

# Double-Stranded RNA-Dependent Protein Kinase Is Not Required for Double-Stranded RNA-Induced Nitric Oxide Synthase Expression or Nuclear Factor- $\kappa$ B Activation by Islets

Libby A. Blair, Monique R. Heitmeier, Anna L. Scarim, Leonard B. Maggi, Jr., and John A. Corbett

Environmental factors, such as viral infection, have been implicated in the destruction of  $\beta$ -cells during the development of autoimmune diabetes. Double-stranded RNA (dsRNA), produced during viral replication, is an active component of a viral infection that stimulates antiviral responses in infected cells. Previous studies have shown that treatment of rat islets with dsRNA in combination with  $\gamma$ -interferon (IFN- $\gamma$ ) results in a nitric oxide-dependent inhibition of glucose-stimulated insulin secretion. This study examines the role of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and the dsRNA-dependent protein kinase (PKR) in dsRNA + IFN- $\gamma$ -induced nitric oxide synthase (iNOS) expression and nitric oxide production by rat, mouse, and human islets. Treatment of rat and human islets with dsRNA in the form of polyinosinic-polycytidylic acid (poly IC) and IFN- $\gamma$  resulted in iNOS expression and nitric oxide production. Inhibitors of NF- $\kappa$ B activation—the proteasome inhibitor MG-132 and the antioxidant pyrrolidinedithiocarbamate (PDTC)—prevented poly IC + IFN- $\gamma$ -induced iNOS expression and nitric oxide production. Incubation of rat islets for 3 h or human islets for 2 h with poly IC alone or poly IC + IFN- $\gamma$  resulted in NF- $\kappa$ B nuclear translocation and degradation of the NF- $\kappa$ B inhibitor protein, I $\kappa$ B, events that are prevented by MG-132. PKR has been shown to participate in dsRNA-induced NF- $\kappa$ B activation in a number of cell types, including mouse embryonic fibroblasts. However, poly IC stimulated NF- $\kappa$ B nuclear translocation and I $\kappa$ B degradation to similar levels in islets isolated from mice devoid of PKR (PKR<sup>-/-</sup>) and wild-type mice (PKR<sup>+/+</sup>). Furthermore, the genetic absence of PKR did not affect dsRNA + IFN- $\gamma$ -induced iNOS expression, nitric oxide production, or the inhibitory actions of these agents on glucose-stimulated insulin secretion.

From the Edward A. Doisy Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to Dr. John A. Corbett, St. Louis University School of Medicine, Department of Biochemistry and Molecular Biology, 1402 South Grand Blvd., St. Louis, MO 63104. E-mail: corbettj@slu.edu.

Received for publication 25 January 2000 and accepted in revised form 4 October 2000.

AG, aminoguanidine; dsRNA, double-stranded RNA; HRP, horseradish peroxidase; IFN, interferon; I $\kappa$ B, NF- $\kappa$ B inhibitor protein; IKK, I $\kappa$ B kinase; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; JNK, c-Jun NH<sub>2</sub>-terminal kinase; KRBB, Krebs-Ringer bicarbonate buffer; MAPK, mitogen-activated protein kinase; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NOS-2, nitric oxide synthase-2; PDTC, pyrrolidinedithiocarbamate; PKR, dsRNA-dependent protein kinase; poly IC, polyinosinic-polycytidylic acid; RT-PCR, reverse transcriptase-polymerase chain reaction.

These results suggest that 1) NF- $\kappa$ B activation is required for dsRNA + IFN- $\gamma$ -induced iNOS expression, 2) PKR is not required for either dsRNA-induced NF- $\kappa$ B activation or dsRNA + IFN- $\gamma$ -induced iNOS expression by islets, and 3) PKR is not required for dsRNA + IFN- $\gamma$ -induced inhibition of glucose-stimulated insulin secretion by islets. *Diabetes* 50:283–290, 2001

**A**utoimmune diabetes is characterized by a local inflammatory reaction in and around the pancreatic islets, followed by selective destruction of insulin-producing  $\beta$ -cells (1). Viral infection has been implicated as one environmental factor that may trigger the initial autoimmune reaction that targets and destroys  $\beta$ -cells in genetically susceptible individuals (2–5). Viruses have been isolated from the pancreata of acutely diabetic deceased patients, and viral-specific IgM responses have been identified in newly diagnosed diabetic patients (2,5). Autoimmune diabetes can also be induced in genetically susceptible strains of rats and mice by infection with Kilham rat virus (6) and encephalomyocarditis virus (7), respectively. In these animal models, viral-induced diabetes is associated with increased cytokine expression and nitric oxide production (6,7). Although this evidence supports a role for viral infection in the development of diabetes, the mechanisms by which viruses initiate  $\beta$ -cell damage have been difficult to determine because multiple viruses from both RNA and DNA viral families have been implicated in this disease. One common feature of a viral infection is the formation of double-stranded RNA (dsRNA), which accumulates during replication. dsRNA is an active component of a viral infection that stimulates host antiviral responses (8). The synthetic dsRNA molecule polyinosinic-polycytidylic acid (poly IC) also activates the antiviral response (8), and has been shown to stimulate the development of diabetes in diabetes-resistant BB rats and accelerate disease development in diabetes-prone BB rats (9,10).

One cellular target activated in response to dsRNA is the transcriptional regulator nuclear factor- $\kappa$ B (NF- $\kappa$ B) (11). dsRNA has been shown to stimulate NF- $\kappa$ B nuclear translocation in endothelial cells (12) and murine macrophages (13). NF- $\kappa$ B is a heterodimer comprised of p50 and p65 (RelA) subunits, and is found sequestered in the cytoplasm of unstimulated cells as an inactive complex with the NF- $\kappa$ B inhibitory protein (I $\kappa$ B). Upon stimulation, I $\kappa$ B is phosphorylated and

degraded in a ubiquitin-dependent manner. Free of I $\kappa$ B, the NF- $\kappa$ B heterodimer translocates to the nucleus and activates mRNA transcription (14). NF- $\kappa$ B is activated by several cytokines, including interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (15), and appears to play a primary role in the regulation of inducible nitric oxide synthase (iNOS) gene expression (16–18). NF- $\kappa$ B activation is required for IL-1 $\beta$  and IL-1 $\beta$  + IFN- $\gamma$ -induced iNOS expression by rat and human islets, respectively (19,20).

The dsRNA-dependent protein kinase (PKR) is an important regulator of the antiviral response. NF- $\kappa$ B activation in response to dsRNA appears to be dependent on functional PKR. dsRNA-induced NF- $\kappa$ B activation is attenuated in mouse embryonic fibroblasts isolated from PKR-deficient mice (21,22). PKR has been shown to phosphorylate I $\kappa$ B in vitro (11), although recent evidence suggests that PKR may mediate dsRNA-induced NF- $\kappa$ B nuclear localization by activating the I $\kappa$ B kinase (IKK) (23,24). PKR is a 65–68 kDa serine/threonine kinase whose expression is induced by interferons (IFNs) (25). Binding to dsRNA, an event that leads to dimerization and autophosphorylation, activates PKR (26). Once activated, PKR participates in the antiviral response by inhibiting translation through phosphorylation of eukaryotic initiation factor-2 $\alpha$  (25). However, PKR is also thought to participate in other cellular and antiviral responses, such as transcription factor activation, cell cycle control, and apoptosis (21,27,28).

In this study, we examined the roles of NF- $\kappa$ B and PKR in dsRNA + IFN- $\gamma$ -induced iNOS expression and nitric oxide production by rat, mouse, and human islets. We show that dsRNA (in the form of poly IC) stimulates the activation of NF- $\kappa$ B in rat and human islets. Pyrrolidinedithiocarbamate (PDTc) and MG-132, inhibitors of NF- $\kappa$ B, prevent dsRNA and dsRNA + IFN- $\gamma$ -induced NF- $\kappa$ B nuclear localization and I $\kappa$ B degradation as well as dsRNA + IFN- $\gamma$ -induced iNOS expression and nitric oxide production by islets. Furthermore, the genetic absence of PKR does not adversely affect dsRNA-induced NF- $\kappa$ B activation or dsRNA + IFN- $\gamma$ -induced iNOS expression by mouse islets. These findings suggest that 1) NF- $\kappa$ B activation is required for dsRNA + IFN- $\gamma$ -induced iNOS expression by islets, and 2) dsRNA-induced NF- $\kappa$ B activation and dsRNA + IFN- $\gamma$ -induced iNOS expression by islets occur by PKR-independent mechanisms.

