitochondria that contain iron-sulfur centers or clusters. Intraislet release of IL-1 also inhibits β-cell function by this same cellular mechanism involving the overproduction of NO. In addition, the cytokine, IL-1, induces the co-expression of both iNOS and the cytokine-inducible isoform of cyclooxygenase, COX-2. The expression of COX-2 results in the overproduction of the proinflammatory prostaglandins and thromboxanes. Furthermore, NO produced by iNOS directly stimulates the activities of both constitutive and inducible isoforms of COX, further augmenting the overproduction of these proinflammatory mediators, NO and prostaglandins, which may be important in initiating or maintaining the inflammatory response and destruction of the β cell associated with autoimmune diabetes.** [P.S.E.B.M. 1996, Vol 211]

Insulin-dependent diabetes mellitus (IDDM) is characterized by an initial inflammatory response or cellular infiltration in and around the islets of Langerhans (1). Cellular components of this inflammation include both $CD4^+$ and $CD8^+$ T cells, monocytes, and macrophages (2, 3). Cytokines, released by infiltrating lymphocytes and macrophages, have been implicated in autoimmune diabetes by exerting both direct effects on β-cell function and by recruitment of lymphocytes and macrophages to the site of inflammation. Our studies indicate that the ability of cytokines, in particular interleukin-1 (IL-1), to produce deleterious effects on pancreatic β cells is mediated by the expression of the inducible isoform of nitric oxide synthase (iNOS) and the overproduction of the free radical nitric oxide (NO) (4). More recent findings have revealed that IL-1 simultaneously expresses the inducible isoform of cyclooxygenase (COX-2) that results in production of the proinflammatory prostaglandins and thromboxanes (5). Moreover, NO produced by iNOS potently activates both the constitutive and inducible isoforms of cyclooxygenase to further augment the production of these proinflammatory mediators. The focus of this review will be (i) to provide a brief background describing the ability of exogenous IL-1 to inhibit β-cell function by the overproduction of NO, (ii) to provide evidence that activation of intraislet macrophages and the endogenous release of IL-1 inhibits β-cell function by this same mechanism involving the free radical NO, and (iii) to discuss briefly our more recent results indicating that IL-1–induced NO is directly linked to the overproduction of the proinflammatory prostaglandins.

Background

Nerup *et al.* first observed that culture media derived from activated human mononuclear cells both inhibited insulin secretion and caused a loss of insulin content from pancreatic islets (6). The active component of this culture media was later determined to be the cytokine IL-1. This group, ourselves, and others showed that brief exposures (1–3 hr) of rat islets to

[1] To whom requests for reprints should be addressed at Box 8118, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110-1010.

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IL-1 augment glucose stimulated insulin secretion, whereas longer exposures (15–18 hr) inhibit insulin secretion by 80%–90% (7–9). Furthermore, more prolonged exposures of islets to IL-1 result in ultrastructural damage and β-cell destruction. A summary of IL-1's effects as described in these early studies on β-cell dysfunction by a number of investigators includes the following: (i) a time-dependent inhibition of glucose-stimulated insulin secretion that requires more than 5 hr to develop; (ii) inhibition of insulin secretion requires both gene transcription and mRNA translation, suggesting a requirement for new protein synthesis; (iii) inhibition of β-cell function is also associated with significantly impaired glucose oxidation by islet-cell mitochondria, and (iv) destruction of islets occurs after prolonged exposure to IL-1. These observations raised the question as to whether the free radical NO may mediate IL-1's inhibitory effects on the pancreatic β cell.

Nitric Oxide Synthase and NO Production

A number of recent findings have revealed that the cellular mechanism responsible for IL-1's inhibitory effects on β-cell function and viability involves the formation of the free radical NO. As illustrated in Figure 1, the enzyme NO synthase (NOS) catalyzes the oxidation of one of the chemically equivalent guanidino nitrogens of L-arginine to produce NO and L-citrulline (see Refs. 10 and 11 for reviews). NO is unstable and is rapidly oxidized to the stable end products nitrite and nitrate. NO is also a potent activator of soluble guanylate cyclase which results in increased accumulation of cGMP. Both the formation of nitrite and cGMP serve as indirect indicators of NO formation. Inhibitors of NOS have also been used to define a role for NO in a number of cellular models. The compounds L-NMMA (NG-monomethyl-L-arginine) and aminoguanidine are potent inhibitors of NOS and at appropriate concentrations block completely the formation of NO and NO-dependent nitrite, nitrate, and cGMP accumulation. Aminoguanidine has an additional important property in that it is a selective inhibitor of the inducible isoform of NO synthase (iNOS) and has minimal effects on constitutive NOS (12, 13).

