Double-stranded RNA Inhibits β-Cell Function and Induces Islet Damage by Stimulating β-Cell Production of Nitric Oxide*  

Monique R. Heitmeier, Anna L. Scarim, and John A. Corbett‡

From the Edward A. Doisy Department of Biochemistry and Molecular Biology, St. Louis University School of Medicine, St. Louis, Missouri 63104

Viral infection has been implicated as a triggering event that may initiate β-cell damage during the development of autoimmune diabetes. In this study, the effects of the viral replicative intermediate, double-stranded RNA (dsRNA) (in the form of synthetic polyinosinic-polycytidylic acid (poly IC)) on islet expression of inducible nitric oxide synthase (iNOS), production of nitric oxide, and islet function and viability were investigated. Treatment of rat islets with poly(IC) + interferon-γ (IFN-γ) stimulates the time- and concentration-dependent expression of iNOS and production of nitrite by rat islets. iNOS expression and nitrite production by rat islets in response to poly(IC) + IFN-γ correlate with an inhibition of insulin secretion and islet degeneration, effects that are prevented by the iNOS inhibitor aminoguanidine (AG). We have previously shown that poly(IC) + IFN-γ activates resident macrophages, stimulating iNOS expression, nitric oxide production and interleukin-1 (IL-1) release. In addition, in response to tumor necrosis factor-α (TNF-α) + lipopolysaccharide, activated resident macrophages mediate β-cell damage via intrasilet IL-1 release followed by IL-1-induced iNOS expression by β-cells. The inhibitory and destructive effects of poly(IC) + IFN-γ, however, do not appear to require resident macrophages. Treatment of macrophage-depleted rat islets for 40 h with poly(IC) + IFN-γ results in the expression of iNOS, production of nitrite, and inhibition of insulin secretion. The destructive effects of dsRNA + IFN-γ on islets appear to be mediated by a direct interaction with β-cells. Poly IC + IFN-γ stimulates iNOS expression and inhibits insulin secretion by primary β-cells purified by fluorescence-activated cell sorting. In addition, AG prevents the inhibitory effects of poly(IC) + IFN-γ on glucose-stimulated insulin secretion by β-cells. These results indicate that dsRNA + IFN-γ interacts directly with β-cells stimulating iNOS expression and inhibiting insulin secretion in a nitric oxide-dependent manner. These findings provide biochemical evidence for a novel mechanism by which viral infection may directly mediate the initial destruction of β-cells during the development of autoimmune diabetes.

Insulin-dependent diabetes mellitus is an autoimmune disease characterized by an inflammatory reaction in and around pancreatic islets followed by selective destruction of insulin secreting β-cells (1). The mechanisms that lead to the development of autoimmune diabetes are unknown; however, the expression of the inducible form of nitric oxide synthase (iNOS) by β-cells and the resulting production of nitric oxide may be one factor that mediates β-cell dysfunction and eventual β-cell death. We and others (2–4) have shown that treatment of isolated rat islets for 18 h with IL-1 stimulates the time- and concentration-dependent expression of iNOS and production of nitrite that correlates with a potent inhibition of glucose-stimulated insulin secretion. In the presence of IFN-γ, the concentration of IL-1 required to induce iNOS expression by β-cells is reduced 10-fold (5). Recently, Okamoto and co-workers (6) have examined the development of diabetes in transgenic mice that express iNOS under control of the rat insulin promoter (6). These mice spontaneously develop diabetes in a nitric oxide-dependent manner that occurs in the absence of insulin. Administration of the iNOS inhibitor, aminoguanidine (AG) (200 mg/kg/2 times daily) prevents the spontaneous development of diabetes in these mice (6). These results support an effector role for nitric oxide in mediating β-cell damage during the development of autoimmune diabetes.