## RESEARCH DESIGN AND METHODS

**Materials and animals.** CMRL-1066 tissue culture medium, L-glutamine, penicillin, streptomycin, and rat recombinant IFN- $\gamma$  were obtained from Gibco BRL-Life Technologies (Grand Island, NY). Fetal calf serum was obtained from Hyclone (Logan, UT); human recombinant IL-1 $\beta$  from Cistron Biotechnology (Pine Brook, NJ); human IFN- $\gamma$  from Boehringer Mannheim (Indianapolis, IN); and mouse IFN- $\gamma$  from R & D Systems (Minneapolis, MN). Poly IC, PDTc, MG-132, and collagenase type XI were obtained from Sigma (St. Louis, MO). [ $\gamma$ - $^{32}$ P]ATP and enhanced chemiluminescence reagents were obtained from Amersham Pharmacia Biotech (Arlington Heights, IL). Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA), and rabbit antiserum specific for the C-terminal 27 amino acids of mouse macrophage iNOS was a generous gift from Dr. Thomas Misko (G.D. Searle, St. Louis, MO). NF- $\kappa$ B consensus oligonucleotide, rabbit anti-human I $\kappa$ B $\alpha$ , and rabbit anti-human nitric oxide synthase-2 (NOS-2) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Male SD rats (250–300 g) and male C57BL/6J mice (7–8 wks) were purchased from Harlan (Indianapolis, IN). PKR $^{-/-}$  mice in a C57BL/6  $\times$  129 background (22) were a generous gift from Dr. Randal J. Kaufman (University of Michigan Medical Center, Ann Arbor, MI). C57BL/6  $\times$  129 mice were obtained from Jackson Laboratories (Bar Harbor, ME). Human islets were obtained from the Juvenile

Diabetes Federation International Islet Isolation Centers at Washington University School of Medicine (St. Louis, MO), the Diabetes Research Institute at the University of Miami (Miami, FL), and the Diabetes Institute for Immunology and Transplantation (University of Minnesota). All other reagents were from commercially available sources.

**Islet isolation.** Islets were isolated from male SD rats, male or female PKR $^{-/-}$  mice, male C57BL/6J or male C57BL/6  $\times$  129 (PKR $^{+/+}$ ) mice by collagenase digestion as previously described (29). After isolation, islets were cultured overnight in complete CMRL-1066 (containing 2 mmol/L L-glutamine, 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, and 10  $\mu$ g/ml streptomycin) under an atmosphere of 95% air and 5% CO $_2$  at 37°C. Human islets were incubated for 48 h at 37°C in complete CMRL-1066 before the initiation of experiments. Before each experiment, islets were cleaned by hand picking, washed three times in complete CMRL-1066, counted, and cultured for an additional 2 h at 37°C. No differences in islet function or cytokine signaling were observed between male and female PKR $^{-/-}$  mice. Data presented in Figs. 5 and 6 were obtained using islets isolated from C57BL/6J mice. To control for potential strain differences, the effects of dsRNA + IFN- $\gamma$  on iNOS expression, nitric oxide production, and NF- $\kappa$ B activation were compared among islets isolated from C57BL/6  $\times$  129 and C57BL/6J mice; no differences were observed (data not shown).

**Nitrite determination.** Nitrite formation was determined by mixing 50  $\mu$ l of culture medium with 50  $\mu$ l of Griess reagent (30). Absorbance was measured at 540 nm, and nitrite concentrations were calculated from a sodium nitrite standard curve.

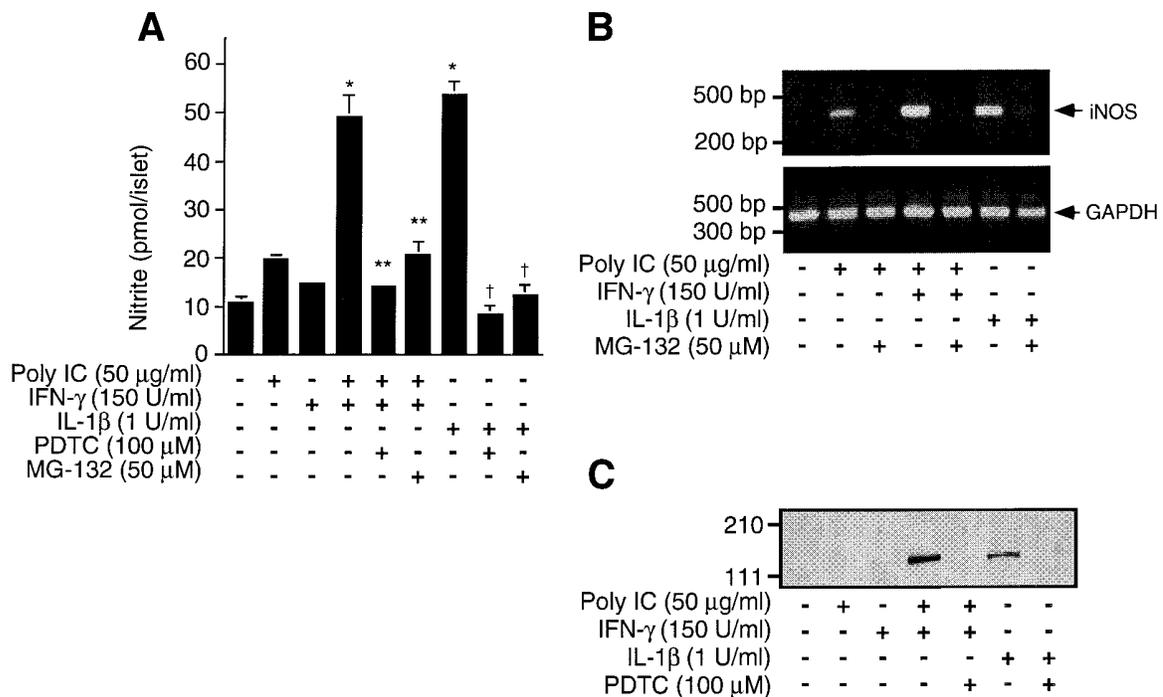
**Western blot analysis.** Western blot analysis of iNOS and I $\kappa$ B was performed as previously described (31), using the following antibody dilutions: rabbit anti-mouse iNOS (1:2,000 dilution), rabbit anti-human I $\kappa$ B $\alpha$  (1:1,500 dilution), rabbit anti-human NOS-2 (1:1,000 dilution), and HRP-conjugated donkey anti-rabbit IgG (1:7,000 dilution). Antigen was detected by enhanced chemiluminescence according to manufacturer's specifications.

**Insulin secretion.** Glucose-stimulated insulin secretion was performed as previously described (29). In brief, islets isolated from PKR $^{-/-}$  and PKR $^{+/+}$  mice (180/ml of complete CMRL-1066) were cultured for 40 h with the indicated concentrations of poly IC, IFN- $\gamma$ , IL-1, and aminoguanidine (AG). The islets were washed three times in Krebs-Ringer bicarbonate buffer (KRBB; 25 mmol/L HEPES, 115 mmol/L NaCl, 24 mmol/L NaHCO $_3$ , 5 mmol/L KCl, 1 mmol/L MgCl $_2$ , 2.5 mmol/L CaCl $_2$ , and 0.1% bovine serum albumin, pH 7.4) containing 3 mmol/L D-glucose, and then preincubated for 30 min in 200  $\mu$ l KRBB containing 3 mmol/L glucose. The preincubation buffer was removed and the islets were incubated for 30 min in 200  $\mu$ l of KRBB containing either 3 or 20 mmol/L glucose. The incubation buffer was removed and insulin content was determined by radioimmunoassay (32).