At present, three different isoforms of NOS have been described (14, 15). These isoforms of NOS include endothelial (eNOS or NOS III) and neuronal

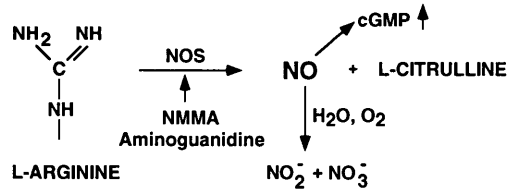


Figure 1. L-Arginine–dependent nitric oxide pathway.

(nNOS or NOS I) that are constitutively expressed, and an inducible NOS (iNOS or NOS II). The constitutive isoforms are expressed in tissues including endothelial cells, brain, and also the pancreatic β cell (16) and are Ca^{2+} and calmodulin dependent. The other isoform is referred to as iNOS and is induced by cytokines and lipopolysaccharide. iNOS is Ca^{2+} independent and was originally isolated from activated macrophages (15). Nitric oxide produced by constitutive isoforms eNOS and nNOS serves as a signaling molecule associated with diverse functions including facilitation of neuronal synaptic transmission and regulation of vascular tone (see Refs. 10 and 17 for reviews). Cytokines and endotoxin stimulate the expression of iNOS, and NO produced by this isoform functions as both a cytostatic and cytotoxic molecule (10, 18). Our studies have focused primarily on the cytokine-inducible isoform of NOS that results in overproduction of NO for extended periods of time.

NO Mediates IL-1–Induced Inhibition of Insulin Secretion

Our laboratory and others first demonstrated that IL-1–induced inhibition of insulin secretion depends on metabolism of L-arginine to NO (19–21). NMMA, a competitive inhibitor of NOS, completely prevents IL-1–induced inhibition of insulin secretion as well as nitrite formation, a stable oxidation product of NO. The cellular mechanisms responsible for the ability of NO to inhibit β-cell function as well as exert its cytotoxic effects are believed to be mediated by inactivation of enzymes that contain iron-sulfur centers or clusters. Our studies demonstrated that IL-1 induces NO formation from islets as evidenced by an electron paramagnetic resonance (EPR) axial feature at $g = 2.04$ which is identical to previously reported iron-nitrosyl complexes (21, 22). Inhibitors of NOS or cycloheximide, an inhibitor of protein synthesis, prevent formation of this EPR signal (23). We and others have further demonstrated that treatment of rat islets for 18 hr with IL-1 results in significant inhibition of aconitase activity that is completely prevented by NMMA (20, 24). These results indicate that NO mediates its inhibitory effects on β-cell function by destruction of iron-sulfur centers of enzymes localized to the islet-cell mitochondria. Since the ability of D-glucose to stimulate insulin secretion from the β cell requires metabolism of this fuel secretagogue by glycolysis and oxidative phosphorylation, inactivation by NO of enzymes specifically localized to the β-cell mitochondria is mirrored temporally by inhibition of insulin secretion.

Our previous studies also demonstrated that a brief exposure of islets to IL-1 for 1 hr or less followed by the complete removal of IL-1 and the continued incubation of islets in the absence of IL-1 was sufficient to inhibit β-cell function to the same extent as

observed with the continuous exposure to IL-1 (9). We recently also demonstrated that a 1-hr exposure of islets with IL-1 followed by an 8- or 18-hr incubation in the absence of this cytokine results in inhibition of glucose stimulated insulin secretion which is completely prevented by the NO synthase inhibitor NMMA. The production of NO under these pulse conditions was demonstrated by both IL-1 induced nitrite and EPR-detectable iron-nitrosyl complex formation, and are prevented by NMMA (25). These results are important since they indicate that a brief exposure of β cells to transient increases of cytokines that may occur *in vivo* is sufficient to initiate the signal transduction cascade leading to the expression of iNOS and overproduction of NO.

