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Upregulation of Mitochondrial Peripheral Benzodiazepine Receptor Expression by Cytokine-Induced Damage of Human Pancreatic Islets

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Abstract Cytokines produced by immune system cells infiltrating pancreatic islets are candidate mediators of islet beta-cell destruction in autoimmune insulin-dependent diabetes mellitus. After 72 h exposure of human pancreatic islets to a cytotoxic cytokine combination of interleukin 1 beta (50 U/ml), tumor necrosis factor alpha (1,000 U/ml), and interferon gamma (1,000 U/ml), an increase of cell death vs. control islets was demonstrated by TUNEL and cell death detection ELISA method. Islet death was associated with apoptosis and mitochondrial swelling as evidenced by electron microscopy. This effect was correlated with a marked decrease of Bcl-2 mRNA expression (without any major change of Bax mRNA) and a marked increase of inducible nitric oxide synthase mRNA. Since peripheral benzodiazepine receptors constitute the aspecific mitochondrial permeability transition pore, and that it has been suggested to be involved in cytokine-induced cell death, we evaluated the effects of the cytotoxic cytokines on PBR density and mRNA expression. We demonstrated that cytokine treatment of human islets induced an increase of maximum density of ^{3}H1-(2-chlorophenyl-*N*-methyl-1-methylpropyl)-3- isoquinolinecarboxamide binding sites, ($5{,}110 \pm 193$ vs. $3{,}421 \pm 336$ fmol/mg proteins, $P < 0.05$) with no significant change in the affinity constant value (9.45 ± 0.869 vs. 8.7 ± 1.159 nM). Moreover, an increase of the expression of peripheral benzodiazepine receptor mRNA was observed, suggesting an increased transcription from the coding gene. These results suggest a possible role of peripheral benzodiazepine receptors in the organism response to tissue damage associated with inflammatory mediator production. J. Cell. Biochem. 84: 636–644, 2002.

Key words: diabetes mellitus; human islets; cytokines; peripheral benzodiazepine receptors

Type I (or insulin-dependent) diabetes (IDDM) results from a selective autoimmune destruction of pancreatic islet insulin-producing beta cells, which develops in genetically predisposed subjects [Kukreja and Maclaren, 1999].

Studies on IDDM animal models have shown that macrophages are the first cells to infiltrate the islets of Langerhans, followed by lymphocytes [Yoon et al., 1998]. An increased expression of cytokines in the insulitis has been reported, and different combinations of cytokines have been shown to decrease the function and viability of cells in vitro, partially through the production of nitric oxide and/or reactive oxygen species by the beta cells themselves or by cells in their vicinity, such as macrophages and endothelial cells [Mandrup-Poulsen, 1996].

It has been previously observed that exposure of human pancreatic islets to a combination of cytokines, interleukin 1-beta (IL-1beta) tumor necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma) severely impairs

Abbreviations used: IFN-gamma, interferon-gamma; TNF-alpha, tumor necrosis factor-alpha; IL-1beta, interleukin 1-beta; PBR, peripheral benzodiazepine receptors; ^{3}H PK11195, [3H] 1-(2-chlorophenyl-*N*-methyl-1-methylpropyl)-3-isoquinolinecarboxamide.

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beta cell function affecting DNA integrity and viability of human islet cells [Delaney et al., 1997; Piro et al., 2001] and eventually causing beta cell death by apoptosis or necrosis [Saldeen, 2000]. It has now become clear that mitochondria do exhibit major changes in their structure and function during apoptosis [Green and Kroemer, 1998; Kroemer et al., 1998; Lemasters et al., 1998]. In most cases of apoptosis, changes in mitochondrial permeability transition (MPT) precedes the major changes in cell morphology and biochemistry including nuclear DNA fragmentation. This change is indicative of enhanced permeability of membrane that has been related to the opening of a dynamic multiprotein pore formed in the contact site between the inner and outer mitochondrial membranes. Although the molecular elements constituting this pore have not been definitively established, they are presumed to derive from well-known membrane proteins, including the peripheral benzodiazepine receptor (PBR) complex which is constituted by the adenine nucleotide translocator (ANC), the porin molecule, and the 18 kDa isoquinoline binding protein [Gunter and Pfeiffer, 1990; Bernardi et al., 1994; Gunter et al., 1994; Zoratti and Szabo, 1995].

PBR seems to be involved in a wide spectrum of activities within the cells [Gavish et al., 1999], and its density is dramatically increased in a variety of pathological states also involving the release of cytokines [Bourdiol et al., 1991; Oh et al., 1992; Agnello et al., 2000; Rey et al., 2000].

It has been demonstrated that proinflammatory cytokines activate intracellular phosphorylation cascades involved in the transcriptional and translational modulation of gene expression, cell cycle arrest and cell death [Salituro et al., 1999].

