Antioxidants, NFκB Activation, and Diabetogenesis (44445)

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Abstract. **Although many risk factors can trigger the development of insulin-dependent diabetes (IDDM), it is likely that reactive oxygen species (ROS) play a central role in β-cell death and disease progression. This review will focus on the role of antioxidant defense systems in the susceptibility to IDDM and on ROS as cellular messengers that regulate the expression of genes leading to β-cell death. Accumulating evidence indicates that increased antioxidant defense systems reduce the susceptibility to IDDM in animal models or in human study. It is suggested that pancreas-specific ROS productions play a critical role in signaling the cellular autoimmune/inflammatory response by activating the transcription factor, NFκB. Various diabetogenic factors may lead to an increase in ROS production, which activates the redox-sensitive NFκB. This may be the initial event for the expression of cytokines and chemotactic agents involved in the autoimmune/inflammatory response. It is believed that this cascade results in a cyclic amplification of ROS and eventually leads to apoptosis and/or necrosis of β cells. The specificity of antioxidants to inhibit NFκB activation and the hyperglycemic response emphasizes the importance of selectivity in antioxidant therapy. Research in this area will contribute significantly to our understanding of the cellular and mechanistic role of ROS in the etiology of IDDM and will lead to the development of better prevention strategies.** [P.S.E.B.M. 1999, Vol 222]

Type I diabetes or insulin-dependent diabetes mellitus (IDDM) is a complex, multifactorial disease involving severe destruction of the insulin-producing β cells. Occurring predominantly in young children, IDDM has an acute onset and often progresses to numerous secondary health complications. Multiple risk factors such as genetics, environmental stresses, viral infection, and diet can predispose an individual to IDDM. Regardless of the triggering factors, the disease is always characterized by a progressive destruction of the insulin-producing β cells in the pancreas. Although the sequence of events at the cellular level is still unknown, a growing body of research suggests that reactive oxygen species (ROS) are involved in β cell destruction (1–3). The finding that ROS production is associated with the same risk factors that cause susceptibility to IDDM increases the likelihood that ROS play an important role in the pathogenesis of IDDM.

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0037-9727/99/2223-0205$14.00/0

The link between multiple trigger factors for IDDM and the production of ROS is illustrated as a unified pathway in our proposed model (Fig. 1). Our model allows us to predict that certain risk factors will result in increased ROS production, which in turn will lead to the destruction of β cells. Involvement of free radicals in the etiology of IDDM and in the pathophysiology of Type 2 diabetes has been extensively reviewed by others (4–11). This review will focus on the role of antioxidant defense systems in the susceptibility to IDDM and on ROS as cellular messengers that regulate the expression of genes leading to β cell death.

ROS and the Development of IDDM

Growing evidence points to the involvement of ROS in the pathogenesis of IDDM. As illustrated in Figure 1, several known diabetogenic factors can be linked to ROS generation. For example, although IDDM has traditionally been described as an autoimmune disorder, the autoimmune response may be mediated by ROS. Insulitis, a prediabetic stage in which islets are infiltrated with immune cells, is accompanied by the release of cytotoxic ROS, such as superoxide ($O_2\cdot^-$) and hydrogen peroxide (H_2O_2), and reactive nitrogen species (RNS), such as nitric oxide (NO•) (12). It is known that certain viruses are associated with the onset of IDDM (13, 14). Viral infections, either through general in-

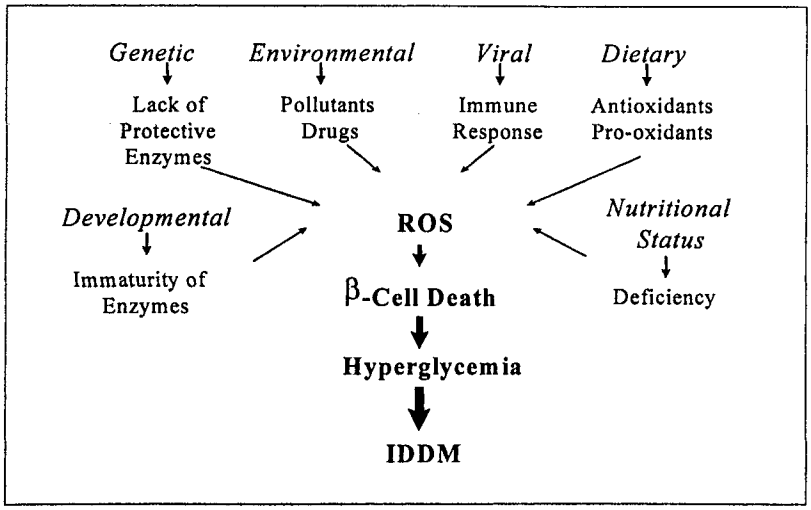

Figure 1. Linking various risk factors to ROS generation in the development of IDDM. ROS, reactive oxygen species; IDDM, insulin-dependent diabetes mellitus.

flammation or direct damage to the β cells, also initiate an immune response that culminates in the release of ROS. In addition to immunologic factors, accidental chemical induction of diabetes, particularly *via* N-nitroso compounds, is well documented in humans (15). Many investigators have demonstrated that poor nutritional status and diets high in nitrites and nitrates are correlated with an increased incidence of IDDM (5, 16, 17). Poor nutritional status produces an environment in which antioxidant defenses are often low resulting in a higher susceptibility to oxidative damage. In all these cases, the link between ROS and pathogenesis of IDDM is apparent; however, it is still unclear if ROS production directly causes β-cell death or is simply the consequence of the disease progression.

Antioxidant Status in IDDM Patients

Patients with IDDM appear to have significant defects in antioxidant protection compared to healthy, nondiabetic controls. Several studies have demonstrated a significant reduction in total antioxidant status in both plasma and serum samples from IDDM patients compared to age-matched controls (18). Diabetic children have also shown significant decreases in erythrocyte glutathione peroxidase (GPx), total glutathione, plasma α-tocopherol, and plasma β-carotene (19). This reduction in antioxidant activity is coupled with significant increases in lipid hydroperoxides, conjugated dienes, and protein carbonyls, which are markers for oxidative stress (20). This suggests that low antioxidant defenses predispose IDDM patients to enhanced oxidative stress. More recent studies have established that in the prediabetic condition, antioxidant status appears to be compromised (21). Islet cell antibodies (ICA) serve as a serological marker for risk for IDDM and have been used in population studies for the prediction of IDDM. Total plasma antioxidant status was assessed in both ICA-positive and ICA-negative, first-degree relatives of patients with IDDM. Antioxidant status was significantly lower in ICA-positive subjects compared to ICA-negative relatives and healthy unrelated subjects. Hence, disturbances in antioxidant defenses appear even in the prediabetic state. These observations suggest that antioxidant status is another contributing risk factor in the development of IDDM.