**Nuclear extraction and gel shift analysis.** After treatment with poly IC and cytokines, islets were dispersed into single cells by trypsin treatment (33) and nuclear proteins were extracted as previously described (13). The probe consisted of a double-stranded oligonucleotide containing the consensus binding sequence for NF- $\kappa$ B (5'-AGTTGAGGGGAC TTTCCAGGC-3') end-labeled with [ $\gamma$ - $^{32}$ P]ATP using T4 polynucleotide kinase (Promega; Madison, WI). Binding reactions consisted of 10  $\mu$ g of nuclear protein extract, 0.5 ng of DNA probe, and 1  $\mu$ g/ml of poly (dI-dC) in a buffer containing 10 mmol/L HEPES (pH 7.8), 50 mmol/L NaCl, 1 mmol/L EDTA, 10% glycerol, and 1 mmol/L dithiothreitol. Reactions were incubated at 30°C for 30 min, and the NF- $\kappa$ B/oligonucleotide complex was separated on 4.5% Tris-glycine nondenaturing polyacrylamide gels in a 2 $\times$  Tris-glycine (50 mmol/L Tris-HCl [pH 8.3], 0.38 mol/L glycine, and 2 mmol/L EDTA) buffer system (34). Gels were dried on Whatman paper and subjected to autoradiography.

**Polymerase chain reaction.** Total RNA was isolated from islets using the RNeasy RNA isolation kit (QIAGEN, Santa Clarita, CA) and was used to prepare first-strand cDNA by reverse transcription using the SuperScript Pre-amplification System (Life Technologies, Grand Island, NY) as described (35). iNOS, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and PKR primers were designed according to these published sequences: 1) iNOS forward primer 5'-CCAACCGGAGAAGGGGACGAAC-3', reverse primer 5'-GGAGGG TGTTGCGGCTGGAC-3' (PCR product size = 297 bp); 2) GAPDH forward primer 5'-GCTGGGGCTCACGTAAGGG-3', reverse primer 5'-GGATGACCT TGCCAGCC-3' (PCR product size = 343 bp); and 3) murine PKR forward primer 5'-GCCAGATGCACGGAGTAGCC-3', reverse primer 5'-GAAAACCTTG CCAAATCCACC-3' (PCR product size = 722 bp). A standard 25- $\mu$ l PCR reaction of 30 cycles was performed (35), and PCR products were separated on 1.5% agarose gels containing 0.5  $\mu$ g/ml ethidium bromide and visualized by ultraviolet light exposure.

**Statistical analysis.** Statistical comparisons were made between groups using a one-way analysis of variance. Significant differences between groups ( $P < 0.05$ ) were determined by Scheffe's  $F$  test post-hoc analysis.



**FIG. 1.** MG-132 and PDTC inhibit poly IC + IFN- $\gamma$ -induced nitrite formation and iNOS expression by rat islets. Rat islets (120/400  $\mu$ l of complete CMRL-1066) were treated with the indicated concentrations of poly IC, IFN- $\gamma$ , or IL-1 $\beta$  for 40 h at 37°C; 100  $\mu$ mol/l PDTC was added to islets 2 h before the addition of cytokines. After treatment, medium was removed for nitrite determination (A). iNOS expression by islets was examined by Western blot analysis (C), as described in RESEARCH DESIGN AND METHODS. **B:** Rat islets (100/400  $\mu$ l of complete CMRL-1066) were treated with the indicated concentrations of poly IC, IFN- $\gamma$ , or IL-1 $\beta$  for 18 h at 37°C; 50  $\mu$ mol/l MG-132 was added to islets 90 min before the addition of cytokines. cDNA was generated from total RNA isolated from islets, and iNOS and GAPDH mRNA expression was examined by RT-PCR, as described in RESEARCH DESIGN AND METHODS. Results for nitrite are averages  $\pm$  SE of three independent experiments, and iNOS mRNA accumulation and protein expression are representative of three independent experiments. \* $P$  < 0.05 vs. untreated control; \*\* $P$  < 0.05 vs. poly IC + IFN- $\gamma$  treatment; † $P$  < 0.05 vs. IL-1 $\beta$  treatment.

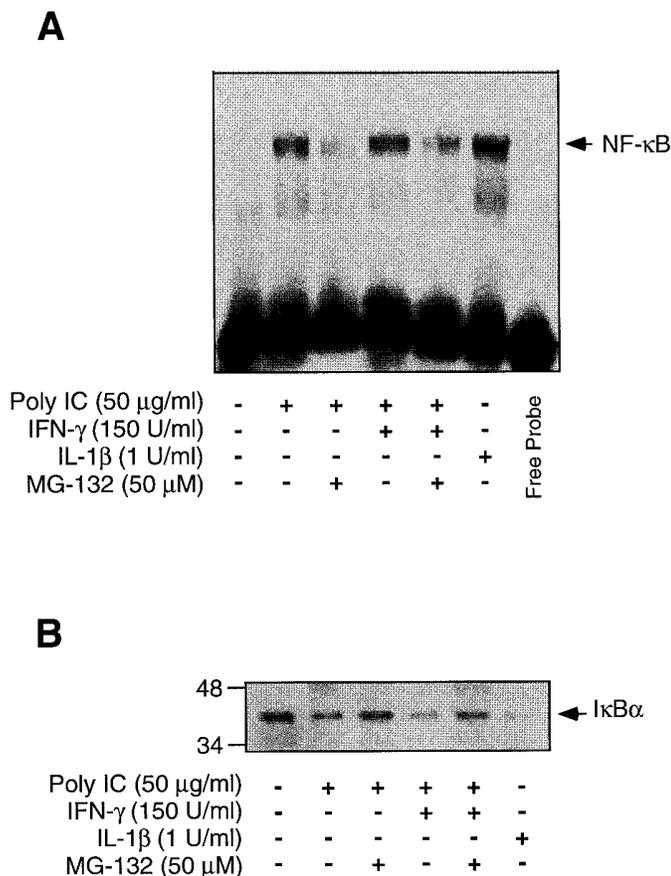
## RESULTS

**Effects of PDTC and MG-132 on poly IC + IFN- $\gamma$ -induced nitrite formation and iNOS expression by rat islets.** Recently we showed that dsRNA + IFN- $\gamma$  stimulates iNOS expression and nitrite production by rat islets, and that nitric oxide mediates the inhibitory actions of these agents on glucose-stimulated insulin secretion (36). The role of NF- $\kappa$ B in dsRNA + IFN- $\gamma$ -stimulated iNOS expression and nitric oxide production was evaluated using PDTC and MG-132, which are inhibitors of NF- $\kappa$ B activation (37,38). As shown in Fig. 1A, the combination of 50  $\mu$ g/ml poly IC and 150 U/ml IFN- $\gamma$  stimulated an approximately fivefold increase in nitrite formation by rat islets. Alone, neither poly IC nor IFN- $\gamma$  stimulated nitrite production by rat islets. Pretreatment of rat islets for 2 h with 100  $\mu$ mol/l PDTC or for 90 min with 50  $\mu$ mol/l MG-132 inhibited dsRNA + IFN- $\gamma$ -induced nitrite formation by ~90%. At these concentrations, PDTC (19) and MG-132 (39; data not shown) have been shown to maximally inhibit IL-1-induced NF- $\kappa$ B activation by rat islets without adversely affecting islet total protein synthesis or oxidative metabolism (19,39). As a control, the inhibitory effects of PDTC and MG-132 on IL-1-induced nitrite production are also shown. In addition, neither PDTC nor MG-132 alone stimulated nitrite production by rat islets, and DMSO, the vehicle control for both inhibitors, neither stimulated nor inhibited nitrite production in response to dsRNA + IFN- $\gamma$  (data not shown).

We previously reported that poly IC + IFN- $\gamma$  stimulates maximal iNOS mRNA accumulation in rat islets after an 18-h

incubation (36). As shown in Fig. 1B, pretreatment of rat islets for 90 min with MG-132 (50  $\mu$ mol/l) prevented poly IC- and poly IC + IFN- $\gamma$ -induced iNOS mRNA accumulation, as determined by reverse transcriptase-polymerase chain reaction (RT-PCR). Similar to iNOS mRNA accumulation, MG-132 (50  $\mu$ mol/l; data not shown) and PDTC (100  $\mu$ mol/l) also prevented poly IC + IFN- $\gamma$ -induced iNOS expression at the protein level, as determined by Western blot analysis (Fig. 1C). These findings indicate that NF- $\kappa$ B activation may be required for dsRNA + IFN- $\gamma$ -induced iNOS expression by rat islets. It is also clear from these experiments that poly IC alone stimulates iNOS mRNA accumulation, but does not induce iNOS protein expression or nitrite formation by rat islets. Previously, we were unable to detect poly IC-induced iNOS mRNA accumulation in rat islets by Northern blot analysis (36). The detection of iNOS mRNA accumulation in response to dsRNA in these studies appears to reflect the increased sensitivity of PCR over Northern blot analysis in the evaluation of mRNA expression.