Viruses have been implicated as one environmental factor that may initiate or trigger an autoimmune reaction that targets and destroys β-cells in genetically susceptible individuals (7–11). Mouse models of virus-induced autoimmune diabetes implicate increased cytokine and iNOS expression and nitric oxide production in the development of the disease. Encephalomyocarditis virus-induced diabetes in DBA/2 mice correlates with an increased level of IL-1γ and TNF mRNA expression in islets as determined by in situ hybridization of pancreatic sections (12). Daily administration of antiserum specific for IL-1β or TNF-α (0.5 mg/mouse) starting on the day of viral infection attenuates encephalomyocarditis virus-induced diabetes in DBA/2 mice (12). In addition, encephalomyocarditis virus stimulates iNOS mRNA expression in islets at early stages of infection, and iNOS expression persists until the onset of diabetes in DBA/2 mice. Daily administration of the iNOS inhibitor, AG, (at 2 mg/mouse/day) significantly attenuates the development of encephalomyocarditis virus-induced diabetes (12). These findings implicate cytokines and nitric oxide in the development of viral-induced diabetes.

dsRNA, formed during viral replication, is an active component of a viral infection that stimulates antiviral activities in infected cells (13). In vivo, dsRNA (in the form of poly(IC))

* This work was supported by a Research Scholar Award from the Tobacco Research Council, National Institutes of Health Grants DK-52194 and AI44455, and a Career Development Award from the Juvénile Diabetes Foundation International (to J. A. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, St. Louis University School of Medicine, 1402 South Grand Blvd., St. Louis, MO 63104. Fax: 314-577-8156; E-mail: corbett@wpogate.slu.edu.

1 The abbreviations used are: iNOS, inducible nitric oxide synthase; IL, interleukin; IFN-γ, interferon-γ; AG, aminoguanidine; poly(IC), polyinosinic-polycytidylic acid; TNF, tumor necrosis factor; FACS, fluorescence-activated cell sorting; ds, double-stranded; LPS, lipopolysaccharide.  

© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.  

This paper is available on line at http://www.jbc.org
stimulates the development of diabetes in diabetes-resistant BioBreeding (BB) rats, and accelerates the development of diabetes in diabetes prone BB rats (14–16). These findings indicate that viral infection and dsRNA modulate islet function; however, the mechanisms associated with viral-induced β-cell dysfunction are unknown.

The goal of these studies was to determine whether dsRNA directly modulates islet function and viability. We show that in combination with IFN-γ, dsRNA stimulates the time- and concentration dependent expression of iNOS and production of nitric oxide by rat islets. We provide evidence that the islet cellular source of iNOS in response to poly(IC) + IFN-γ is the β-cell. In addition, we show that poly(IC) + IFN-γ induces islet degeneration and inhibits insulin secretion by rat islets and β-cells purified by fluorescence-activated cell sorting (FACS), and that these effects are mediated by increased nitric oxide production. These results indicate, for the first time, that the viral replicative intermediate, dsRNA (in combination with IFN-γ) directly modulates islet viability and β-cell function by a mechanism that involves β-cell production of nitric oxide.

**EXPERIMENTAL PROCEDURES**

**Materials and Animals—**CMRL-1066 tissue culture medium, α-glutamine, penicillin, streptomycin, and rat recombinant IFN-γ were from Life Technologies, Inc. Fetal calf serum was obtained from Hyclone (Logan, UT). Male Sprague-Dawley rats (250–300 g) were purchased from Harlan (Indianapolis, IN). Polyinosinopolyclcytidylic acid (poly(IC)), aminoguanidine hemisulfate (AG), and collagenase type XI were from Sigma Chemical Co. [α-32P]dCTP and enhanced chemiluminescence reagents were purchased from Amersham Pharmacia Biotech. Human recombinant IL-1β was from Cistron Biotechnology (Pine Brook, NJ). Horseradish peroxidase-conjugated donkey anti-rabbit IgG was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Rabbit antisera specific for the C-terminal 27 amino acids of mouse macrophage iNOS was a gift from Dr. Thomas Misko (G. D. Searle, St. Louis, MO). iNOS and cyclophilin cDNAs were gifts from Dr. Charles Rodi (Monsanto Corporate Research, St. Louis, MO) and Dr. Steve Carroll (Dept. of Pathology, University of Alabama, Birmingham, AL), respectively. All other reagents were from commercially available sources.