In the past, the proposed genetic component of IDDM has focused on diabetes-susceptible HLA-haplotypes found in the major histocompatibility complex (MHC) (22, 23). Individuals who possess these susceptible alleles are more predisposed to developing the autoimmune response resulting in IDDM. However, not all diabetics possess a susceptible haplotype, and not all individuals with this haplotype go on to develop the disease (24). In fact, twin studies have only shown 30% concordance in developing diabetes (25). This suggests that both environmental and genetic factors play a role in the development of IDDM. If ROS contribute to β-cell death and dysfunction, individuals that possess relatively lower endogenous levels of key antioxidant enzymes may be more susceptible to developing IDDM.

ROS Production in Animal Models of IDDM

In animal models of IDDM, the involvement of free radicals in the development of the disease has been clearly demonstrated. For example, alloxan, a derivative of uric acid, is a known diabetogenic agent and has been used for over 50 years to induce diabetes in laboratory animals (26). Alloxan-induced diabetes was the first described chemical model for the disease, yet still little is known about its precise cytotoxic mechanism or its selectivity for the pancreatic β cells. Research has suggested that alloxan-induced β-cell damage is mediated through the generation of ROS (27, 28). During the metabolism of alloxan, it is reduced to dialuric acid. ROS species are produced during alloxan metabolism *via* auto-oxidation of dialuric acid back to alloxan. The production of $O_2\cdot$ can lead to the production of other ROS such as H_2O_2 and hydroxyl radical (·OH) *via* Fenton reactions (29).

Another commonly used diabetogenic agent is the N-nitroso compound, streptozotocin (STZ). Several lines of research suggest that NO• production contributes to the cytotoxicity of STZ to the pancreatic β cells. Biochemical evidence for nitric oxide formation in pancreatic islets has also been demonstrated (6, 30, 31). An increase in nitrite, a stable end product of NO•, can be detected in islets treated with STZ. In STZ-induced diabetes, the incidence of hyperglycemia is decreased when NO synthase (NOS) inhibitors are used (32). In addition, transgenic mice deficient in the inducible isoform of NOS (iNOS) have also demonstrated a reduced sensitivity to STZ-induced diabetes (33), suggesting that NO• is involved in the mechanism of the disease.

In diabetes-prone animals such as the nonobese diabetes (NOD) mouse and the Biobreeding (BB) rat, islet cell infiltration of immune and inflammatory cells occurs prior to β-cell death. Both of these strains are thought to mimic the autoimmune destruction of β cells seen in the human disease. Destruction of the insulin-producing β cells may result from direct exposure to free radicals produced by the immune cells (34). In addition, free radicals may be produced due to production of pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and γ-interferon (IFN-γ), which can be cytotoxic to cells through mechanisms involving free radical production (12, 35).

Antioxidant Defense and Protection Against-Diabetogenesis. ROS and RNS scavengers have been shown to prevent β-cell death induced by diabetogenic drugs such as alloxan and STZ and prevent β-cell death induced by pro-inflammatory cytokines. *In vitro* addition of scavenging agents such as SOD, catalase, hydroxyl radical scavengers, and metal chelators prior to alloxan (36–38), STZ (39–42), and cytokine exposure (43) has prevented β-cell death in isolated islets. Our lab has demonstrated *in vivo*, that overexpression of CuZnSOD in transgenic animals provides some protection against the development of alloxan- and STZ-induced diabetes (44, 45). Transgenic strains that either ubiquitously overexpress CuZnSOD (TgHS mice) or contain β cell–specific CuZnSOD overexpression (RIPSOD mice) have significantly lower fasting blood glucose levels following injection of alloxan or STZ compared with nontransgenic control mice. Supplementation with antioxidants such as N-acetylcysteine (NAC) and α-phenyl-t-butylnitrone (PBN) also protect against the development of experimentally induced diabetes. Dietary supplementation of NAC, a glutathione precursor, effectively attenuated the hyperglycemic response and weight loss associated with alloxan-induced diabetes (46). Co-injection of PBN with multiple STZ injections similarly prevented β-cell death and attenuated hyperglycemia and weight loss in STZ-induced diabetes (47). Similarly, antioxidant supplementation is also beneficial in reducing spontaneous β-cell loss and hyperglycemia in NOD mice and BB rats. A variety of antioxidants including metal chelators, nicotinamide, SOD, and α-tocopherol have been shown to give some protection against the development of IDDM in those diabetes-prone animals (48–54).

Pancreatic Islets Are Highly Susceptible to Oxidative Stress

Several key enzymes in ROS defense are unusually low in pancreatic islets compared with other tissues, suggesting that the islet cells are uniquely susceptible to oxidative stress–induced damage. Gene expression and activity of several key antioxidant enzymes such as CuZnSOD, MnSOD, GPx, and catalase are all markedly decreased compared with other tissues such as liver (55). Studies found that the gene expression levels of the cytoplasmic CuZnSOD and the mitochondrial MnSOD in the pancreatic islet cells were 30%–40% of levels found in liver. GPx expression was 15%, and catalase gene expression was not detectable in pancreatic islets (56). Corresponding protein and activity levels were also found to be markedly lower in the islet cells. The low levels of antioxidant enzyme gene expression may account for the exquisite sensitivity of the β cells to ROS and free radical–induced damage leading to β-cell death and IDDM. For example, the uptake of alloxan by both liver and pancreatic islets has been observed (57). However, cytotoxicity of alloxan is apparent only to pancreatic islets. Research suggests that most chemical-induced β-cell damage is mediated through the generation of ROS (2, 58, 59). If increased ROS production is a causative factor in the destruction of the β cells, why is generalized oxidative stress solely detrimental to the pancreatic islet cells and not other tissues? Some of this specificity may be attributed to the low antioxidant capacity of the pancreatic islet cells.