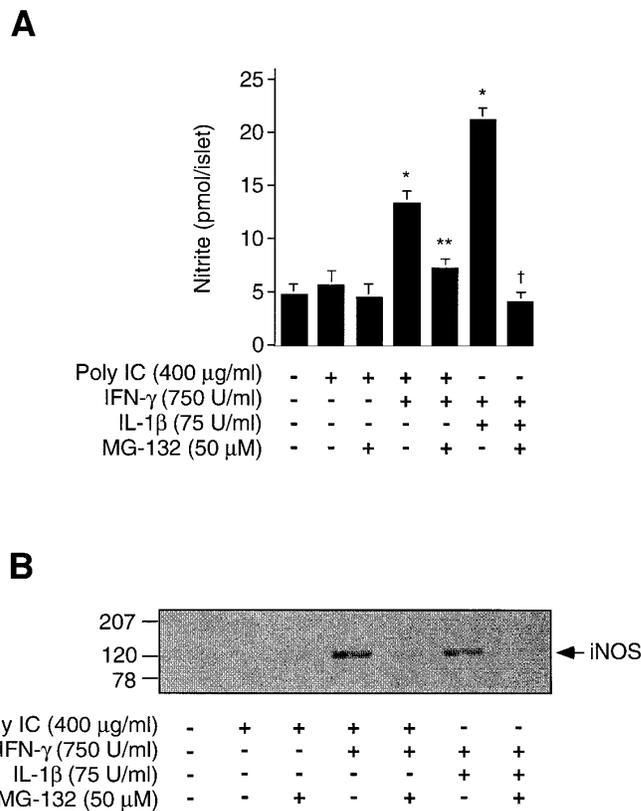
**Effects of poly IC on NF- $\kappa$ B activation in rat islets.** To determine whether dsRNA stimulates NF- $\kappa$ B activation in rat islets, NF- $\kappa$ B nuclear localization was examined by gel shift analysis and I $\kappa$ B degradation was determined by Western blot analysis. Treatment of rat islets for 3 h with 50  $\mu$ g/ml poly IC alone or in combination with 150 U/ml IFN- $\gamma$  resulted in the maximal nuclear translocation of NF- $\kappa$ B, as demonstrated by the reduced mobility of the radio-labeled DNA probe containing the NF- $\kappa$ B consensus sequence (Fig. 2A;



**FIG. 2.** Poly IC induces NF- $\kappa$ B nuclear translocation and I $\kappa$ B $\alpha$  degradation in rat islets. **A:** Rat islets (400/ml of complete CMRL-1066) were treated with indicated concentrations of poly IC or IFN- $\gamma$  for 3 h, or IL-1 $\beta$  for 30 min at 37°C; 50  $\mu$ mol/l MG-132 was added 90 min before the addition of cytokines. Islets were then dispersed by trypsin treatment, nuclear proteins were extracted, and NF- $\kappa$ B activation was determined by gel shift analysis as described in RESEARCH DESIGN AND METHODS. **B:** Rat islets (120/400  $\mu$ l of complete CMRL-1066) were treated with the indicated concentrations of poly IC and IFN- $\gamma$  for 3 h, or IL-1 $\beta$  for 30 min at 37°C; 50  $\mu$ mol/l MG-132 was added 90 min before the addition of cytokines. Islets were isolated and I $\kappa$ B $\alpha$  degradation was evaluated by Western blot analysis. Results are representative of three independent experiments.

data not shown for time-dependence of NF- $\kappa$ B nuclear translocation). As controls, inclusion of antiserum specific for the p65 subunit of NF- $\kappa$ B in the binding reaction further reduced the NF- $\kappa$ B/DNA complex migration (supershift), and the addition of excess unlabeled NF- $\kappa$ B oligonucleotide prevented the NF- $\kappa$ B/DNA complex formation (data not shown). Consistent with NF- $\kappa$ B nuclear localization, treatment of rat islets for 3 h with 50  $\mu$ g/ml poly IC and poly IC + 150 U/ml IFN- $\gamma$  resulted in the degradation of I $\kappa$ B $\alpha$ . Pretreatment of islets for 90 min with MG-132 (50  $\mu$ mol/l) attenuated poly IC- and poly IC + IFN- $\gamma$ -induced NF- $\kappa$ B nuclear localization and I $\kappa$ B $\alpha$  degradation (Fig. 2). Alone, neither MG-132 nor IFN- $\gamma$  stimulated NF- $\kappa$ B nuclear translocation or I $\kappa$ B $\alpha$  degradation (data not shown). As a positive control, the stimulatory effects of IL-1 on NF- $\kappa$ B nuclear translocation and I $\kappa$ B $\alpha$  degradation in rat islets are also shown (Fig. 2).

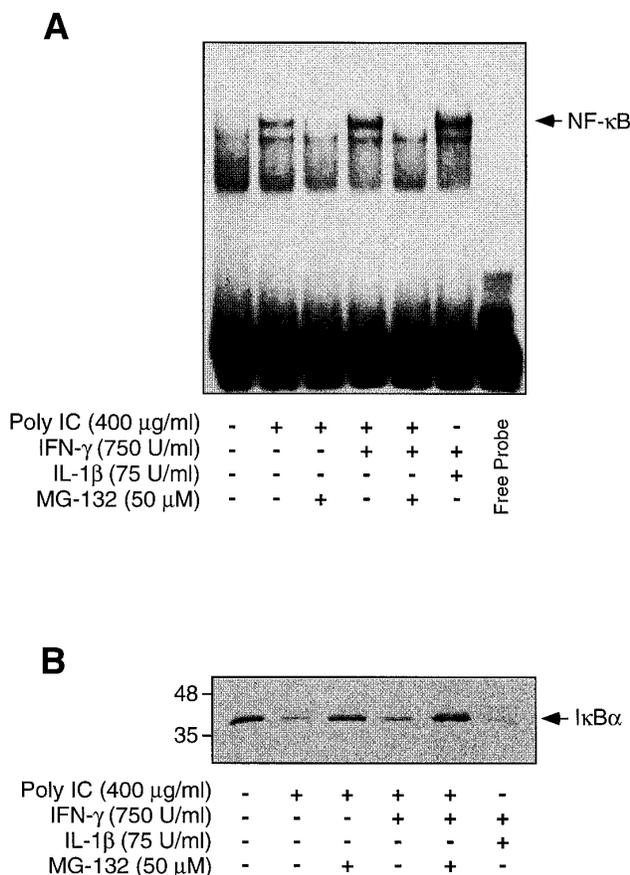
**Effects of MG-132 on poly IC + IFN- $\gamma$ -induced nitrite formation and iNOS expression by human islets.** Treatment of human islets with dsRNA + IFN- $\gamma$  resulted in the con-



**FIG. 3.** MG-132 inhibits poly IC + IFN- $\gamma$ -induced nitrite formation and iNOS expression by human islets. Human islets (220/400  $\mu$ l of complete CMRL-1066) were incubated with the indicated concentrations of poly IC, IFN- $\gamma$ , or IL-1 $\beta$  for 48 h at 37°C; 50  $\mu$ mol/l MG-132 was added to islets 90 min before the addition of cytokines. After treatment, medium was removed for nitrite determination (**A**). iNOS expression by islets was examined by Western blot analysis (**B**). Results for nitrite determination are averages  $\pm$  SE of four independent experiments; results for iNOS protein expression are representative of two independent experiments. \* $P$  < 0.05 vs. untreated control; \*\* $P$  < 0.05 vs. poly IC + IFN- $\gamma$  treatment; † $P$  < 0.05 vs. IL-1 $\beta$  + IFN- $\gamma$  treatment.

centration-dependent expression of iNOS and production of nitric oxide that was maximal at 400  $\mu$ g/ml poly IC and 750 U/ml IFN- $\gamma$  (M.R.H. and J.A.C., unpublished observation). To determine if NF- $\kappa$ B activation is required for dsRNA + IFN- $\gamma$ -induced nitric oxide formation, human islets were pretreated for 90 min with 50  $\mu$ mol/l MG-132, followed by incubation with poly IC + IFN- $\gamma$  for 48 h. Alone, neither poly IC nor IFN- $\gamma$  stimulated nitrite production by human islets (Fig. 3A) (40); however, in combination, poly IC + IFN- $\gamma$  stimulated an approximately threefold increase in nitrite formation. Similar to rat islets, MG-132 (50  $\mu$ mol/l) prevented nitrite formation by human islets in response to poly IC + IFN- $\gamma$ . As a positive control, the inhibitory actions of MG-132 on IL-1 + IFN- $\gamma$ -induced nitrite formation by human islets are also shown (Fig. 3A) (20,40).