An additional key observation was the demonstration that the deleterious effects produced by overproduction of NO are reversible under certain conditions. Our early studies indicated that pretreatment of islets for 18 hr with IL-1 results in almost complete inhibition of insulin secretion and requires ~4 days incubation in the complete absence of IL-1 to restore insulin secretory function (8). With knowledge of the cellular mechanism responsible for cytokine-induced inhibition of β-cell function, we recently determined that inhibition of glucose stimulated insulin secretion is reversed in a time-dependent manner when islets are treated with a NO synthase inhibitor after 18 hr of preexposure to IL-1. In this design, IL-1 is not removed from the incubation media following exposure to IL-1 and complete recovery of insulin secretion is observed 8 hr after inhibition of NO formation by NMMA (26). The destructive effects of IL-1 on islet viability are also prevented if NMMA is added to islet cultures after 24 hr of exposure to IL-1, but destruction is not prevented if NMMA is added after 48 hr of exposure to IL-1. The importance of these findings is that a "window" of intervention exists to rescue islets from the destructive effects of NO, if iNOS is inhibited early after its induction.

β-Cell Expression of iNOS

The islet of Langerhans represents a heterogeneous cell population consisting of both endocrine and nonendocrine cells. Approximately 50%–65% of the cells of an islet are β cells that both synthesize and secrete insulin. The islet also contains other endocrine cells including glucagon secreting α cells and somatostatin secreting δ cells that are located primarily around the periphery of the islet. The islet also contains nonendocrine cells including fibroblasts, endothelial cells, dendritic cells, and macrophages. Our studies have focused on determining the cellular source of IL-1–induced NO production and whether the β cell which is selectively destroyed in autoimmune diabetes could express iNOS, and if the overproduction of NO by the β cell would exert a deleterious effect directly on β-cell function. Using antiserum specific for mouse macrophage iNOS, a 130-kDa protein (iNOS) was immunoprecipitated from primary β cells purified by fluorescent-activated cell sorting (FACS) treated with IL-1 (27). The expression of iNOS was inhibited by actinomycin D, an inhibitor of gene transcription, and not observed in FACS purified α cells, the other major endocrine cell of the islet (27). Recent studies have also addressed by immunohistochemistry the cellular localization of iNOS and insulin using thin sections of cryopreserved islets previously exposed to IL-1 (28). This approach indicates that exogenous IL-1 induces iNOS expression in virtually every insulin containing β cell of the islet. These studies provided the initial evidence that IL-1 has the ability to induce iNOS expression in the β cell which is selectively destroyed in autoimmune diabetes (24).

Based on these studies, our working model to explain the ability of cytokines to both potently inhibit insulin secretion from the pancreatic β cell and also produce cytotoxic effects by the overproduction of NO is shown schematically in Figure 2. In this model, the cytokine IL-1, released by intraislet macrophages, binds to a β cell–specific receptor, a Type 1 IL-1 receptor, which then initiates a series of signal transduction events that include activation of a post-receptor tyrosine kinase activity and a role for the early transcriptional factors c-*fos*, c-*jun*, and NFκB that ultimately culminates in the expression of iNOS. The overproduction of NO by iNOS damages the β-cell mitochondria by inactivating iron-sulfur containing enzymes and also inhibits DNA synthesis that is believed to ultimately contribute to β-cell death. Inhibition of the islet-cell mitochondria by NO is reflected by decreased insulin secretion from β cells that is first detected at >5 hr following exposure to IL-1. Activated intraislet macrophages also play an important role in this cascade by directly producing NO, but more importantly, as will be discussed, by their ability to re-

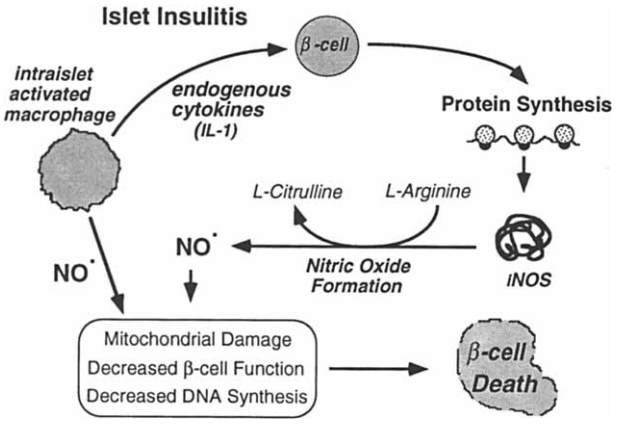


Figure 2. Proposed mechanism of action of cytokines and nitric oxide on pancreatic β cells.

lease endogenous cytokines that following even brief exposures to the β cell is sufficient to initiate this signal transduction cascade which culminates in the overproduction of NO.