**Islet Isolation and Culture—**Islets were isolated from male Sprague-Dawley rats by collagenase digestion as described previously (17). Following isolation, islets were cultured overnight in complete CMRL-1066 (CMRL-1066 containing 2 mM α-glutamine, 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin) under an atmosphere of 95% air and 5% CO2 at 37 °C. Before each experiment, islets were washed three times in complete CMRL-1066, counted, and then cultured for an additional 3 h at 37 °C. Experiments were initiated by the addition of poly(IC), cytokines, and iNOS inhibitors, followed by culture for the indicated times.

**Islet Dispersion and Macrophage Depletion—**Isolated rat islets were dispersed into individual cells by treatment with trypsin (1 mg/ml) in Ca2+- and Mg2+-free Hank's (17) at 37 °C for 3 min as stated previously (5, 17). For pseudoplate formation, dispersed rat islets were cultured for 7 days at 37 °C to allow for endocrine cell reaggregation (18). Alternatively, intact rat islets were cultured for 7 days in complete CMRL-1066 at 24 °C in an atmosphere of 95% air and 5% CO2 (19, 20). Islets were removed from the 24 °C culture, washed three times with fresh complete CMRL-1066, and then cultured for 2 days at 37 °C in complete CMRL-1066 (19, 20). Experiments were conducted as described above for freshly isolated islets.

**Purification of β-Cells by FACS—**Islets isolated from 12 rats were cultured overnight (~1,200/3 ml) in complete CMRL-1066 under an atmosphere of 95% air and 5% CO2 at 37 °C. Islets were then dispersed into individual cells as stated above. Dispersed islet cells were incubated for 60 min at 37 °C in complete CMRL-1066 before cell sorting. Islet cells were purified as described previously (21–23) using a FACStar (Becton Dickinson, San Jose, CA). The cells were illuminated at 488 nm, and emission was monitored at 515–535 nm. The sorting process yielded a 95% pure population of β-cells and an 80–85% pure population of α-cells.

**Insulin Secretion—**Islets (220 ml of complete CMRL-1066) were cultured for 40 h with the indicated concentrations of poly(IC), IL-1, IFN-γ, and AG. The islets were isolated and washed three times in Krebs-Ringer bicarbonate buffer (KR: 25 mM Heps, 115 mM NaCl, 24 mM NaHCO3, 5 mM KCl, 1 mM MgCl2, 2.5 mM CaCl2, and 0.1% bovine serum albumin, pH 7.4) containing 3 mM t-glucose, and insulin secretion was performed as described (5). FACS-purified β-cells were treated for 24 h with the indicated concentrations of IL-1, poly(IC), IFN-γ, and AG. The cells were washed three times with 0.1% bovine serum albumin, and 0.1% bovine serum albumin. β-cells were aliquoted into 96-well microtiter plates (15,000 cells/250 μl of KR containing either 3 mM or 20 mM d-glucose and 0.1% bovine serum albumin). β-cells were aliquoted into 96-well microtiter plates with the indicated concentrations of poly(IC), IL-1, IFN-γ, and AG. Islet degeneration was determined in a double-blind manner by phase-contrast microscopic analysis. Islet degeneration is characterized by the loss of islet integrity, disintegration, and partial dispersion of islets as described previously (4, 5, 19).

**Western Blot Analysis—**Rat islets (120/400 μl of complete CMRL-1066), cultured for the indicated times with poly(IC), IL-1, and rat IFN-γ were isolated, lysed, and proteins were separated by SDS-gel electrophoresis as described (5). Detection of rat iNOS was by enhanced chemiluminescence according to manufacturer’s specifications and as described previously (5).

**Northern Blot Analysis—**Rat islets (900/3 ml of complete CMRL-1066) were cultured for the indicated times at 37 °C with poly(IC) (50 μg/ml), IL-1β (1 unit/ml), and IFN-γ (150 units/ml). After culture, the cells were washed three times with 0.1x phosphate-buffered saline (pH 7.4), and total RNA was isolated using the RNeasy kit (Qiagen, Inc., Chatsworth, CA). Total cellular RNA (5–10 μg) was denatured, fractionated, and transferred to Duralon UV nylon membranes (Stratagene, La Jolla, CA) as described (5). Membranes were hybridized to a32P-labeled probe specific for rat iNOS or cyclophilin (27). The cDNA probe was radiolabeled with [α-32P]dCTP by random priming using the Prime-a-Gene nick translation system from Promega (Madison, WI). cDNA probes correspond to bases 509–1415 of the rat iNOS coding region. Cyclophilin was used as an internal control for RNA loading. Hybridization and autoradiography were performed as described previously (28).