Consistent with its inhibitory actions on nitrite formation, MG-132 prevented poly IC + IFN- $\gamma$ -induced iNOS expression by human islets (Fig. 3B). Treatment of human islets for 48 h with poly IC + IFN- $\gamma$  resulted in the expression of iNOS to levels similar in magnitude to that induced by IL-1 + IFN- $\gamma$ . MG-132 prevented both poly IC + IFN- $\gamma$ - and IL-1 +



**FIG. 4. Poly IC induces NF- $\kappa$ B nuclear translocation and I $\kappa$ B $\alpha$  degradation in human islets. A:** Human islets (500/ml of complete CMRL-1066) were treated with the indicated concentrations of poly IC or IFN- $\gamma$  for 2 h, or IL-1 $\beta$  for 1 h at 37°C; 50  $\mu$ mol/l MG-132 was added 90 min before the addition of cytokines. Islets were then dispersed by trypsin treatment, nuclear proteins were extracted, and NF- $\kappa$ B activation was determined by gel shift analysis as described in RESEARCH DESIGN AND METHODS. **B:** Human islets (150/400  $\mu$ l of complete CMRL-1066) were treated with the indicated concentrations of poly IC and IFN- $\gamma$  for 2 h, or IL-1 $\beta$  for 1 h at 37°C; 50  $\mu$ mol/l MG-132 was added 90 min before the addition of cytokines. Islets were isolated and I $\kappa$ B $\alpha$  degradation was evaluated by Western blot analysis. Results are representative of three independent experiments.

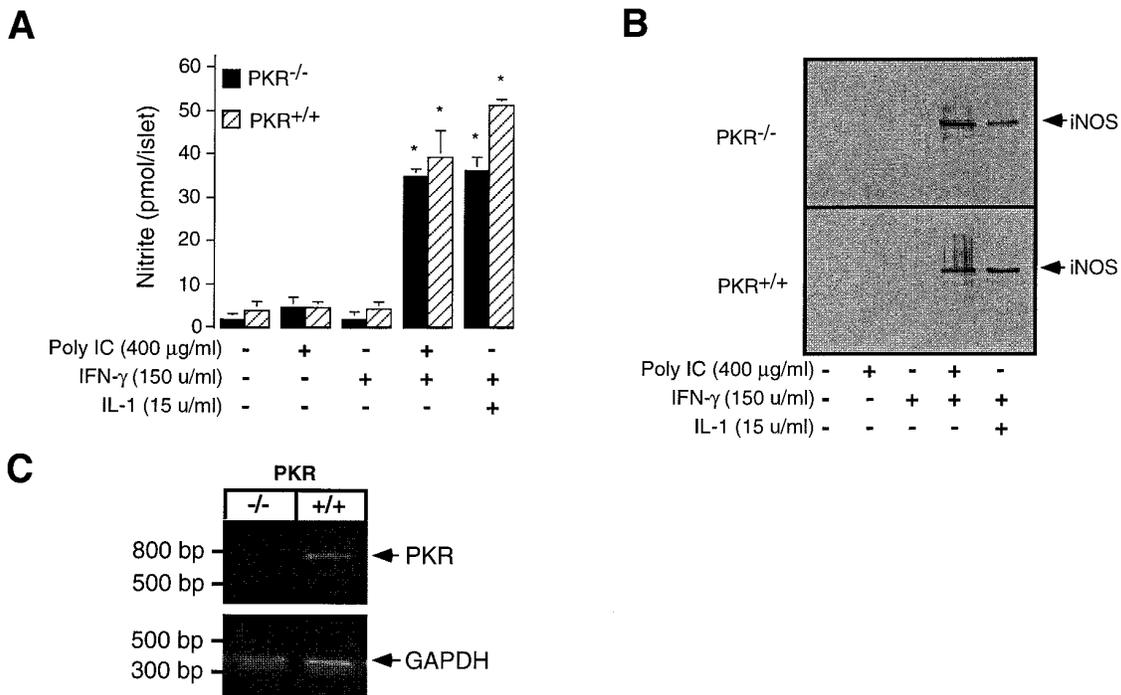
IFN- $\gamma$ -induced iNOS expression by human islets. Alone, neither poly IC (Fig. 3B) nor IFN- $\gamma$  (39; data not shown) stimulated iNOS protein expression by human islets.

**Poly IC stimulates NF- $\kappa$ B activation in human islets.** To determine whether poly IC alone or in combination with IFN- $\gamma$  activates NF- $\kappa$ B in human islets, NF- $\kappa$ B nuclear translocation was examined by gel shift analysis. Treatment of human islets for 2 h with 400  $\mu$ g/ml poly IC or poly IC + 750 U/ml IFN- $\gamma$  stimulated maximal NF- $\kappa$ B nuclear translocation (Fig. 4A; data not shown for time-dependence of NF- $\kappa$ B nuclear translocation). MG-132 attenuated NF- $\kappa$ B nuclear translocation induced by poly IC and poly IC + IFN- $\gamma$ . Alone, neither IFN- $\gamma$  (20) nor MG-132 (data not shown) stimulated NF- $\kappa$ B nuclear localization in human islets. Consistent with the effect of dsRNA on NF- $\kappa$ B nuclear localization, poly IC- and poly IC + IFN- $\gamma$ -induced I $\kappa$ B $\alpha$  degradation was maximal after a 2-h incubation (Fig. 4B; data not shown for time-dependence of I $\kappa$ B $\alpha$  degradation); the addition of MG-132 (50  $\mu$ mol/l) attenuated I $\kappa$ B $\alpha$  degradation under these conditions.

**The genetic absence of PKR does not affect islet expression of iNOS or production of nitrite in response to poly IC + IFN- $\gamma$ .** PKR is believed to regulate dsRNA-induced NF- $\kappa$ B activation by either directly phosphorylating I $\kappa$ B or by activating the upstream I $\kappa$ B kinase, IKK (11,22). As shown in Figs. 1–4, NF- $\kappa$ B activation appears to be required for dsRNA-induced iNOS expression by islets. Therefore, the effects of dsRNA + IFN- $\gamma$  on iNOS expression and nitric oxide production were examined in islets isolated from both PKR<sup>+/+</sup> and PKR<sup>-/-</sup> mice. Islets isolated from either PKR<sup>-/-</sup> or PKR<sup>+/+</sup> mice were treated with poly IC + IFN- $\gamma$ , which resulted in the production of similar levels of nitrite after a 40-h incubation (Fig. 5A). Poly IC + IFN- $\gamma$  also stimulated iNOS expression to similar levels in islets isolated from both PKR<sup>-/-</sup> and PKR<sup>+/+</sup> mice (Fig. 5B). Similar to human islets, a concentration of 400  $\mu$ g/ml poly IC + 150 U/ml IFN- $\gamma$  was required to stimulate maximal iNOS expression and nitrite production by mouse islets. Also, similar to rat and human islets, neither poly IC nor IFN- $\gamma$  alone stimulated nitrite production by islets isolated from PKR<sup>-/-</sup> and PKR<sup>+/+</sup> mice. The level of nitrite produced by mouse islets in response to poly IC + IFN- $\gamma$  was similar to the levels induced in response to 15 U/ml IL-1 + 150 U/ml IFN- $\gamma$ . RT-PCR was used to confirm the lack of PKR mRNA accumulation in islets isolated from PKR<sup>-/-</sup> mice (Fig. 5C).