Intraislet Release of Cytokines and β-Cell Expression of iNOS

Macrophages are believed to play a primary role in development of autoimmune diabetes and destruction of the pancreatic β cell. Kolb *et al.* (29) have shown that administration of silica to young BB rats, a procedure that targets macrophages, prevents spontaneous development of diabetes in the BB rat. Wright *et al.* (30) also examined the effects of essential fatty acid depletion on the incidence and severity of diabetes in CD-1 mice treated with streptozotocin. This study demonstrated that a diet deficient in essential fatty acids, believed to deplete intraislet macrophages, prevents development of diabetes in this animal model. Evidence also indicates that activation of murine peritoneal macrophages *in vitro* with LPS + IFNγ results in NO formation that then mediates the subsequent destruction of macrophages through apoptosis (31). This type of cell death is indicated by nuclear and cytoplasmic alterations characteristic of apoptosis and by specific patterns of internucleosomal DNA fragmentation. There is also recent evidence that cytokine induced β-cell destruction from RINm5F cells and HIT-T15 cells also occurs by apoptosis, and NO mediates this cytotoxicity (32, 33).

As shown schematically in Figure 3, our recent focus has been to test the hypothesis that intraislet or the endogenous release of cytokines, in particular IL-1, by nonendocrine cells induces iNOS expression by β cells (28).

In this experimental approach, conditions have been established whereby the cytokine, tumor necrosis factor–α (TNF-α) and endotoxin (LPS) in combination, conditions known to activate resident macrophages, stimulate intraislet release of IL-1 and NO.

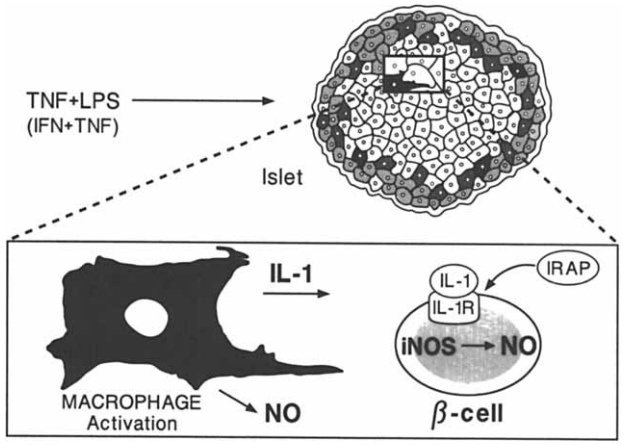


Figure 3. Intraislet release of IL-1 and β-cell iNOS expression.

This experimental model allows an evaluation of the relative importance of the endogenous release of IL-1 from activated macrophages that can then attain sufficient local concentrations within the islet to induce iNOS expression by β cells, and whether IL-1 by this cellular mechanism or the direct production of NO represents the most important effector molecule in the destruction of the β cell. Since an islet contains only ~10–12 macrophages, it was anticipated that the absolute level of NO produced by this relatively small number of activated macrophages would be low. In contrast, IL-1 at picomolar levels is sufficient to induce iNOS expression by the β cells of an islet.

Our first approach was to demonstrate that TNF + LPS induce iNOS expression as determined by quantitative immunoprecipitation from islets that contain macrophages but have no direct effects on iNOS expression from primary β cells purified by FACS. The effects of cytokines and LPS on iNOS expression by islets and purified β cells are shown in Table I. In the upper panel of Table I, IL-1 alone causes maximum iNOS expression by islets, and combinations of LPS + TNF and TNF + IFN induce iNOS expression that is ~50% of that of IL-1 alone. In the lower panel of Table 1, IL-1 also causes maximum iNOS expression from FACS-purified β-cell, but TNF + LPS do not result in any detectable iNOS expression by FACS-purified β cells that do not contain contaminating macrophages.

Our next approach was to correlate these effects of IL-1 and TNF + LPS on iNOS expression by islets and FACS-purified β cells with their functional effects on insulin secretion. As shown in the left panel of Fig-

Table I. Effects of Cytokines and LPS on iNOS Expression by Islets and Purified β Cells

Treatment	Optical density (% of IL-1–induced iNOS)
Islets	
Control	0
IL-1	100
TNF + IFN	47 ± 18
LPS + TNF	50 ± 9
Purified β cells	
Control	0
IL-1	100
TNF + LPS	0

Note. Intact islets and purified β cells obtained by FACS were incubated for 5 hr with 5 U/ml IL-1β, 150 U/ml IFN-γ, 0.7 n*M* TNF-α, and 10 μg/ml LPS alone or in combination as indicated. [^{35}S]Methionine was added and the islets and cells were incubated for an additional 13 hr. iNOS was immunoprecipitated using antiserum specific for the COOH-terminal 27 amino acids of mouse macrophage iNOS. Immunoprecipitates were separated on 8% SDS-polyacrylamide gels, visualized by fluorography, and densitometry was performed. The level of iNOS expression after treatment with IL-1 was set at 100%. Results are the average ± SEM from two experiments with two replicates/condition. (From Ref. 28 with permission.)