**Statistical Analysis—**Results of one way analysis of variance were analyzed. Significant differences between treatment groups compared with untreated controls (indicated by *; p < 0.05) were evaluated using a Scheffe’s F-test post hoc analysis.

**RESULTS**

**dsRNA + IFN-γ Strenuates the Time- and Concentration-dependent Expression of iNOS and Production of Nitrile by Rat Islets—**To determine whether poly(IC) stimulates nitric oxide formation, rat islets were incubated for 40 h with 1–100 μg/ml poly(IC) and 150 units/ml IFN-γ. Alone, neither IFN-γ (5) nor poly(IC) stimulates nitrite production by rat islets; however, in combination with IFN-γ, poly(IC) stimulates the concentration-dependent increase in nitrite formation that is maximal at 50 μg/ml poly(IC) (Fig. 1). The level of nitrite produced in response to 50–100 μg/ml poly(IC) + IFN-γ is similar in magnitude to the levels produced in response to 1 unit/ml IL-1β and 150 units/ml IFN-γ.

The effects of poly(IC) + IFN-γ on iNOS mRNA accumulation and protein expression are shown in Fig. 1, b and c. Alone, poly(IC) and IFN-γ do not induce iNOS mRNA accumulation by rat islets (data not shown); however, in combination, poly(IC) + IFN-γ induce the time-dependent accumulation of iNOS mRNA that is first apparent at 6 h and maximal following an 18-h
Double-stranded RNA + IFN-γ Induces iNOS Expression by β-Cells

Fig. 1. Effects of poly(IC) and IFN-γ on nitrite production, iNOS mRNA, and protein expression by rat islets. Panel a, rat islets (120/400 μl of complete CMRL) were incubated for 40 h with the indicated concentrations of poly(IC), 150 units/ml IFN-γ, and 1 unit/ml IL-1. Nitrite production was determined on the culture medium as described under "Experimental Procedures." Panel b, rat islets (900/3 ml of complete CMRL-1066) were cultured for 6, 12, and 18 h with 50 μg/ml poly(IC) and 150 unit/ml IFN-γ. Total RNA was isolated and probed for iNOS and cyclophilin by Northern analysis as stated under "Experimental Procedures." iNOS mRNA levels were quantitated by phosphorimaging analysis (arbitrary phosphorimaging units) using cy- clophilin as an internal control for RNA loading. Panel c, rat islets (120/400 μl of complete CMRL) were incubated for 40 h with the indicated concentrations of IL-1, poly(IC), IFN-γ, and AG. The islets were isolated and glucose-stimulated insulin secretion was examined. Panel b, rat islets (25/500 μl of CMRL) were incubated for 96 h with the indicated concentrations of IL-1, poly(IC), IFN-γ, and AG and then islet degeneration was examined by phase-contrast microscopy in a double-blind manner as described under "Experimental Procedures." Results for insulin secretion are the average ± S.E. of four independent experiments and islet degeneration are the average ± S.E. of three independent experiments. Statistical significance, p < 0.05 versus control (*) as indicated.

Incubation period (Fig. 1b). Similarly, poly(IC) and IFN-γ alone do not induce iNOS protein expression by rat islets (data not shown, and Ref. 5); however, poly(IC) + IFN-γ induce the time-dependent expression of iNOS protein that is first apparent following a 24-h incubation period and maximal following a 48-h incubation period (Fig. 1c). The stimulatory effects of poly(IC) + IFN-γ on iNOS expression by rat islets are delayed in comparison to IL-1-induced iNOS expression, which is first apparent following a 6-h incubation period and maximal fol-
Treatment of rat islets with IL-1, or the combination of IL-1 + IFN-γ, for 96 h induces islet degeneration that is characterized by the loss of islet integrity, disintegration, and partial dispersion of islets into individual cells (4, 5, 19). The effects of dsRNA on islet viability are shown in Fig. 2b. Rat islets were incubated for 96 h with IL-1, poly(IC), IFN-γ, and poly(IC) + IFN-γ in the presence or absence of AG. Individually, poly(IC) and IFN-γ do not induce islet degeneration; however, the combination of poly(IC) + IFN-γ stimulates islet degeneration to levels comparable with the destructive effects of IL-1 (Fig. 2b). Islet destruction appears to be mediated by nitric oxide as AG completely prevents islet degeneration in response to IL-1 (Ref. 5 and Fig. 2b) and poly(IC) + IFN-γ. These findings provide evidence that dsRNA, in combination with IFN-γ, induces islet degeneration in a nitric oxide-dependent manner.