ROS as Signaling Molecules

What is the mechanism that causes ROS to destroy these cells? The missing piece in the pathway lies in the connection between ROS production and β-cell death. Whereas higher concentrations of ROS may result in massive, indiscriminate oxidative damage, it is possible that low concentrations of ROS are sufficient to activate specific genes and cause the inappropriate activation of autoimmune or inflammatory responses leading to β-cell death. We will present some evidence that production of ROS can activate the transcription factor NFκB. ROS-mediated activation of NFκB may be the key modulator in the pathway that begins with the triggering of ROS production by multiple factors and leads to the ultimate destruction of β cells and the development of IDDM.

The majority of the research has concentrated on how ROS production causes direct cellular damage by oxidizing nucleic acids, protein, and membrane lipids to induce the disease. However, increasing evidence shows that ROS also play roles as physiologically important cellular messengers. ROS appear to play a critical role in regulating the expression of genes that encode for proteins involved in inflammation, immune response, and cell death (60, 61). Excess ROS production may be involved in disease progression

through inappropriate activation of genes involved in cellular defenses, excess NO• production, and apoptosis. These observations have launched a revolution in the understanding of the role of ROS in many human inflammatory and autoimmune diseases. Although IDDM is largely considered to be an autoimmune disease, this link between ROS and cellular response is a relatively unexplored area of research, leaving a large gap in the understanding of the mechanisms that signal β-cell death.

NFκB, a Transcription Factor Sensitive to Oxidative Stress

ROS are produced during normal cellular metabolism. However, under certain stresses, an increase in the production of oxygen free radicals may overwhelm our antioxidant defenses, resulting in oxidative stress. Several researchers have now discovered that there are several "redox sensitive" biological molecules important in cell signaling that are sensitive to low concentrations of ROS. One potential target of ROS activation is the nuclear transcription factor, NFκB.

NFκB was first discovered by Baltimore and Sen as B-cell specific nuclear protein that bound to a site in the immunoglobulin κ light chain gene enhancer (62). Since then, NFκB has gained widespread attention in many fields of research. It is now known that NFκB is present in many cell types and controls the expression of numerous gene products. NFκB appears to play a central role in regulating immune and inflammatory responses (63). For example, many inflammatory response factors such as pro-inflammatory cytokines, chemokines, adhesion molecules, colony-stimulating factors and inflammatory enzymes (Table I) are products of genes regulated by NFκB. Thus, dysregulation or aberrant activation of NFκB could initiate inappropriate autoimmune and inflammatory responses.

Table I. Genes Regulated by NFκB

Class	Target genes
Cytokines	Tumor necrosis factor-α (TNF-α)
	TNF-β
	Interferon-β (IFN-β)
	Interleukin-1β (IL-β)
	IL-2
	IL-6
	IL-12
Chemokines	IL-8
	Gro α,β,γ
	RANTES
	Macrophage chemotactic protein-1 (MCP-1)
Adhesion molecules	ICAM-1
	E-selectin
	V-CAM
Colony stimulating factors	Granulocyte-macrophage colony stimulating factor (GM-CSF)
	Granulocyte colony stimulating factor (G-CSF)
	Macrophage colony stimulating factor (M-CSF)
Immunoreceptors	Ig κ light chain
	Major histocompatibility complex (MHC) class I
	T-cell receptor β chain
Inflammatory enzymes	Inducible nitric oxide synthase (iNOS)
	Cyclooxygenase-2 (COX-2)
	12-Lipoxygenase

Note. Adapted table, see references (101, 103).

Activation of NFκB, Inflammatory Response, and β-Cell Death

NFκB is usually stored in the cytosol in its inactive form bound to the inhibitory unit IκBα, which prevents DNA binding and nuclear uptake of the factor. Degradation of IκBα is critical for NFκB activation. Extracellular stimuli such as ROS signal the degradation and release of the inhibitory unit IκBα through a complex but rapid cascade of events resulting in a rapid translocation of active NFκB to the nucleus (Fig. 2) (64–66). NFκB-inducing agents will initiate the phosphorylation of IκBα on its N-terminal serine residues (Ser32 and Ser36) (67, 68). Currently, the kinase cascade that directly phosphorylates IκBα has not been clearly identified. Recently, NFκB-inducing kinase (NIK) and IκBα kinase (IKK) have been identified (69–72), but the precise pathway from inducing agent to phosphorylation of IκBα still remains unknown.

This phosphorylation of IκBα induces polyubiquination of IκBα at multiple sites, tagging the subunit for degradation by the 26S proteosome complex. The free NFκB unit is now able to translocate into the nucleus and bind to consensus DNA binding sites in target genes. In the nucleus, NFκB binds to DNA and modulates the expression of several genes, including the genes controlling inflammatory and autoimmune process (66, 73, 74). Unlike many other systems, NFκB is already present in the cytosol; thus, no new protein synthesis is required for its activation. This unique activation system allows NFκB to regulate immune and inflammatory processes in a rapid and very efficient manner.

NFκB activation is responsible for both the initiation and amplification of immune and inflammatory responses in the cell. An increase in NFκB activation is followed by an increase in the release of cytokines and other chemotactic factors involved in inflammation (75). NFκB also up-regulates the expression of adhesion molecules critical to leukocyte migration into target tissues (76). NFκB binding sites are also present in the HLA class 1 genes and other immunoreceptors involved in cell-mediated immune responses. At the same time, more ROS and other reactive species such as NO• are generated as a result of the infiltration of immune cells such as macrophages and leukocytes that produce ROS and NO• as a killing or defense mecha-