ure 4, IL-1 inhibits completely glucose-stimulated insulin secretion, which is consistent with the ability of IL-1 to induce maximum iNOS expression by islets. TNF + LPS inhibit insulin secretion by only ~40%, also consistent with reduced levels of iNOS expression induced by TNF + LPS from islets as shown in Table 1. The right panel of Figure 4 also shows that IL-1 inhibits glucose-stimulated insulin secretion from FACS-purified β cells by ~70%, but TNF + LPS neither inhibit insulin secretion nor induce iNOS expression by FACS-purified β cells (see Table 1).

So how does TNF + LPS inhibit insulin secretion from intact islets and can these results be explained by activation of resident macrophages and either the endogenous release of the cytokine IL-1 or direct production of NO? As shown in the left panel of Figure 4, the ability of TNF + LPS to inhibit insulin secretion from the intact islet may be explained by the ability of TNF + LPS to activate resident macrophages that release NO that then diffuses to adjacent β cells and results in inhibition of insulin secretion. Alternatively, these results may be explained by the endogenous release of IL-1 from intraislet macrophages, which then attains sufficient local concentrations within the islet to induce iNOS expression by the β cell. To test this alternative mechanism involving the endogenous release of IL-1, we have used IRAP or IL-1ra, the IL-1 receptor antagonist protein, that binds to the IL-1 receptor present on its target cells, in this case the β cell. The ability of IRAP to block specifically IL-1's inhibitory effects on insulin secretion by islets allows evaluation of whether the endogenous release of IL-1 or the direct production of NO is the most important effector molecule in this destructive process of the β cell.

Figure 5 describes the effects of IRAP on inhibition of insulin secretion and nitrite production by islets. IL-1 results in complete inhibition of insulin secretion following 24 hr of exposure, and as anticipated this inhibition is prevented completely by co-incubation of IL-1 and IRAP. The combination of TNF and LPS also inhibits glucose-stimulated insulin secretion by ~70% in this experiment, and this effect is also completely prevented by co-incubation of TNF + LPS and IRAP. Overall, these results suggest that the intraislet release of endogenous IL-1 is solely responsible for the ability of TNF + LPS to inhibit insulin secretion from intact islets and that the direct production of NO by a limited number of nonendocrine cells has minor if any effects.

To determine more directly the cellular source of iNOS under these same experimental conditions, the effects of TNF + LPS alone and in the presence of IRAP on iNOS protein expression have been determined by immunohistochemistry (see Co-Localization of iNOS and Insulin in Ref. 28). In these studies, TNF + LPS stimulated a relatively low number of islet cells compared with IL-1 that were positive for iNOS and co-localized with insulin-containing β cells. In the presence of TNF + LPS, IRAP reduced the number of iNOS-positive cells to only six cells, and only one of

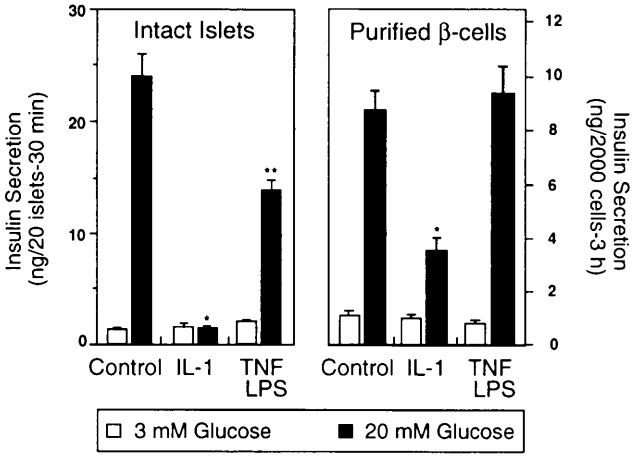
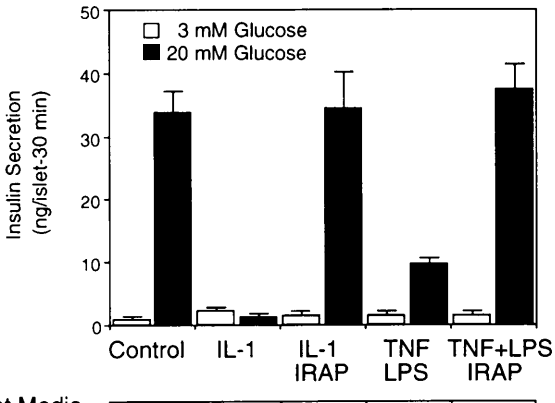