**Poly IC stimulates NF- $\kappa$ B nuclear translocation and I $\kappa$ B degradation in islets isolated from PKR<sup>-/-</sup> and PKR<sup>+/+</sup> mice.** The effects of dsRNA on I $\kappa$ B degradation and NF- $\kappa$ B nuclear translocation in islets isolated from PKR<sup>-/-</sup> and PKR<sup>+/+</sup> mice were examined by Western blot and gel shift analyses, respectively. As shown in Fig. 6A and B, a 1-h incubation with poly IC or poly IC + IFN- $\gamma$  stimulated I $\kappa$ B degradation and NF- $\kappa$ B nuclear localization in islets isolated from PKR<sup>-/-</sup> mice. The effects of poly IC and poly IC + IFN- $\gamma$  on I $\kappa$ B degradation and NF- $\kappa$ B nuclear localization were similar to those observed in islets isolated from PKR<sup>+/+</sup> mice. As a positive control, we also showed that a 30-min incubation with IL-1 + IFN- $\gamma$  stimulated I $\kappa$ B degradation and NF- $\kappa$ B nuclear translocation in islets isolated from both PKR<sup>-/-</sup> and PKR<sup>+/+</sup> mice.

**Poly IC + IFN- $\gamma$  inhibits glucose-stimulated insulin secretion by islets isolated from PKR<sup>-/-</sup> mice.** We have previously shown that dsRNA + IFN- $\gamma$  inhibits glucose-stimulated insulin secretion by rat islets in a nitric oxide-dependent manner (36). To determine if PKR is required for the inhibitory actions of poly IC + IFN- $\gamma$  on insulin secretion, the effects of these agents on glucose-stimulated insulin secretion by islets isolated from PKR<sup>-/-</sup> mice were examined. Treatment of islets isolated from PKR<sup>-/-</sup> mice for 40 h with 400  $\mu$ g/ml poly IC + 150 U/ml IFN- $\gamma$  resulted in ~75% inhibition of glucose-stimulated insulin secretion (Fig. 7). AG, the iNOS selective inhibitor, prevented poly IC + IFN- $\gamma$ -induced inhibition of insulin secretion by islets isolated from PKR<sup>-/-</sup> mice, indicating that nitric oxide mediates the inhibitory actions of these agents on islet function. Alone, neither poly IC nor IFN- $\gamma$  inhibited glucose-stimulated insulin secretion by PKR<sup>-/-</sup> mouse islets (Fig. 7). The inhibitory actions of poly IC + IFN- $\gamma$  were similar in magnitude to the inhibitory effects of 15 U/ml IL-1 + 150 U/ml IFN- $\gamma$  on glucose-stimulated insulin secretion by islets isolated from PKR<sup>-/-</sup> mice. Similar results were obtained in islets isolated from PKR<sup>+/+</sup> mice (data not shown). These results suggest that PKR is not required for the



**FIG. 5.** Poly IC + IFN- $\gamma$  induces nitrite formation and iNOS expression by islets isolated from PKR<sup>-/-</sup> mouse islets. PKR<sup>-/-</sup> or C57BL/6J (PKR<sup>+/+</sup>) mouse islets (120/400  $\mu$ l of complete CMRL-1066) were treated with the indicated concentrations of poly IC, IFN- $\gamma$ , or IL-1 $\beta$  for 40 h at 37°C. After treatment, medium was removed for nitrite determination (A). iNOS expression was examined by Western blot analysis (B), as described in RESEARCH DESIGN AND METHODS. C: Total RNA was isolated from PKR<sup>-/-</sup> or C57BL/6J (PKR<sup>+/+</sup>) mouse islets (120/400  $\mu$ l of complete CMRL-1066), and PKR and GAPDH mRNA expression was examined by RT-PCR, as described in RESEARCH DESIGN AND METHODS. Results for nitrite are averages  $\pm$  SE of three independent experiments; both iNOS protein expression and PKR mRNA accumulation and protein expression are representative of three independent experiments. \* $P < 0.05$  vs. untreated control.

inhibitory actions of dsRNA + IFN- $\gamma$  on glucose-stimulated insulin secretion by mouse islets.

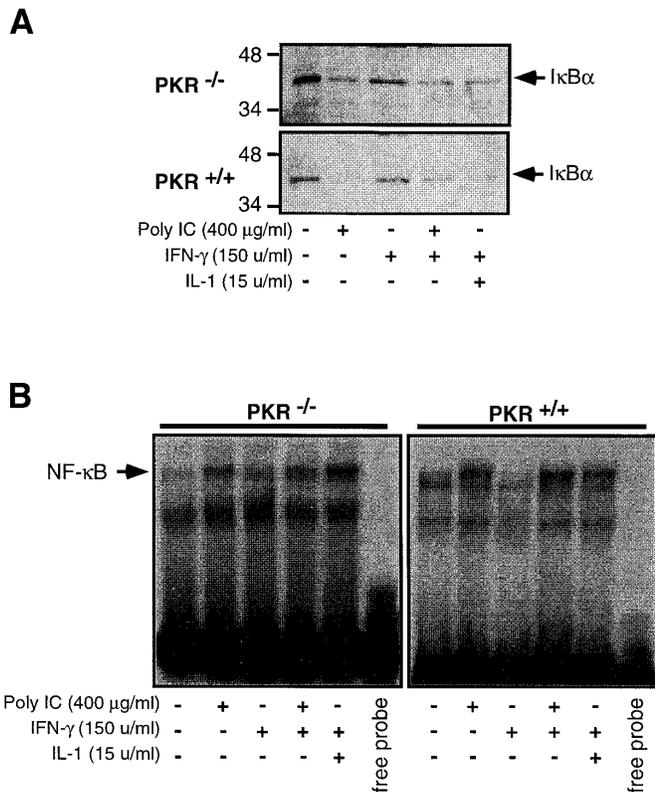
## DISCUSSION

Viral infection has been implicated as one environmental factor that may trigger  $\beta$ -cell destruction during the development of autoimmune diabetes. However, the mechanism by which a viral infection induces  $\beta$ -cell damage has been difficult to examine because multiple viruses from both DNA and RNA genome families have been associated with the development of diabetes (2–5). dsRNA is an active component of a viral infection that stimulates antiviral responses in infected cells (8). Recently we showed that treatment of rat islets and primary  $\beta$ -cells with dsRNA + IFN- $\gamma$  results in a potent inhibition of glucose-stimulated insulin secretion and islet degeneration, events that require  $\beta$ -cell production of nitric oxide (36). In this study, the role of NF- $\kappa$ B in dsRNA + IFN- $\gamma$ -induced iNOS expression and nitric oxide production by islets was examined. We showed that dsRNA (in the form of poly IC), in the presence or absence of IFN- $\gamma$ , stimulated NF- $\kappa$ B nuclear translocation and I $\kappa$ B degradation, and that inhibition of NF- $\kappa$ B activation prevented dsRNA + IFN- $\gamma$ -induced iNOS expression and nitric oxide production by rat and human islets. These findings indicate that NF- $\kappa$ B activation is required for iNOS expression by islets in response to dsRNA, and provide further evidence supporting a key role for NF- $\kappa$ B activation in the regulation of iNOS expression by rat and human islets (19,20).