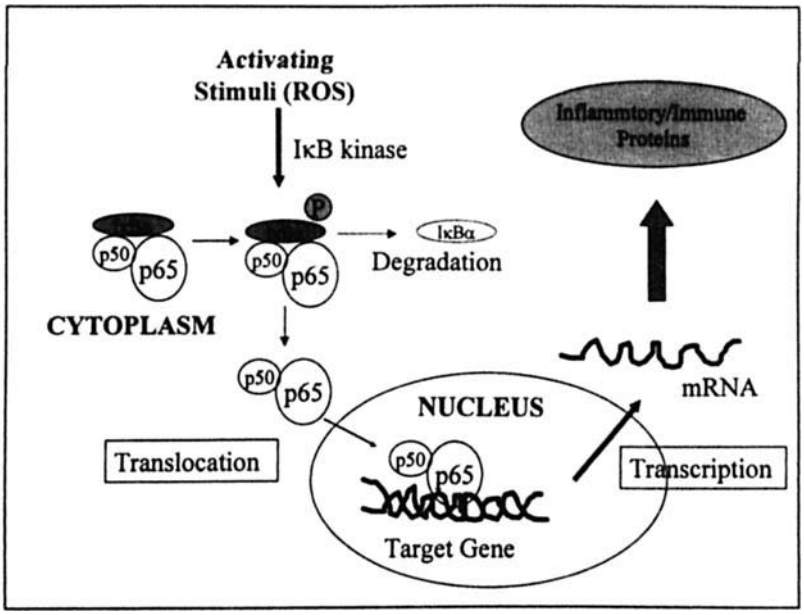

Figure 2. Schematic illustration of ROS-mediated NFκB activation. In response to extracellular inducers, such as ROS, IκB kinase (IKK) is activated and phosphorylates the IκBα subunit associated with the NFκB p50/p65 heterodimer. The phosphorylated IκBα then becomes ubiquinated. This results in subsequent degradation of the IκBα subunit by proteosomes. Degradation of IκBα releases the p50/p65 complex, allowing the complex to translocate into the nucleus where it binds to the κB-binding sites of gene promoters and induces their transcription. Many of the target genes regulated by NFκB include several immune and inflammatory factors.

nism (63). NFκB has also been shown to stimulate the production of NO• by activating the inducible form of NO synthase (iNOS) directly (75). Many of these inflammatory cytokines in turn also activate NFκB, thus amplifying ROS production and creating a vicious cycle. In the development of IDDM, it is possible that ROS-mediated NFκB activation in the pancreas is the central signal that initiates and propagates the inflammation and autoimmune processes responsible for β-cell death (Fig. 3). If this is true, agents that can inhibit this process should protect against the development of the disease.

Although IDDM is largely considered to be an autoimmune disease, this link between ROS and cellular response is a relatively unexplored area of research, leaving a large

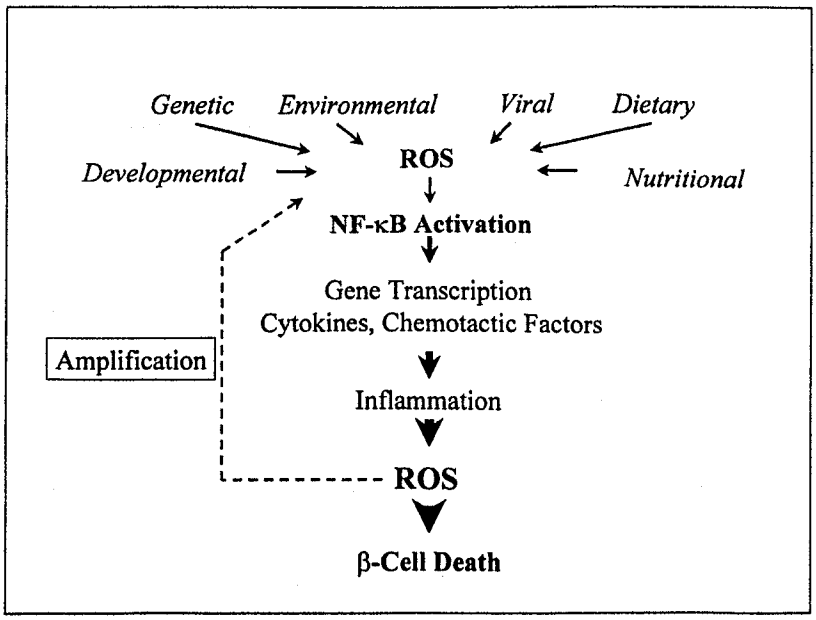

Figure 3. Initiation and amplification of the immune/inflammatory response by ROS-induced NFκB activation in β-cell death and IDDM. ROS production induced by diabetogenic factors cause the activation of NFκB. This in turn induces the transcription of autoimmune and inflammatory factors. Initiation of immune or inflammatory responses results in the production of more ROS and further activation of NFκB. This pathway acts as a positive loop, amplifying ROS production and the immune response, ultimately destroying the pancreatic β cells.

gap in the understanding of the mechanisms that signal β-cell death. More recently, NFκB activation has been detected in rat insulinoma cell lines following exposure to cytokines such as IL-1 (77, 78). These studies confirm the possible link between NFκB activation, inflammation, and iNOS production in a cell culture model system of diabetes.

Several lines of evidence suggest that NFκB is also a redox-sensitive transcription factor. Many different agents can activate NFκB, including phorbol esters, inflammatory cytokines, UV light, γ rays, viral and bacterial proteins, and lipopolysaccharide (75, 79). All of these agents produce oxidative stress. Thus, despite the diverse stimuli, ROS appear to serve as the common intracellular agents involved in the activation of NFκB (80). Secondly, direct exposure to oxidants such as H_2O_2 activates NFκB (81). Antioxidants have also been known to have the ability to inhibit NFκB activation, both *in vitro* and *in vivo*. *In vitro*, NFκB activation can be inhibited by addition of various antioxidants including lipoic acid (61), NAC (82), vitamin E derivatives (83), pyrrolidine dithiocarbamate (PTDC) (84), selenoproteins (85), and vitamin C (86). Antioxidant supplementation in animals has also been shown to inhibit NFκB activation *in vivo*. Administration of NAC can inhibit NFκB activation by systemic endotoxin treatment in the lung of rats (87). NAC has also been shown to inhibit NFκB activation by the diabetogenic drug alloxan in the pancreas of CD1 mice (46). The production of ROS appears to be the key factor in initiating NFκB activation. Thus, strategies to enhance antioxidant status may be beneficial in inhibiting inappropriate activation of NFκB and preventing any downstream pathological events.

We have demonstrated in our laboratory, that in alloxan-induced diabetes, there appears to be a specific activation of NFκB in the pancreas, not in the liver, within 30 min of alloxan injection. In addition, supplementation with NAC can inhibit NFκB activation in pancreas by alloxan and concurrently provide some protection against the development of the disease. NAC-supplemented animals have significantly reduced hyperglycemia and weight loss compared to unsupplemented controls. NAC supplementation also inhibited the expression of the inducible nitric oxide synthase (iNOS), a downstream inflammatory enzyme under regulation by NFκB. These results confirm that the role of ROS in the pathogenesis of IDDM may be far more complex than originally thought. The benefit of antioxidant therapy may not be simply to protect the β cells from oxidative damage as a result of ROS produced in the immune response. Instead antioxidant therapy may be used to stop the initiation and propagation of immune and inflammatory responses directly through inhibition of NFκB activation. If this is true, the efficacy of antioxidant therapy will depend on the ability of antioxidants to modulate cellular response pathways that have been activated inappropriately by excess ROS.