Figure 4. Effects of IL-1β and TNF + LPS on glucose-stimulated insulin secretion by intact islets and purified β cells. Intact islets and β cells purified by FACS were incubated for 18 hr with or without 5 U/ml IL-1β, or 0.7 n*M* TNF and 10 μg/ml LPS as indicated. Islets and β cells were then isolated for subsequent measurement of glucose-stimulated insulin secretion. Results for intact islets are the average ± SEM of four individual experiments containing three replicates per condition. Results for FACS-purified β cells are the average ± SEM of two individual experiments containing four replicates per condition. Statistically significant inhibition of insulin secretion as compared with controls are indicated (*$P < 0.001$; and **$P < 0.05$). (Modified from Ref. 28, with permission.)



Overnight Media Nitrite (pmol/islet-24 h)					
	10.8	46.7	15.7	35.4	18.5
	0.3	1.3	2.5	1.0	0.6

Figure 5. Effects of IRAP on IL-1β– and TNF + LPS–induced inhibition of glucose-stimulated insulin secretion by islets. Islets were pretreated for 1 hr ± 1 μg/ml human recombinant IRAP. The islets were then incubated for an additional 24 hr ± IRAP with 5 U/ml IL-1β, or 0.7 n*M* TNF and 10 μg/ml LPS as indicated. After the 24-hr incubation, the culture media was removed for nitrite determination and glucose stimulated insulin secretion was subsequently measured. Results are the average ± SEM of an individual experiment containing three replicates per condition, and are representative of three individual experiments. (From Ref. 28, with permission).

these six cells co-localized with insulin (28). The remaining five cells that stained positive for iNOS are believed to be resident macrophages that comprise 0.5% (~10–12 macrophages/islet) and are known to express iNOS following treatment with TNF + LPS. These results raise the important question as to whether intraislet activation of macrophages could represent the initiation or triggering event in autoimmune diabetes (28). In this context, our studies suggest that activation of a relatively small number of intraislet macrophages results in the endogenous release of IL-1 that can achieve local concentrations within the islet sufficient to induce iNOS expression by β cells. Induction of iNOS by β cells and the overproduction of NO would result in inhibition of insulin secretion and eventually β-cell death. This would be accompanied by the release of β-cell autoantigens that could be both processed and presented by macrophages which would initiate or drive the T cell–dependent process that ultimately destroys all the β cells in the islet.

IL-1 Co-Expresses iNOS and Cyclooxygenase (COX-2) Protein Expression and NO Activates COX-2 Enzyme Activity

Cytokines released by T lymphocytes and activated macrophages produce a variety of proinflammatory mediators that have been implicated in inflammation. Autoimmune diabetes is characterized both by the selective destruction of the β cell and by an early insulitis or inflammatory response. The production of the proinflammatory prostaglandins have been implicated in both acute and chronic inflammation. Our previous studies indicated that recombinant human IL-1 induces significant accumulation of prostaglandins of the E-series, in particular prostaglandin E_2 (PGE_2), by isolated islets (34). IL-1 stimulated a 5-fold increase in PGE_2 accumulation that was first detected at ~10 hr, and PGE_2 accumulation increased in a time-dependent manner thereafter. The cellular mechanism responsible for IL-1–induced increases in prostaglandin production by islets were unknown at that time. However, the possibilities that IL-1 may modulate either phospholipase-mediated release of arachidonic acid or cyclooxygenase enzyme activity and/or synthesis were considered.

A general scheme of the cyclooxygenase and lipoxygenase metabolic pathways for arachidonic acid by islets is described in Figure 6. In isolated islets, the major cyclooxygenase-derived prostaglandins are PGE_2, $PGF_{2\alpha}$, and thromboxane A_2. In rat islets the predominant prostaglandin is PGE_2. The 12-lipoxygenase is also expressed by β cells but not by glucagon containing α cells of the islet or by exocrine cells. Although the 5-lipoxygenase is not expressed by the β cell, intraislet macrophages present in the islet express