One mechanism by which dsRNA may activate NF- $\kappa$ B is by PKR-dependent phosphorylation of I $\kappa$ B, an event that results

in I $\kappa$ B degradation by the proteasome complex (11). In addition, PKR has been shown to activate IKK in mouse embryonic fibroblasts, and this activation results in I $\kappa$ B degradation and NF- $\kappa$ B nuclear localization (23,24). In this study, the role of PKR in both dsRNA + IFN- $\gamma$ -induced iNOS expression and NF- $\kappa$ B activation was examined using islets isolated from PKR-deficient mice. In contrast to previous studies (21,22), the genetic absence of PKR did not prevent dsRNA or dsRNA + IFN- $\gamma$ -induced NF- $\kappa$ B nuclear translocation or I $\kappa$ B degradation in islets isolated from PKR<sup>-/-</sup> mice. In addition, dsRNA + IFN- $\gamma$  stimulated iNOS expression and nitric oxide production to similar levels in islets isolated from PKR<sup>-/-</sup> and PKR<sup>+/+</sup> mice. Consistent with iNOS expression and nitric oxide production, treatment of islets isolated from PKR<sup>-/-</sup> mice with poly IC + IFN- $\gamma$  resulted in a nitric oxide-dependent inhibition of glucose-stimulated insulin secretion. These findings indicate that PKR is not required for dsRNA-induced NF- $\kappa$ B activation or dsRNA + IFN- $\gamma$ -induced iNOS expression by isolated mouse islets. The mechanisms responsible for the differences in dsRNA responsiveness of mouse embryonic fibroblasts as compared to islets are unknown. However, in support of our findings in islets, we recently showed that dsRNA + IFN- $\gamma$ -induced iNOS expression and NF- $\kappa$ B activation in mouse peritoneal macrophages occur by PKR-independent mechanisms (41).

Although our findings indicate that PKR is not required for dsRNA-induced NF- $\kappa$ B activation, the signaling pathways activated by dsRNA in islets have yet to be identified. The mitogen-activated protein kinases (MAPKs) p38 and c-Jun NH<sub>2</sub>-terminal kinase (JNK), along with their upstream MAPK

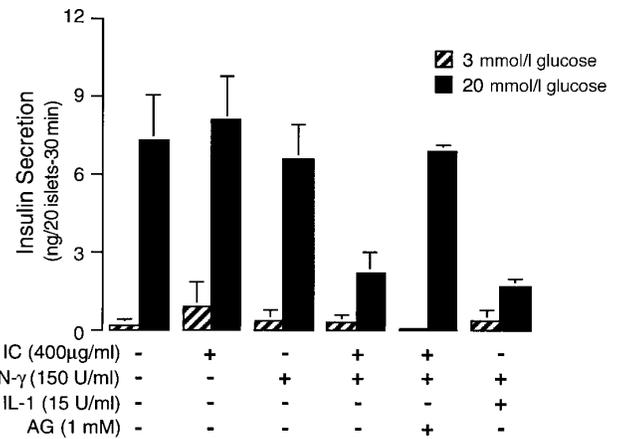


**FIG. 6.** Poly IC induces NF- $\kappa$ B nuclear translocation and I $\kappa$ B $\alpha$  degradation in islets isolated from PKR<sup>-/-</sup> mouse islets. **A:** PKR<sup>-/-</sup> or C57BL/6J (PKR<sup>+/+</sup>) mouse islets (120/400  $\mu$ l of complete CMRL-1066) were treated with indicated concentrations of poly IC and IFN- $\gamma$  for 1 h, or IL-1 $\beta$  for 30 min at 37°C. Islets were isolated and I $\kappa$ B $\alpha$  degradation was evaluated by Western blot analysis. **B:** PKR<sup>-/-</sup> or C57BL/6J (PKR<sup>+/+</sup>) mouse islets (500/ml of complete CMRL-1066) were treated with indicated concentrations of poly IC or IFN- $\gamma$  for 1 h, or IL-1 $\beta$  for 30 min at 37°C. Islets were then dispersed by trypsin treatment, nuclear proteins were extracted, and NF- $\kappa$ B nuclear translocation was determined by gel shift analysis, as described in RESEARCH DESIGN AND METHODS. Results are representative of three independent experiments.

kinases (MKK3, MKK4, and MKK6), are activated by dsRNA and viral infection in mouse embryonic or immortalized 3T3 fibroblasts, HeLa, and Rat-1 cell lines (23,42). Importantly, dsRNA-induced p38 and JNK activation appears to be PKR-independent; however, these stress kinases do not appear to directly activate NF- $\kappa$ B in response to dsRNA (23,42). It is also possible that dsRNA may interact with other cellular proteins, distinct from PKR, that contain dsRNA-binding motifs (43), and that this interaction may result in the activation of NF- $\kappa$ B. A number of novel candidate dsRNA-binding proteins have been identified; however, a protein that contains dsRNA-dependent kinase activity has yet to be described (44–47). Although we have not identified the kinase(s) that regulate dsRNA-induced NF- $\kappa$ B activation in islets, our findings provide novel evidence that dsRNA + IFN- $\gamma$ -induced iNOS expression and nitric oxide production require NF- $\kappa$ B activation and occur by mechanisms that are independent of PKR.

#### ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants DK-52194 and AI-44458, and a Career Development Award from the Juvenile Diabetes Foundation International (J.A.C.).



**FIG. 7.** Poly IC + IFN- $\gamma$  inhibit glucose-stimulated insulin secretion by islets isolated from PKR<sup>-/-</sup> mice. Islets isolated from PKR<sup>-/-</sup> mice were treated for 40 h with the indicated concentrations of poly IC, IFN- $\gamma$ , IL-1, and AG. The islets were isolated and glucose-stimulated insulin secretion was examined. Results are averages  $\pm$  SE of three independent experiments.

We thank Jessica Gorman, Joseph Martin, and Tracey Baird for expert technical assistance and Dr. Randal Kaufman for providing the PKR-deficient mice. We also thank the Juvenile Diabetes Federation International Islet Isolation Centers at Washington University School of Medicine, the Diabetes Research Institute at the University of Miami, and the Diabetes Institute for Immunology and Transplantation (University of Minnesota) for providing human islets used in these studies.

#### REFERENCES

- Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14:619–633, 1965
- Yoon J-W: A new look at viruses in type 1 diabetes. *Diabetes Metab Rev* 11:83–107, 1995
- von Herrath MG, Evans CF, Horwitz MS, Oldstone MB: Using transgenic models to dissect the pathogenesis of virus-induced autoimmune disorders of the islets of Langerhans and the central nervous system. *Immunol Rev* 152:111–143, 1996
- von Herrath MG, Oldstone MB: Virus-induced autoimmune disease. *Curr Opin Immunol* 8:878–885, 1996
- Bach J-F: Insulin-dependent diabetes mellitus as an autoimmune disease. *Endocr Rev* 15:516–541, 1994
- Chung Y-H, Jun H-S, Kang Y, Hirasawa K, Lee B-R, Rooijen NV, Joon J-W: Role of macrophages and macrophage-derived cytokines in the pathogenesis of Kilham rat virus-induced autoimmune diabetes in diabetes-resistant BioBreeding rats. *J Immunol* 159:466–471, 1997
- Hirasawa K, Tsutsui S, Takeda M, Mizutani M, Itagaki S, Doi K: Depletion of Mac1-positive macrophages protects DBA/2 mice from encephalomyocarditis virus-induced myocarditis and diabetes. *J Gen Virol* 77:737–741, 1996
- Jacobs BL, Langland JO: When two strands are better than one: the mediators and modulators of the cellular responses to double-stranded RNA. *Virology* 219:339–349, 1996
- Ewel CH, Sobel DO, Zeligs BJ, Bellanti JA: Poly I:C accelerates development of diabetes mellitus in diabetes-prone BB rat. *Diabetes* 41:1016–1021, 1992
- Sobel DO, Newsome J, Ewel CH, Bellanti JA, Abbassi V, Creswell K, Blair O: Poly I:C induces development of diabetes mellitus in BB rat. *Diabetes* 41:515–520, 1992
- Kumar A, Haque J, Lacoste J, Hiscott J, Williams BRG: Double-stranded RNA-dependent protein kinase activates transcription factor NF- $\kappa$ B by phosphorylating I $\kappa$ B. *Proc Natl Acad Sci U S A* 91:6288–6292, 1994
- Faruqi TR, Erzurum SC, Kaneko FT, DiCorleto PE: Role of nitric oxide in poly(I-C)-induced endothelial cell expression of leukocyte adhesion molecules. *Am J Physiol* 273:H2490–H2497, 1997
- Heitmeier MR, Scarim AL, Corbett JA: Double-stranded RNA-induced inducible nitric oxide synthase expression and interleukin-1 release by