**Resident Macrophages Are not Required for the Inhibitory Actions of dsRNA + IFN-γ on Islet Function**—We have recently shown that poly(IC) + IFN-γ activate resident mouse macrophages, stimulating iNOS expression, nitric oxide formation, and IL-1 release (35). In addition, activation of resident macrophages by treatment of islets with TNF + LPS results in the expression of iNOS, production of nitric oxide, and inhibition of insulin secretion by a mechanism associated with the intracellular release of IL-1 (20, 24). Because poly(IC) + IFN-γ activates macrophages, stimulating nitric oxide production and IL-1 release, and resident islet macrophage IL-1 release (in response to TNF + LPS) results in the inhibition of insulin secretion, we examined the role of islet macrophages in poly(IC) + IFN-γ-induced iNOS expression, nitric oxide formation, and inhibition of insulin secretion by depleting rat islets of this cell population. Rat islets were dispersed into individual cells and then allowed to reaggregate during a 7 day culture at 37°C. These reaggregated islet cells, termed pseudoirlets, are composed of only endocrine cells (18). As shown in Fig. 3, the combination of poly(IC) + IFN-γ stimulates the production of nitrite and expression of iNOS to levels comparable with IL-1-induced nitrite formation and iNOS expression by rat pseudoirlets (Fig. 3, a and b, respectively). As a control for macrophage depletion, we show that TNF + LPS fail to stimulate iNOS expression or nitrite production by rat pseudoirlets. We have confirmed these findings using rat islets depleted of resident macrophages by culturing for 7 days at 24°C. Previous studies have shown that these culture conditions deplete greater than 95% of islet macrophages (19, 20). As shown in Fig. 3c, a 40 h incubation period of macrophage-depleted rat islets (by culturing for 7 days at 24°C with poly(IC) + IFN-γ) results in an 3-fold increase in nitrite production and a potent inhibition of insulin secretion. The stimulatory effects of poly(IC) + IFN-γ on nitrite formation and inhibitory effects on insulin secretion are similar in magnitude to the actions of IL-1. Importantly, TNF + LPS fails to inhibit insulin secretion or induce nitrite formation, indicating that resident macrophages have been depleted by the 7 day culture at 24°C. These results suggest that resident islet macrophages are not required for poly(IC) + IFN-γ-induced iNOS expression, nitrite formation, or the inhibition of insulin secretion by rat islets.

dsRNA + IFN-γ Stimulate iNOS Expression by FACS-purified β-Cells—Because resident macrophages are not required for dsRNA + IFN-γ-induced iNOS expression by rat islets, the effects of dsRNA + IFN-γ on α- and β-cell expression of iNOS were examined. α- and β-cells purified by FACS were treated for 40 h with IL-1, poly(IC), IFN-γ, and poly(IC) + IFN-γ. As shown in Fig. 4, poly(IC) + IFN-γ induce β-cell expression of iNOS to levels that are 2-fold higher than the levels of iNOS expressed in response to 1 unit/ml IL-1. Alone, neither poly(IC) nor IFN-γ induces iNOS expression by primary β-cells, and poly(IC) + IFN-γ (alone or in combination) do not induce iNOS expression by primary α-cells. These results indicate that the β-cell is one islet cellular source of iNOS in response to poly(IC) + IFN-γ.