The Future of Antioxidant Therapy in IDDM

In vitro studies using defined chemical or enzymatic sources of NO• or ROS have demonstrated that islet cells are very susceptible to free radical–induced islet cell death (88–90). Furthermore, addition of antioxidants and NO• inhibitors protect against this damage (38, 91). Because this inhibition of β-cell death occurs *in vitro* with antioxidants and NO• inhibitors, we expect to see a protective effect *in vivo*. However, the translation of this phenomenon to *in vivo* studies is less clear. *In vivo* antioxidant intervention studies in both human and animal models have shown conflicting results (92). For example, in some studies, supplementation with NO synthase inhibitors such as N-nitro-L-arginine-methylester (NAME) has reduced the hyperglycemic response in STZ-induced diabetes (6, 93). However, other studies have shown no effect with inhibitors specific to the iNOS (94, 95). It is clear that excess production of free radical species (both ROS and RNS) is detrimental to the pancreatic islet cells. However, attempts to use antioxidant therapy in a clinical setting have shown questionable results. Currently, large scale, multinational, randomized, placebo-controlled intervention studies are underway to test the efficacy of nicotinamide to prevent IDDM. Generation of free radicals, DNA strand breaks, activation of polyADP-ribose polyermase (PARP), and depletion of NAD appear to be common events in β-cell death (96). Nicotinamide at high doses has shown to be a free radical scavenger, an inhibitor, and protector against depletion of intracellular NAD. Based on these functions, and promising *in vitro* and animal studies, nicotinamide appeared to be an excellent candidate for clinical studies in ICA-positive first-degree relatives and children at high risk for IDDM. So far, results indicate only a modest decrease or delay in diabetes development with nicotinamide (97, 98). In some cohorts, no benefit in disease progression was seen at all (99). It is possible that nicotinamide supplementation targets the repair of oxidative damage that occurs late in the diabetogenic process. A more effective therapy may need to inhibit oxidative signaling processes much earlier in the diabetogenic pathway to be effective.

An important research goal is to understand how the balance between ROS and antioxidants determines susceptibility to the disease. We hypothesize that this critical balance is defined by the ability of ROS to activate cellular responses, such as inflammation and other immune processes. Specifically, an imbalance between ROS and antioxidants (i.e., an excess of ROS or inadequacy of antioxidants) induces the activation of the transcription factor, NFκB. This activation results in an increase in inflammatory and immune responses and leads to an amplification of ROS and NO• production which, in turn, ultimately leads to the destruction of the β cells, hyperglycemia, and the development of IDDM.

Currently, inhibition of NFκB activation to diminish the expression of pro-inflammatory and immune response

genes has been discussed as a therapeutic approach in several other immune or inflammatory-related diseases including inflammatory bowel disease, inflammatory response syndrome, septic shock, asthma, and rheumatoid arthritis (100, 101). Despite the obvious link of NFκB to regulation of immune responses and the link between immune dysregulation and the development of IDDM, the link between NFκB activation and diabetogenesis has still not been fully established. Strategies to limit inappropriate activation of NFκB may prove to be a very effective approach in preventing the disease. We have focused primarily on the role of antioxidants in preventing NFκB activation. Several other compounds also have the ability to inhibit NFκB activation. These include proteosome inhibitors, corticosteroids, and agents that can block NIK and IKK. However, use of these compounds may have limited therapeutic effect. Proteosome degradation is critical for normal protein turnover and regulates normal cellular function and cell cycling. Thus, the use of proteosome inhibitors clinically may have serious side effects *in vivo*. Long-term glucocorticoid therapy also has limited benefit due to side effects associated with its influence on endocrine function and metabolism. Currently, agents that can block NIK and IKK selectively, and thus block IκBα degradation, have not been identified. Phosphorylation cascades are a widespread regulatory modification throughout cellular metabolism. So far, less specific kinase inhibitors have shown conflicting results.

The key to successful antioxidant therapy in IDDM will rely both on effective targeting to the islet cells and on dose. Studies investigating the effect of NAC in animal models of acute respiratory disease (ARDS) have found that NAC is beneficial in ARDS possibly through inhibition of NFκB activation. However, the effect of NAC only proved to be beneficial at lower supplementation levels, whereas at high doses lung injury was exacerbated (102). Thus, dose response relationships with antioxidant therapy will also need to be considered.

Summary

Although there is substantial evidence that free radicals are involved in the etiology of diabetes, the precise cellular mechanisms leading to β-cell death and the development of IDDM remains unknown. The understanding of the pathways leading to IDDM is essential before effective treatment and prevention strategies can be developed. Since IDDM is primarily a childhood disease, developing effective preventative strategies will produce considerable savings in terms of long-term health care costs, and immeasurable savings in the human anguish associated with this disease. Currently, there is little understanding of the cellular events leading to the development of diabetes. Activation of NFκB by acute oxidative stress may be the critical signal initiating the cascade of events leading to β-cell death and IDDM. Thus, understanding these ROS-induced signal pathways in the immune and inflammatory response becomes essential in finding preventive treatments. Antioxidant therapy that can target this activation of NFκB may prove to be an effective therapeutic tool in finding treatments to prevent IDDM.