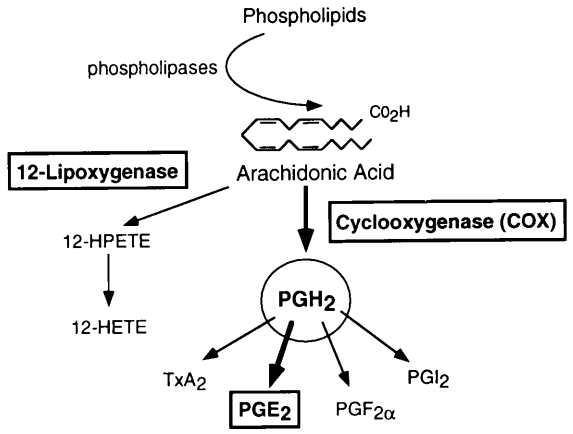


Figure 6. Arachidonic acid metabolism by pancreatic islets.

the 5-lipoxygenase that produces the chemotactic leukotrienes (35).

Similar to the different isoforms that have been identified for NOS, two different isoforms of cyclooxygenase (COX) have also been characterized. One isoform (COX-1) is constitutively expressed, and expression of the other isoform (COX-2) is stimulated by cytokines and mitogens (36, 37). Importantly, COX-2 is believed to exert a significant role in both acute and chronic inflammation by the overproduction of the proinflammatory prostaglandins. Also of importance is that both isoforms of cyclooxygenase, COX-1 and COX-2, contain an iron-heme prosthetic group at their active site. Whereas the cytotoxic effects produced by NO are believed to be mediated, in part, by the destruction of iron-sulfur centers of enzymes, the signaling mechanism of NO is believed to be facilitated by the ability of NO to interact with iron-heme centers to form an iron-heme complex. The ability of NO to potently stimulate soluble guanylate cyclase, an iron-heme containing enzyme, is believed to be mediated by this latter mechanism. Figure 7 describes in its upper panel the time-dependent effects of IL-1 on nitrite production as a reflection of iNOS expression and, in its lower panel, PGE_2 formation as a reflection of COX-2 expression (5). As indicated in the upper panel of Figure 7, exposure of islets to IL-1 displays a 5-hr lag period before significant increases in nitrite, an indicator of NO, is observed. As expected, NMMA, a potent inhibitor of NOS, completely inhibits nitrite formation. In the lower panel of Figure 7, IL-1 also displays a 5-hr lag period before significant increases in PGE_2 formation are observed. Also, quite unexpectantly, NMMA blocks IL-1–induced increases in PGE_2 formation. Our interest was 2-fold: to explain these functional effects of IL-1 on time-dependent increases in nitrite and PGE_2, and to understand the ability of NMMA to prevent IL-1–induced PGE_2 formation by pancreatic islets. As illustrated in Figure 8, we wanted to determine if IL-1 co-expresses iNOS and COX-2,

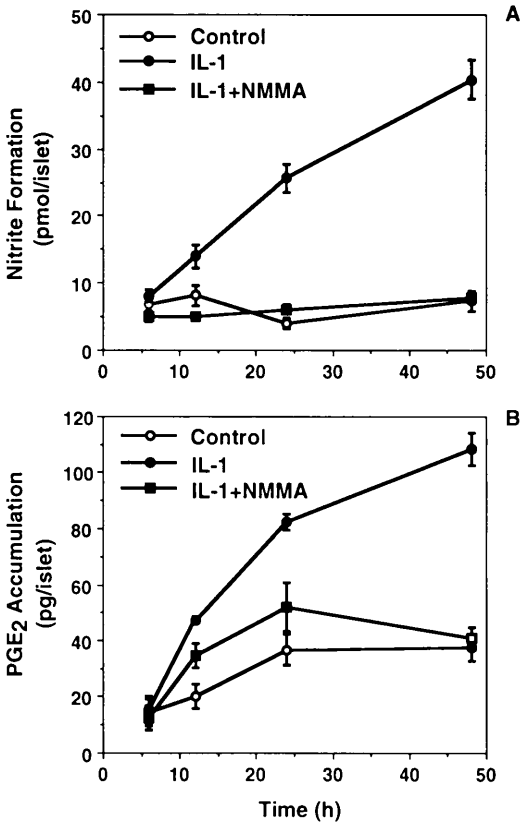


Figure 7. IL-1β induction of time-dependent production of both PGE_2 and nitrite by rat islets. Isolated islets were cultured for the indicated times in complete CMRL-1066 in the presence or absence of 5 U/ml IL-1β or IL-1β + 0.5 m*M* NMMA. At the indicated times, the medium was removed and both nitrite (A) and PGE_2 production (B) were determined. Results are the average ± SEM of two or five individual experiments containing two replicates per condition for both PGE_2 and nitrite, respectively. (From Ref. 5, with permission.)