In rat insulin-producing cells, it has been suggested that cytokines induce both necrosis and apoptosis via a common Bcl-2 inimitable pathway [Saldeen, 2000], which might involve MPT. In fact, the principal role of the anti-apoptotic protein Bcl-2 in preventing cell death was recently shown to be the inhibition of the MPT-pore opening induced by death receptor activation, such as TNF receptors [Adams and Cory, 1998; Ashkenazi and Dixit, 1998]. In addition, it is now ascertained that the citodestructive effects of cytokines on islet beta-cells involve the production of oxygen free radicals and ceramide [Sjoholm, 1998] that, during the premitochondrial phase of apoptosis, play an important role as endogenous effectors in the induction of MPT [Susin et al., 1998].

In a previous work we characterized PBRs in human pancreatic islets and demonstrated their role in the insulin release [Giusti et al., 1997]. In the present study, we evaluated the cytokine-induced damage of human pancreatic islets (monitored by electron microscopy and death marker expression levels) and, in parallel, PBR density and mRNA expression levels.

Based on the present results, we demonstrated that cytotoxic cytokine mediated human islet death and increased PBR site levels. The cytokines-induced upregulation of PBR expression levels may represent an amplifying process in mediating cytotoxic effects of cytokines at mitochondrial level or may compensate for the decrease in Bcl-2 levels to prevent the opening of the MPT pore.

MATERIALS AND METHODS

Islet Isolation

Pancreatic islets were prepared by collagenase digestion and density gradient purification with a yield of approximately 70% [Marchetti et al., 2000; Rosati et al., 2000]. To complete this study, 10 human pancreases (from donors aged 22–56 years) were used. At the end of the isolation procedure, the islets were resuspended in M199 culture medium and cultured at 37°C in a CO_2 incubator for 4–6 days. All the protocols had been approved by our local Ethics Committee.

Islet Survival and Bcl2, Bax and iNOS mRNA Expression Studies

Human islets were incubated for 72 h with a combination of 50 U/ml human recombinant IL-1beta+1,000 U/ml IFN-gamma+1,000 U/ml TNF-alpha, as previously described [Eizirik et al., 1994; Delaney et al., 1997].

The amount of dead cells was evaluated by the TUNEL technique (TUNEL DNA fragmentation kit, Roche, Germany), and the Cell Death Detection ELISA method (Roche), according to the procedure indicated by the manufacturer.

For RT-PCR studies, total RNA was extracted from purified human pancreatic islets with Trizol (Gibco-BRL, Grand Island, NY). First-strand cDNA synthesis was performed using

2 μg of each RNA sample primed with random examers with 200 U of Superscript II (Gibco-BRL, Gaithersburg, MD); 200 ng aliquots of cDNA were subsequently amplified in 100 μl reaction volume containing 20 pmol of upstream and downstream specific primers, 2.5 U of Taq DNA polymerase (Gibco-BRL), and 200 μM of each deoxynucleoside triphosphate and 1.5 mM $MgCl_2$.

The specific primer for human iNOS amplified a 461-bp product (sense: 5′-TCC GAG GCA AAC AGC ACA TTC A-3′; antisense: 5′-GGG TTG GGG GTG TGG TGA TGT-3′). The human Bcl2 primer pair (5′-ACA ACA TCG CCC TGT GGA TGA C-3′ and 5′-ATA GCT GAT TCG ACG TTT TGC C-3′) and human Bax primer pair (5′-GGC CCA CCA GCT CTG AGC AGA-3′ and 5′-GCC ACG TGG GCG TCC CAA AGT-3′) generated a 408-bp and a 477-bp product respectively.

Electron Microscopy

Control and cytokine treated human pancreatic islets were pelletted by centrifugation at 1,300*g* and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 for 1 h at 4°C. After rinsing in cacodylate buffer, islet pellets were postfixed in 1% cacodylate buffered osmium tetroxide for 2 h at room temperature, then dehydrated in a graded series of ethanol, briefly transferred to propylene oxide and embedded in Epon–Araldite. Ultrathin sections (60–80 nm thick) were cut with a diamond knife, placed on formvar-carbon coated copper grids (200 mesh), stained with uranyl acetate and lead citrate, and observed with a Jeol 100 SX transmission electron microscope.

Islet Membrane Preparations

Membranes were prepared from human islets as described in a previous work [Giusti et al., 1997]. Briefly, after washing twice with phosphate-buffered saline, pH 7.4, the islets were resuspended in 10 ml of 5 mM Tris-buffer (pH 8.0), supplemented with protease inhibitors (1 mmol/l phenylmethanesulfonyl-fluoride, soybean trypsin inhibitor (20 μg/ml), benzamidine (160 μg/ml), bacitracin (200 μg/ml), and then homogenized with an Ultra-Turrax homogenizer. The homogenate was then centrifuged at 48,000*g* for 15 min at 4°C and the supernatant was discarded. This washing step was repeated twice. The pellets from the second centrifugation were resuspended in 50 mM, pH 7.4, rehomogenized and centrifuged again at 48,000*g* for 15 min at 4°C. The resulting crude membrane fraction (50 μg of protein/ml) was used for binding assays. Membrane protein concentration was determined according to the Lowry method modified by Peterson using bovine serum albumin as standard [Peterson, 1977].