1. Oberley LW. Free radicals and diabetes. Free Radic Biol Med **5**:113–124, 1988.
2. Rabinovitch A. Free radicals as mediators of pancreatic islet β-cell injury in autoimmune diabetes [editorial; comment]. J Lab Clin Med **119**:455–456, 1992.
3. Ludvigsson J. Intervention at diagnosis of type I diabetes using either antioxidants or photopheresis. Diabetes Metab Rev **9**:329–336, 1993.
4. Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension, and cardiovascular disease: Which role for oxidative stress? Metabolism **44**:363–368, 1995.
5. Virtanen SM, Aro A. Dietary factors in the aetiology of diabetes. Ann Med **26**:469–478, 1994.
6. Corbett JA, McDaniel ML. Does nitric oxide mediate autoimmune destruction of β cells? Possible therapeutic interventions in IDDM. Diabetes **41**:897–903, 1992.
7. Doly M, Droy-Lefaix MT, Braquet P. Oxidative stress in diabetic retina. EXS **62**:299–307, 1992.
8. Chisolm GM, Irwin KC, Penn MS. Lipoprotein oxidation and lipoprotein-induced cell injury in diabetes. Diabetes **41**(Suppl 2):S61–S66, 1992.
9. Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med **10**:339–352, 1991.
10. Nerup J, Mandrup-Poulsen T, Molvig J, Helqvist S, Wogensen L, Egeberg J. Mechanisms of pancreatic β-cell destruction in type I diabetes. Diabetes Care **11**(Suppl 1):S16–S23, 1988.
11. Gillery P, Monboisse JC, Maquart FX, Borel JP. Does oxygen free radical increased formation explain long-term complications of diabetes mellitus? Med Hypotheses **29**:47–50, 1989.
12. Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet β-cell destruction and insulin-dependent diabetes mellitus. Biochem Pharmacol **55**:1139–1149, 1998.
13. Foulis AK, McGill M, Farquharson MA, Hilton DA. A search for evidence of viral infection in pancreases of newly diagnosed patients with IDDM. Diabetologia **40**:53–61, 1997.
14. Taylor KW. Conference report: CIBA Foundation meeting on enteroviruses and early childhood diabetes. Diabet Med **13**:910–911, 1996.
15. Helgason T, Jonasson MR. Evidence for a food additive as a cause of ketosis-prone diabetes. Lancet **2**:716–720, 1981.
16. Dahlquist GG, Blom LG, Persson LA, Sandstrom AI, Wall SG. Dietary factors and the risk of developing insulin-dependent diabetes in childhood. BMJ **300**:1302–1306, 1990.
17. Scott FW, Marliss EB. Conference summary: Diet as an environmental factor in development of insulin-dependent diabetes mellitus. Can J Physiol Pharmacol **69**:311–319, 1991.
18. Maxwell SR, Thomason H, Sandler D, Leguen C, Baxter MA, Thorpe GH, Jones AF, Barnett AH. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. Eur J Clin Invest **27**:484–490, 1997.
19. Dominguez C, Ruiz E, Gussinye M, Carrascosa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. Diabetes Care **21**:1736–1742, 1998.
20. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G. Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. Diabetes **46**:1853–1858, 1997.
21. Rocic B, Vucic M, Knezevic-Cuca J, Radica A, Pavlic-Renar I, Profozic V, Metelko Z. Total plasma antioxidants in first-degree relatives of patients with insulin-dependent diabetes. Exp Clin Endocrinol Diabetes **105**:213–217, 1997.
22. Zonana J, Rimoin DL. Inheritance of diabetes mellitus. N Engl J Med **295**:603–605, 1976.
23. Adams DD, Adams YJ, Knight JG, McCall J, White P, Horrocks R, van Loghem E. A solution to the genetic and environmental puzzles of insulin-dependent diabetes mellitus. Lancet **1**:420–424, 1984.
24. Suciu-Foca N, Nicholson JF, Reemtsma K, Rubinstein P. The HLA