Our previous studies have shown that treatment of FACS-purified β-cells for 18 h with IL-1 results in an inhibition of glucose-stimulated insulin secretion that is attenuated by co-incubation with AG (22, 24). Treatment of primary β-cells with poly(IC) + IFN-γ for 24 h results in a complete inhibition of glucose-stimulated insulin secretion (Fig. 5). AG prevents
poly(IC) + IFN-γ-induced inhibition of insulin secretion suggesting that the inhibitory effects are mediated by nitric oxide. In addition, we have previously shown that AG does not inhibit insulin secretion by purified β-cells in the absence of cytokine treatment (22, 24). These results show that dsRNA, in combination with IFN-γ, is able to act directly on the β-cell, stimulating iNOS expression and inhibiting insulin secretion in a nitric oxide-dependent manner.

DISCUSSION

The biochemical events that precipitate the initial destruction of pancreatic β-cells during the development of autoimmune diabetes have remained elusive. It has been proposed that viral infection of islets may “trigger” or initiate an autoimmune process in genetically predisposed individuals by stimulating the initial destruction of β-cells (7–11). However, few studies have examined the effects of viral infection on the function and viability of isolated islets. In this study, we have examined the effects of the viral replicative intermediate, dsRNA, on islet function and viability. dsRNA is the active component of a viral infection that stimulates antiviral responses in infected cells (13). We show that treatment of rat islets with dsRNA + IFN-γ results in the time- and concentration-dependent expression of iNOS and production of nitric oxide. dsRNA + IFN-γ-induced nitric oxide production results in a potent inhibition of insulin secretion and islet degeneration. One islet cellular source of iNOS appears to be the β-cell, because poly(IC) + IFN-γ induces iNOS expression and inhibits insulin secretion by primary β-cells purified by FACS. In addition, treatment of primary β-cells with dsRNA + IFN-γ results in the inhibition of insulin secretion. AG prevents the inhibitory effects of dsRNA + IFN-γ on β-cell function indicating that the destructive effects are mediated by β-cell production of nitric oxide. These results suggest that one potential mechanism by which a viral infection may mediate the initial damage to β-cells during the development of autoimmune diabetes is by the induction of iNOS expression by β-cells followed by nitric oxide-mediated inhibition of β-cell function and eventual islet damage. In support of this hypothesis, we have recently shown that incubation of rat islet cells with dsRNA + IFN-γ stimulates an ~3–4-fold increase in both β-cell DNA damage (colocalization of insulin with DNA damage assessed by immunohistochemistry and TdT-mediated dUTP nick-end labeling staining) and islet cell necrosis (determined by acridine orange/ethidium bromide staining), and that both effects are attenuated by NMMMA.

Viral infection and poly(IC) are classic activators of macrophages, stimulating antiviral responses such as cytokine production and nitric oxide production (36–44). We have recently shown that activation of resident mouse macrophages, by treatment with poly(IC) + IFN-γ, results in the induction of iNOS, the production of nitric oxide, and the release of IL-1 (35). In addition, treatment of rat islets with TNF + LPS (conditions known to activate macrophages) results in a potent inhibition of insulin secretion (20, 24). The inhibitory effects of TNF + LPS on insulin secretion are mediated by intracellular IL-1β release followed by IL-1β-induced iNOS expression by β-cells. Resident macrophages appear to be the source of IL-1β, as a 7-day culture of rat islets at 24 °C (conditions known to deplete islets of class II+ lymphoid cells, see Refs. 19 and 20) prevents TNF + LPS-induced iNOS expression, nitric oxide formation, and the inhibitory effects on insulin secretion. Immunocytochemical colocalization of IL-1β with the macrophage surface marker, ED1, directly supports the resident islet macrophage as the islets cellular source of IL-1β (20). Similar to the effects of TNF + LPS, poly(IC) + IFN-γ may activate resident macrophages, stimulating nitric oxide production and IL-1β release; however, our studies indicate that resident macrophages are not required for the inhibitory effects of poly(IC) + IFN-γ on islet function and viability. Removal of resident macrophages by culture for 7 days at 24 °C or pseudoislet formation does not inhibit poly(IC) + IFN-γ-induced iNOS expression, nitric oxide production, and the inhibition of insulin secretion. In addition, poly(IC) + IFN-γ stimulates iNOS expression and inhibit insulin secretion by β-cells purified by FACS. These results indicate that the inhibitory and destructive effects of dsRNA on rat islet function are not dependent upon resident macrophage production of nitric oxide or IL-1β but appear to be associated with a direct interaction of dsRNA with the β-cell. These findings provide evidence for a novel biochemical mechanism by which a viral infection may trigger the initial damage to β-cells leading to the development of autoimmune diabetes. Viral infection of β-cells and the accumulation of the viral replicative intermediate, dsRNA, in combination with IFN-γ supplied by peri-insulitic T-cells, would result in the induction