- murine macrophages requires NF- $\kappa$ B activation. *J Biol Chem* 273:15301–15307, 1998
14. Baldwin AS Jr: The NF- $\kappa$ B and I $\kappa$ B proteins: new discoveries and insights. *Annu Rev Immunol* 14:649–681, 1996
  15. Baeuerle PA, Henkel T: Function and activation of NF- $\kappa$ B in the immune system. *Annu Rev Immunol* 12:141–179, 1994
  16. Xie QW, Kashiwabara Y, Nathan C: Role of transcription factor NF- $\kappa$ B/Rel in induction of nitric oxide synthase. *J Biol Chem* 269:4705–4708, 1994
  17. Kim H, Lee HS, Chang KT, Ko TH, Baek KJ, Kwon NS: Chloromethyl ketones block induction of nitric oxide synthase in murine macrophages by preventing activation of nuclear factor- $\kappa$ B. *J Immunol* 154:4741–4748, 1995
  18. Beck K-F, Sterzel RB: Cloning and sequencing of the proximal promoter of the rat iNOS gene: activation of NF- $\kappa$ B is not sufficient for transcription of the iNOS gene in rat mesangial cells. *FEBS Lett* 394:263–267, 1996
  19. Kwon G, Corbett JA, Rodi CP, Sullivan P, McDaniel ML: IL-1 $\beta$  induces the expression of nitric oxide synthase by rat islets of Langerhans and RINm5F cells: evidence for the involvement of NF- $\kappa$ B in the signaling mechanism. *Endocrinology* 136:4790–4795, 1995
  20. Flodstrom M, Welsh N, Eizirik DL: Cytokines activate the nuclear factor  $\kappa$ B (NF- $\kappa$ B) and induce nitric oxide production by human pancreatic islets. *FEBS Lett* 385:4–6, 1996
  21. Kumar A, Yang Y-L, Flati V, Der S, Kadereit S, Deb A, Haque J, Reis L, Weissmann C, Williams BRG: Deficient cytokine signaling in mouse embryo fibroblasts with a targeted deletion in the PKR gene: roles of IRF-1 and NF- $\kappa$ B. *EMBO J* 16:406–416, 1997
  22. Yang Y-L, Reis LFL, Pavlovic J, Aguzzi A, Schafer R, Kumar A, Williams BRG, Aguet M, Weissman C: Deficient signaling in mice devoid of double-stranded RNA-dependent protein kinase. *EMBO J* 14:6095–6106, 1995
  23. Chu WM, Ostertag D, Li ZW, Chang L, Chen Y, Hu Y, Williams B, Perrault J, Karin M: JNK2 and IKK $\beta$  are required for activating the innate response to viral infection. *Immunity* 11:721–731, 1999
  24. Zamanian-Daryoush M, Mogensen TH, DiDonato JA, Williams BRG: NF- $\kappa$ B activation by double-stranded-RNA-activated protein kinase (PKR) is mediated through NF- $\kappa$ B-inducing kinase and I $\kappa$ B kinase. *Mol Cell Biol* 20:1278–1290, 2000
  25. Stark GR, Kerr IM, Williams BRG, Silverman RH, Schreiber RD: How cells respond to interferons. *Annu Rev Biochem* 67:227–264, 1998
  26. Wu S, Kaufman RJ: A model for the double-stranded RNA (dsRNA)-dependent dimerization and activation of the dsRNA-activated protein kinase PKR. *J Biol Chem* 272:1291–1296, 1997
  27. Kirchhoff S, Koromilas AE, Schaper F, Martina G, Sonenberg N, Hauser H: IRF-1 induced cell growth inhibition and interferon induction requires the activity of the protein kinase PKR. *Oncogene* 11:439–445, 1995
  28. Kaufman RJ: Double-stranded RNA-activated protein kinase mediates virus-induced apoptosis: a new role for an old actor. *Proc Natl Acad Sci U S A* 96:11693–11695, 1999
  29. McDaniel ML, Colca JR, Kotagal N, Lacy PE: A subcellular fractionation approach for studying insulin release mechanisms and calcium metabolism in islets of Langerhans. *Methods Enzymol* 98:182–200, 1983
  30. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrate, nitrite, and [ $^{15}$ N] nitrate in biological fluids. *Anal Biochem* 126:131–138, 1982
  31. Heitmeier MR, Scarim AL, Corbett JA: Interferon- $\gamma$  increases the sensitivity of islets of Langerhans for inducible nitric-oxide synthase expression induced by interleukin-1. *J Biol Chem* 272:13697–13704, 1997
  32. Wright PH, Makulu DR, Vichick D, Sussman KE: Insulin immunoassay by back-titration: some characteristics of the technic and the insulin precipitant action of alcohol. *Diabetes* 20:33–45, 1971
  33. Ono J, Takaki R, Fukuma M: Preparation of single cells from pancreatic islets of adult rat by the use of dispase. *Endocrinol Jpn* 24:265–270, 1977
  34. Buratowski S, Chodosh LA: Mobility shift DNA-binding assay using gel electrophoresis. In *Current Protocols in Molecular Biology*. Vol. 2. Ausubel FM, Brent R, Kingston R, Moore DD, Seidman JG, Smith JA, Struhl K, Eds. New York, Wiley-Interscience, 1991, p 12.2.1–12.2.11
  35. Arnush M, Scarim AL, Heitmeier MR, Kelly CB, Corbett JA: Potential role of resident islet macrophage activation in the initiation of autoimmune diabetes. *J Immunol* 160:2684–2691, 1998
  36. Heitmeier MR, Scarim AL, Corbett JA: Double-stranded RNA inhibits  $\beta$ -cell function and induces islet damage by stimulating  $\beta$ -cell production of nitric oxide. *J Biol Chem* 274:12531–12536, 1999
  37. Schreck R, Albermann K, Baeuerle PA: Nuclear factor  $\kappa$ B: an oxidative stress-responsive transcription factor of eukaryotic cells. *Free Radic Res Commun* 17:221–237, 1992
  38. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T: The ubiquitin-proteasome pathway is required for processing the NF- $\kappa$ B1 precursor protein and the activation of NF- $\kappa$ B. *Cell* 78:773–785, 1994
  39. Kwon G, Corbett JA, Hauser S, Hill JR, Turk J, McDaniel ML: Evidence for involvement of the proteasome complex (26S) and NF- $\kappa$ B in IL-1 $\beta$ -induced nitric oxide and prostaglandin production by rat islets and RINm5F cells. *Diabetes* 47:583–591, 1998
  40. Corbett JA, Sweetland MA, Wang JL, Lancaster JR Jr, McDaniel ML: Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. *Proc Natl Acad Sci U S A* 90:1731–1735, 1993
  41. Maggi LB Jr, Heitmeier MR, Scheuner D, Kaufman RJ, Buller ML, Corbett JA: Potential role of PKR in double-stranded RNA-induced macrophage activation. *EMBO J* 19:3630–3638, 2000
  42. Iordanov MS, Paranjape JM, Zhou A, Wong J, Williams BRG, Meurs EF, Silverman RH, Magun BE: Activation of p38 mitogen-activated protein kinase and c-Jun NH $_2$ -terminal kinase by double-stranded RNA and encephalomyocarditis virus: involvement of RNase L, protein kinase R, and alternative pathways. *Mol Cell Biol* 20:617–627, 2000
  43. St Johnston D, Brown NH, Gall JG, Jantsch M: A conserved double-stranded RNA-binding domain. *Proc Natl Acad Sci U S A* 89:10979–10983, 1992
  44. Patel RC, Sen GC: PACT, a protein activator of the interferon-induced protein kinase, PKR. *EMBO J* 17:4379–4390, 1998
  45. Ito T, Mingli Y, May WS: RAX, a cellular activator for double-stranded RNA-dependent protein kinase during stress signaling. *J Biol Chem* 274:15427–15432, 1999
  46. Satoh M, Shaheen VM, Kao PN, Okano T, Shaw M, Yoshida H, Richards HB, Reeves WH: Autoantibodies define a family of proteins with conserved double-stranded RNA-binding domains as well as DNA binding activity. *J Biol Chem* 274:34598–34604, 1999
  47. Patel RC, Vestal DJ, Xu Z, Bandyopadhyay S, Guo W, Erme SM, Williams BRG, Sen GC: DRBP76, a double-stranded RNA-binding nuclear protein, is phosphorylated by the interferon-induced protein kinase, PKR. *J Biol Chem* 274:20432–20437, 1999