system and the genetics of juvenile diabetes mellitus. Diabetes Metab 3:193–198, 1977.
25. Barnett AH, Eff C, Leslie RD, Pyke DA. Diabetes in identical twins: A study of 200 pairs. Diabetologia 20:87–93, 1981.
26. Lazarow A. Protective effect of glutathione and cysteine against alloxan diabetes in the rat. Proc Soc Exp Biol Med 61:441–447, 1946.
27. Heikkila RE, Winston B, Cohen G. Alloxan-induced diabetes: Evidence for hydroxyl radical as a cytotoxic intermediate. Biochem Pharmacol 25:1085–1092, 1976.
28. Malaisse WJ. Alloxan toxicity to the pancreatic β-cell: A new hypothesis. Biochem Pharmacol 31:3527–3534, 1982.
29. Cohen G, Heikkila RE. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotoxic agents. J Biol Chem 249:2447–2452, 1974.
30. Fehsel K, Kroncke KD, Kolb-Bachofen V. The action of NO and its role in autoimmune diabetes mellitus. Res Immunol 146:711–715, 1995.
31. Kroncke KD, Fehsel K, Sommer A, Rodriguez ML, Kolb-Bachofen V. Nitric oxide generation during cellular metabolization of the diabetogenic N-methyl-N-nitroso-urea streptozotozin contributes to islet cell DNA damage. Biol Chem Hoppe Seyler 376:179–185, 1995.
32. Kolb H, Kiesel U, Kroncke KD, Kolb-Bachofen V. Suppression of low-dose streptozotocin induced diabetes in mice by administration of a nitric oxide synthase inhibitor. Life Sci 49:L213–L217, 1991.
33. Flodstrom M, Tyrberg B, Eizirik DL, Sandler S. Reduced sensitivity of inducible nitric oxide synthase-deficient mice to multiple low-dose streptozotocin-induced diabetes. Diabetes 48:706–713, 1999.
34. Nathan CF, Tsunawaki S. Secretion of toxic oxygen products by macrophages: Regulatory cytokines and their effects on the oxidase. Ciba Found Symp 118:211–230, 1986.
35. Rabinovitch A, Suarez-Pinzon WL, Strynadka K, Lakey JR, Rajotte RV. Human pancreatic islet β-cell destruction by cytokines involves oxygen free radicals and aldehyde production. J Clin Endocrinol Metab 81:3197–3202, 1996.
36. Fischer LJ, Hamburger SA. Inhibition of alloxan action in isolated pancreatic islets by superoxide dismutase, catalase, and a metal chelator. Diabetes 29:213–216, 1980.
37. Fischer LJ, Hamburger SA. Dimethylurea: A radical scavenger that protects isolated pancreatic islets from the effects of alloxan and dihydroxyfumarate exposure. Life Sci 26:1405–1409, 1980.
38. Gandy SE, Buse MG, Crouch RK. Protective role of superoxide dismutase against diabetogenic drugs. J Clin Invest 70:650–658, 1982.
39. Sandler S, Welsh M, Andersson A. Streptozotocin-induced impairment of islet β-cell metabolism and its prevention by a hydroxyl radical scavenger and inhibitors of poly(ADP-ribose) synthetase. Acta Pharmacol Toxicol (Copenh) 53:392–400, 1983.
40. Sandler S, Andersson A. The partial protective effect of the hydroxyl radical scavenger dimethyl urea on streptozotocin-induced diabetes in the mouse *in vivo* and *in vitro*. Diabetologia 23:374–378, 1982.
41. Mendola J, Wright JR Jr., Lacy PE. Oxygen free-radical scavengers and immune destruction of murine islets in allograft rejection and multiple low-dose streptozocin-induced insulitis. Diabetes 38:379–385, 1989.
42. Robbins MJ, Sharp RA, Slonim AE, Burr IM. Protection against streptozotocin-induced diabetes by superoxide dismutase. Diabetologia 18:55–58, 1980.
43. Sumoski W, Baquerizo H, Rabinovitch A. Oxygen free-radical scavengers protect rat islet cells from damage by cytokines. Diabetologia 32:792–796, 1989.
44. Kubisch HM, Wang J, Bray TM, Phillips JP. Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic β-cells against oxidative stress. Diabetes 46:1563–1566, 1997.
45. Kubisch HM, Wang J, Luche R, Carlson E, Bray TM, Epstein CJ, Phillips JP. Transgenic copper/zinc superoxide dismutase modulates susceptibility to type I diabetes. Proc Natl Acad Sci U S A 91:9956–9959, 1994.
46. Ho E, Chen G, Bray TM. Supplementation of N-acetylcysteine inhibits NFkB activation and protects against alloxan-induced diabetes in CD-1 mice. FASEB J 13:1845–1854, 1999.
47. Tabatabaie T, Kotake Y, Wallis G, Jacob JM, Floyd RA. Spin trapping agent phenyl N-tert-butylnitrone protects against the onset of drug-induced insulin-dependent diabetes mellitus. FEBS Lett 407:148–152, 1997.
48. Kim JY, Chi JK, Kim EJ, Park SY, Kim YW, Lee SK. Inhibition of diabetes in non-obese diabetic mice by nicotinamide treatment for 5 weeks at the early age. J Korean Med Sci 12:293–297, 1997.
49. Nomikos IN, Wang Y, Lafferty KJ. Involvement of O_2 radicals in autoimmune diabetes. Immunol Cell Biol 67:85–87, 1989.
50. Nomikos IN, Prowse SJ, Carotenuto P, Lafferty KJ. Combined treatment with nicotinamide and desferrioxamine prevents islet allograft destruction in NOD mice. Diabetes 35:1302–1304, 1986.
51. Behrens WA, Scott FW, Madere R, Trick K, Hanna K. Effect of dietary vitamin E on the vitamin E status in the BB rat during development and after the onset of diabetes. Ann Nutr Metab 30:157–165, 1986.
52. Hayward AR, Shriber M, Sokol R. Vitamin E supplementation reduces the incidence of diabetes but not insulitis in NOD mice [see comments]. J Lab Clin Med 119:503–507, 1992.
53. Rabinovitch A, Suarez WL, Power RF. Lazaroid antioxidant reduces incidence of diabetes and insulitis in nonobese diabetic mice. J Lab Clin Med 121:603–607, 1993.
54. Roza AM, Slakey DP, Pieper GM, Van Ye TM, Moore-Hilton G, Komorowski RA, Johnson CP, Hedlund BE, Adams MB. Hydroxyethyl starch deferoxamine, a novel iron chelator, delays diabetes in BB rats. J Lab Clin Med 123:556–560, 1994.
55. Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. Free Radic Biol Med 20:463–466, 1996.
56. Tiedge M, Lortz S, Drinkgern J, Lenzen S. Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes 46:1733–1742, 1997.
57. Gorus FK, Malaisse WJ, Pipeleers DG. Selective uptake of alloxan by pancreatic β-cells. Biochem J 208:513–515, 1982.
58. Malaisse WJ, Malaisse-Lagae F, Sener A, Pipeleers DG. Determinants of the selective toxicity of alloxan to the pancreatic β cell. Proc Natl Acad Sci U S A 79:927–930, 1982.
59. Ferner RE. Drug-induced diabetes. Baillieres Clin Endocrinol Metab 6:849–866, 1992.
60. Palmer HJ, Paulson KE. Reactive oxygen species and antioxidants in signal transduction and gene expression. Nutr Rev 55:353–361, 1997.
61. Sen CK, Packer L. Antioxidant and redox regulation of gene transcription [see comments]. FASEB J 10:709–720, 1996.
62. Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-κB by a post-translational mechanism. Cell 47:921–928, 1986.
63. Baeuerle PA, Henkel T. Function and activation of NFκB in the immune system. Annu Rev Immunol 12:141–179, 1994.
64. Baeuerle PA, Baltimore D. Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NFκB transcription factor. Cell 53:211–217, 1988.
65. Baeuerle PA, Baltimore D. IκB: A specific inhibitor of the NFκB transcription factor. Science 242:540–546, 1988.
66. Baldwin AS Jr. The NFκB and IκB proteins: New discoveries and insights. Annu Rev Immunol 14:649–683, 1996.
67. Brown K, Gerstberger S, Carlson L, Franzoso G, Siebenlist U. Control of IκB-α proteolysis by site-specific, signal-induced phosphorylation. Science 267:1485–1488, 1995.
68. Traenckner EB, Pahl HL, Henkel T, Schmidt KN, Wilk S, Baeuerle PA. Phosphorylation of human IκB-α on serines 32 and 36 controls IκB-α proteolysis and NFκB activation in response to diverse stimuli. EMBO J 14:2876–2883, 1995.
69. Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NFκB activation. Cell 91:243–252, 1997.
70. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. A cytokine-responsive IκB kinase that activates the transcription factor NFκB [see comments]. Nature 388:548–554, 1997.
71. Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IκB kinase-β: NFκB activation and complex formation with IκB kinase-α and NIK [see comments]. Science 278:866–869, 1997.
72. Regnier CH, Song HY, Gao X, Goeddel DV, Cao Z, Rothe M. Identification and characterization of an IκB kinase. Cell 90:373–383, 1997.