**Fig. 4.** Effects of poly(IC) and IFN-γ on iNOS expression by FACS-purified α- and β-cells. α- and β-cells (200,000 cells/200 μl of complete CMRL-1066) purified by FACS were incubated with the indicated concentrations of IL-1, poly(IC), and IFN-γ for 40 h. α- and β-cell iNOS expression was determined by Western blot analysis as described under “Experimental Procedures.” Results are representative of three independent experiments.

**Fig. 5.** Effects of poly(IC) and IFN-γ on glucose-stimulated insulin secretion by FACS-purified β-cells. β-cells purified by FACS were incubated for 24 h with the indicated concentrations of IL-1, poly(IC), IFN-γ, and AG. The cells were then isolated, and glucose-stimulated insulin secretion was performed as described under “Experimental Procedures.” Results are the average ± S.E. of four independent experiments containing three replicates/condition. Statistical significance, p < 0.05 versus control (*) as indicated.

A. L. Scarim, M. R. Heitmeier, and J. A. Corbett, manuscript in preparation.
of iNOS and the production of nitric oxide by β-cells, followed by nitric oxide-mediated inhibition of β-cell function and eventual β-cell damage.

Acknowledgments—we thank Jessica Gorman and Colleen Kelly for expert technical assistance.

REFERENCES

1. Gepts, W. (1965) Diabetes 14, 619–633
2. Welsh, N., Eizirik, D. L., Bendtzen, K., and Sandler, S. (1991) Endocrinology 129, 3167–3173
3. Corbett, J. A., Wang, J. L., Hughes, J. H., Wolf, B. A., Sweetland, M. A., Lancaster, J. R., Jr., and McDaniel, M. L. (1992) Biochem. J. 287, 229–235
4. Corbett, J. A., and McDaniel, M. L. (1994) Biochem. J. 309, 719–724
5. Heitmeier, M. R., Scarim, A. L., and Corbett, J. A. (1997) J. Biol. Chem. 272, 1397–1404
6. Takamura, T., Kato, I., Kimura, N., Nakazawa, T., Yonekura, H., Takasawa, S., and Okamoto, H. (1998) J. Biol. Chem. 273, 2485–2496
7. Yoon, J.-W. (1996) in Textbook of Diabetes Mellitus, pp. 14.1–14.14, Blackwell Science Ltd. Cambridge, MA
8. Yoon, J.-W. (1997) in Textbook of Diabetes Mellitus, pp. 14.1–14.14, Blackwell Science Ltd. Cambridge, MA
9. Yoon, J.-W. (1997) in Textbook of Diabetes Mellitus, pp. 14.1–14.14, Blackwell Science Ltd. Cambridge, MA
10. Yoon, J.-W. (1997) in Textbook of Diabetes Mellitus, pp. 14.1–14.14, Blackwell Science Ltd. Cambridge, MA
11. Yoon, J.-W. (1997) in Textbook of Diabetes Mellitus, pp. 14.1–14.14, Blackwell Science Ltd. Cambridge, MA
12. Hirasawa, K., Tasutsui, S., Takeda, M., Mizutani, M., Itagaki, S., and Doi, K. (1997) J. Gen. Virol. 77, 737–741
13. Jacobs, B. L., and Langland, J. O. (1996) Virology 219, 339–349
14. Ewel, C. H., Sobel, D. O., Zeligs, B. J., and Bellanti, J. A. (1992) Diabetes 41, 1016–1021
15. Sobel, D. O., Newsome, J., Ewel, C. H., Bellanti, J. A., Abbassi, V., Creswell, K., and Blair, O. (1992) Diabetes 41, 515–520
16. Huang, X., Hultgren, B., Dybdal, N., and Stewart, T. A. (1994) Immunity 1, 469–478