and if this would explain the parallel increases in nitrite and PGE_2 formation. Also, we wanted to determine if by a completely separate mechanism whether NO directly activates COX-2 enzyme activity that would explain the ability of NMMA to inhibit increases in PGE_2 formation. Our studies have demonstrated that IL-1 induces the co-expression of iNOS

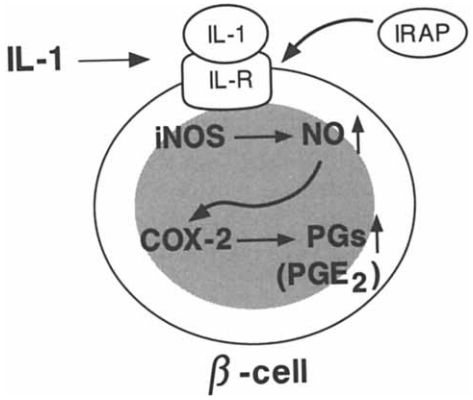


Figure 8. IL-1 induces coexpression of iNOS and cyclooxygenase (COX-2) and nitric oxide activates COX-2 activity.

and COX-2 from rat islets by immunoprecipitation of iNOS and COX-1 protein, which explains the similar time dependency for the accumulation of both nitrite and PGE_2 following exposure of islets to IL-1 (5). Furthermore, NO stimulates formation of PGE_2 by direct activation of both COX-1 and COX-2 enzyme activities, which also explains the ability of NO inhibitors such as NMMA as described in the lower panel of Figure 7 to prevent IL-1–induced accumulation of PGE_2. Salvemini *et al.* (38) also made the observation that NO directly activates both COX-1 and COX-2 in fetal fibroblasts and murine macrophage RAW 264.7 cells, respectively. These results indicated that under conditions where both NOS and COX are present, NO mediates increases in the production of proinflammatory prostaglandins that may result in an exacerbated inflammatory response. Salvemini *et al.* have also shown that endogenous NO enhances prostaglandin production in a model of renal inflammation, further indicating that COX isozymes are receptor targets for NO (39). Rettori *et al.* (40) have also reported that NMMA inhibits norepinephrine stimulated PGE_2 production from hypothalamic fragments, and more recently this same group has proposed a role for NO in eicosanoid synthesis and uterine motility in estrogen-treated rat uteri (41).

Significance

A proposed role for iNOS and COX-2 in autoimmune diabetes is described in Figure 9. Autoimmune diabetes is characterized by an early insulitis or inflammation that is followed by the selective destruction of the pancreatic β cell. Our studies indicate that cytokines released during this islet insulitis from T lymphocytes and activated macrophages can induce iNOS expression by β cells that results in the overproduction of NO that may play an important role, in part, in the selective destruction of the β cell. Our studies also indicate that cytokines can co-express COX-2, the rate-limiting enzyme responsible for the overproduction of the prostaglandins. Also by a separate mechanism, NO produced by iNOS can directly activate COX-2 enzyme activity that further augments the production of these proinflammatory prostaglandins that may be important in either initiating or maintaining the inflammatory response that is a hallmark of autoimmune diabetes. These findings are important in attempting to design strategies of intervention in animal models of chronic and immunologically mediated inflammation since they indicate that both cytokine inducible isoforms of iNOS and COX-2 may significantly contribute to overproduction of these proinflammatory mediators. An evaluation of the roles of these cytokine-induced proinflammatory mediators in animal models of diabetes will require the use of selective inhibitors of iNOS and COX-2.

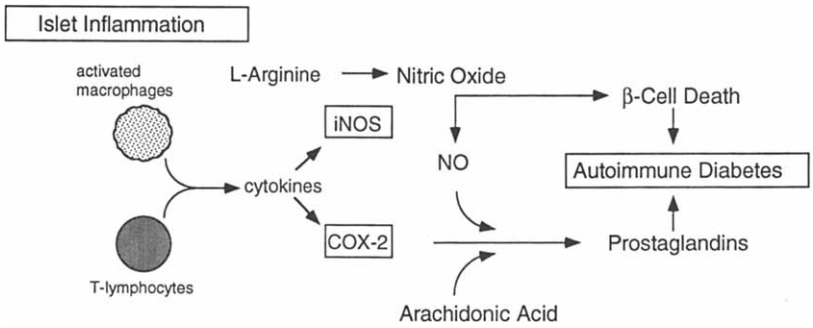


Figure 9. Proposed role for iNOS and COX-2 in autoimmune diabetes.

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