73. Nakamura H, Nakamura K, Yodoi J. Redox regulation of cellular activation. Annu Rev Immunol **15:**351–369, 1997.
74. Winyard PG, Blake DR. Antioxidants, redox-regulated transcription factors, and inflammation. Adv Pharmacol **38:**403–421, 1997.
75. Siebenlist U, Franzoso G, Brown K. Structure, regulation, and function of NFκB. Annu Rev Cell Biol **10:**405–455, 1994.
76. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T. Transcriptional regulation of endothelial cell adhesion molecules: NFκB and cytokine-inducible enhancers. FASEB J **9:**899–909, 1995.
77. Kwon G, Corbett JA, Rodi CP, Sullivan P, McDaniel ML. Interleukin-1 β-induced nitric oxide synthase expression by rat pancreatic β-cells: Evidence for the involvement of nuclear factor kappa B in the signaling mechanism. Endocrinology **136:**4790–4795, 1995.
78. Kwon G, Corbett JA, Hauser S, Hill JR, Turk J, McDaniel ML. Evidence for involvement of the proteasome complex (26S) and NFκB in IL-1β-induced nitric oxide and prostaglandin production by rat islets and RINm5F cells. Diabetes **47:**583–591, 1998.
79. Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: An oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic Res **17:**221–237, 1992.
80. Muller JM, Rupec RA, Baeuerle PA. Study of gene regulation by NFκB and AP-1 in response to reactive oxygen intermediates. Methods **11:**301–312, 1997.
81. Schreck R, Rieber P, Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NFκB transcription factor and HIV-1. EMBO J **10:**2247–2258, 1991.
82. Aillet F, Gougerot-Pocidalo MA, Virelizier JL, Israel N. Appraisal of potential therapeutic index of antioxidants on the basis of their *in vitro* effects on HIV replication in monocytes and interleukin 2–induced lymphocyte proliferation. AIDS Res Hum Retroviruses **10:**405–411, 1994.
83. Suzuki YJ, Packer L. Inhibition of NFκB activation by vitamin E derivatives. Biochem Biophys Res Commun **193:**277–283, 1993.
84. Offermann MK, Lin JC, Mar EC, Shaw R, Yang J, Medford RM. Antioxidant-sensitive regulation of inflammatory-response genes in Kaposi's sarcoma cells. J Acquir Immune Defic Syndr Hum Retrovirol **13:**1–11, 1996.
85. Makropoulos V, Bruning T, Schulze-Osthoff K. Selenium-mediated inhibition of transcription factor NFκB and HIV-1 LTR promoter activity. Arch Toxicol **70:**277–283, 1996.
86. Harakeh S, Jariwalla RJ. NFκB-independent suppression of HIV expression by ascorbic acid. AIDS Res Hum Retroviruses **13:**235–239, 1997.
87. Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW. *In vivo* antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J Immunol **157:**1630–1637, 1996.
88. Cominacini L, Garbin U, Lo Cascio V. The need for a "free radical initiative" [comment]. Diabetologia **39:**364–366, 1996.
89. Asayama K, Nyfeler F, English D, Pilkis SJ, Burr IM. Alloxan-induced free radical production in isolated cells: Selective effect on islet cells. Diabetes **33:**1008–1011, 1984.
90. Rabinovitch A, Suarez WL, Thomas PD, Strynadka K, Simpson I. Cytotoxic effects of cytokines on rat islets: Evidence for involvement of free radicals and lipid peroxidation. Diabetologia **35:**409–413, 1992.
91. McDaniel ML, Kwon G, Hill JR, Marshall CA, Corbett JA. Cytokines and nitric oxide in islet inflammation and diabetes. Proc Soc Exp Biol Med **211:**24–32, 1996.
92. Heller B, Burkart V, Lampeter E, Kolb H. Antioxidant therapy for the prevention of type I diabetes. Adv Pharmacol **38:**629–638, 1997.
93. Wu G. Nitric oxide synthesis and the effect of aminoguanidine and NG-monomethyl-L-arginine on the onset of diabetes in the spontaneously diabetic BB rat. Diabetes **44:**360–364, 1995.
94. Holstad M, Jansson L, Sandler S. Inhibition of nitric oxide formation by aminoguanidine: An attempt to prevent insulin-dependent diabetes mellitus. Gen Pharmacol **29:**697–700, 1997.
95. Hammes HP, Ali SS, Uhlmann M, Weiss A, Federlin K, Geisen K, Brownlee M. Aminoguanidine does not inhibit the initial phase of experimental diabetic retinopathy in rats. Diabetologia **38:**269–273, 1995.
96. Kolb H, Burkart V. Nicotinamide in type 1 diabetes: Mechanism of action revisited. Diabetes Care **22**(Suppl 2):B16–B20, 1999.
97. Pozzilli P, Visalli N, Cavallo MG, Signore A, Baroni MG, Buzzetti R, Fioriti E, Mesturino C, Fiori R, Romiti A, Giovannini C, Lucentini L, Matteoli MC, Crino A, Teodonio C, Paci F, Amoretti R, Pisano L, Suraci C, Multari G, Suppa M, Sulli N, De Mattia G, Faldetta MR, Suraci MT. Vitamin E and nicotinamide have similar effects in maintaining residual β-cell function in recent onset insulin-dependent diabetes (the IMDIAB IV study) [published erratum appears in Eur J Endocrinol **137:**558, 1997] Eur J Endocrinol **137:**234–239, 1997.
98. Pozzilli P, Visalli N, Signore A, Baroni MG, Buzzetti R, Cavallo MG, Boccuni ML, Fava D, Gragnoli C, Andreani D et al. Double-blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). Diabetologia **38:**848–852, 1995.
99. Lampeter EF, Klinghammer A, Scherbaum WA, Heinze E, Haastert B, Giani G, Kolb H. The Deutsche Nicotinamide Intervention Study: An attempt to prevent type 1 diabetes. DENIS Group. Diabetes **47:**980–984, 1998.
100. Christman JW, Lancaster LH, Blackwell TS. Nuclear factor kappa B: A pivotal role in the systemic inflammatory response syndrome and new target for therapy [see comments]. Intensive Care Med **24:**1131–1138, 1998.
101. Chen F, Castranova V, Shi X, Demers LM. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. Clin Chem **45:**7–17, 1999.
102. Sprong RC, Winkelhuyzen-Janssen AM, Aarsman CJ, van Oirschot JF, van der Bruggen T, van Asbeck BS. Low-dose N-acetylcysteine protects rats against endotoxin-mediated oxidative stress, but high-dose increases mortality. Am J Respir Crit Care Med **157:**1283–1293, 1998.
103. Lee JI, Burckart GJ. Nuclear factor kappa B: Important transcription factor and therapeutic target. J Clin Pharmacol **38:**981–993, 1998.