17. McDaniel, M. L., Colea, J. R., Katagai, N., and Lacy, P. E. (1983) Methods Enzymol. 20, 182–200
18. Hopcroft, D. W., Mason, D. R., and Scott, R. S. (1985) In Vitro Cell. Dev. Biol. 21, 421–427
19. Lacy, P. E., and Finke, E. H. (1991) Am. J. Pathol. 138, 1183–1190
20. Arnath, M., Scarim, A. L., Heitmeier, M. R., Kelly, C. B., and Corbett, J. A. (1998) J. Immunol. 160, 2684–2691
21. Corbett, J. A., Kwon, G., Misko, T. P., Rodi, C. P., and McDaniel, M. L. (1994) Am. J. Physiol. 267, C48–C54
22. Corbett, J. A., Wang, J. L., Sweetland, M. A., Lancaster, J. R., Jr., and McDaniel, M. L. (1999) J. Clin. Invest. 105, 965–971
23. Pilpel, D., and Engert, V. (1992) J. Biol. Chem. 267, 15067–15074
24. Corbett, J. A., and McDaniel, M. L. (1995) J. Exp. Med. 181, 559–568
25. Wang, J. L., Corbett, J. A., Marshall, C. A., and McDaniel, M. L. (1993) J. Biol. Chem. 268, 7785–7791
26. Wright, P. H., Makulu, D. R., Vichick, D., and Sussman, K. E. (1971) Diabetes 20, 33–45
27. Selden, R. F. (1991) in Current Protocols in Molecular Biology (Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K., eds), Vol. 1, pp. 4.2.1–4.2.6, Green Publishing Associates and Wiley-Interscience, New York
28. Burd, P. R., Rogers, H. W., Gordon, J. R., Martin, C. A., Jayaraman, S., Wilson, S. D., Dvorak, A. M., Galli, S. J., and Dorf, M. E. (1989) J. Exp. Med. 170, 245–257
29. Green, L. C., Wagner, D. A, Glosowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. (1982) Anal. Biochem. 126, 131–138
30. Southern, C., Schulster, D., and Green, I. C. (1990) FEBS Lett. 276, 42–44
31. Nerup, J., Mandrup-Poulsen, T., Helqvist, S., Andersen, H. U., Pociot, F., Reimers, J. I., Cuartero, B. G., Karlsen, A. E., Ijera, U., and Lorenzen, T. (1994) Diabetologia 37, Suppl. 2, 82–89
32. Corbett, J. A., Kwon, G., Hill, J. R., and McDaniel, M. L. (1995) In The Diabetes Annual 9 (Marshall, S. M., Home, P. D., and Rizza, R. A., eds), pp. 265–294, Elsevier Science B. V., Amsterdam, The Netherlands
33. Corbett, J. A., Wang, J. L., Sweetland, M. A., Lancaster, J. R., Jr., and McDaniel, M. L. (1999) J. Clin. Invest. 106, 2134–2139
34. Scarr, A. L., Heitmeier, M. R., and Corbett, J. A. (1997) Endocrinology 138, 5301–5307
35. Heitmeier, M. R., Scarr, A. L., and Corbett, J. A. (1998) J. Biol. Chem. 273, 15301–15307
36. Kerr, I. M., and Stark, G. R. (1982) J. Interferon Res. 12, 237–240
37. Samuel, C. E. (1991) Virology 183, 1–11
38. Lengyel, P. (1987) J. Interferon Res. 7, 511–519
39. Nathan, C. (1992) FASEB J. 5, 1216–1225
40. Faruqi, T. R., Erzurum, S. C., Kaneko, F. T., and DiCorleto, P. E. (1997) J. Biol. Chem. 272, H2490–H2497
41. Alexander, P., and Evans, R. (1971) Nature 229, 235–237
42. Hibbs, J. B., Jr., Taintor, R. R., and Vavrin, Z. (1987) Science 235, 473–476
43. Snell, J. C., Chernyshev, O., Gilbert, D. L., and Colton, C. A. (1997) J. Leukocyte Biol. 62, 369–373
44. Karupiah, G., Xie, Q.-W., Buller, R. M. L., Nathan, C., Duarte, C., and Mac-Micking, J. D. (1993) Science 261, 1445–1448