Binding Studies

Human islet crude membrane preparations from control islets and islets exposed to the cytokine combination were incubated in the presence of increasing ^3H PK11195 (DuPont de Nemours, Germany) concentrations. The binding assays were performed in 50 mM TRIS-HCl buffer, pH 7.4 in a final volume of 0.5 ml. Binding was terminated by rapid filtration through Whatman GF/C fiberglass filters followed by three washes with 5 ml ice-cold buffer. Radioactivity was counted on a Packard 1600 TR scintillation counter. Incubation was stopped by the addition of ice-cold buffer, and membrane-bound radioligand was collected onto Whatman GF/C fiberglass filters by vacuum filtration. The radioactivity trapped on the filters was counted using an LS 1800 scintillation counter. Displacement studies were performed with each single cytokine, used at concentrations ranging, respectively, from 10 to 500 U/ml for IL-1 beta, and 100–10,000 U/ml for TNF-alpha and IFN-gamma.

PBR mRNA Expression

The expression levels of PBR in human islets, following cytokine treatment, were evaluated by PCR-studies on total extracted RNA as described above. The specific primers for human 18 kDa PBRs sequence (sense: 5′-ACAGA-GAAGGCTGTGGTTCC-3′; antisense: 5′-CTC-ACTCTGGCAGCCGCCGTCC-3′) gave a 289-bp product [Riond et al., 1991]. Expression of beta-actin as RNA control was analyzed employing the following primers, generating a 354-bp product: 5′-ACC AAC TGG GAG GAG ATG GAG-3′ and 5′-CGT GAG GAT CTT CAT GAG GTA AGT C-3′.

Analysis of Results

The results obtained by the TUNEL and the ELISA methods were expressed as mean ± SD, and the data were compared by the Student's *t*-test. The statistical analysis and curve-fitting of the binding results were carried using GraphPad Instat.

RESULTS

By the TUNEL technique, it was demonstrated that the amount of dead cells was significantly ($P < 0.05$) higher in the islets cultured for 72 h with the cytokine combination ($37.4 \pm 2.8\%$) than in control islets ($13.8 \pm 1.7\%$). This was confirmed by the ELISA data that showed an increased ($P < 0.05$) amount of oligonucleosomes in cytokine treated cells (OD: 2.6 ± 0.4) compared to control ones (OD: 0.8 ± 0.2). The semiquantitative RT-PCR experiments demonstrated a marked decrease of Bcl-2 mRNA expression and a marked increase of iNOS mRNA expression in cytokine exposed islets with respect to control islets (without any major change of Bax mRNA) (Fig. 1).

At the electron microscope level, beta cells from untreated pancreatic islets showed the typical ultrastructure characterized by a nucleus with finely granular and uniformly dispersed chromatin, a cytoplasm containing randomly distributed organelles, the characteristic granules, and a well-preserved plasma membrane (Fig. 2–4(2)). In cytokine exposed pancreatic islets signs of apoptosis were observed in beta cells after 48 h treatment. The morphological changes observed in these cells included progressive margination and condensation of chromatin (Fig. 2–4(3)), nucleus fragmentation, cytoplasmic shrinkage (Figs. 2–4(4) and 5–6(5)) and the eventual formation of membrane-bound cell fragments (apoptotic bodies) of different size in which the nuclear material and cell organelles were randomly distributed (Figs. 5–6(6)). In early stages of apoptosis, mitochondria appeared to maintain their integrity (Figs. 2–4(3&4)) while in later stages, after DNA fragmentation, they showed some alterations (Fig. 5–6(5)).

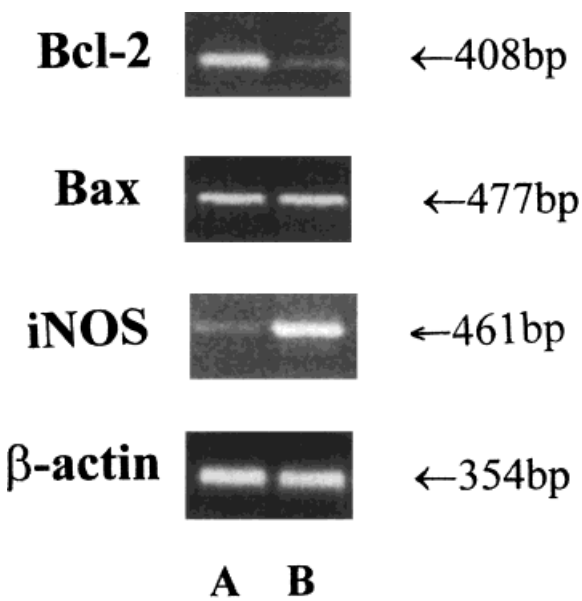


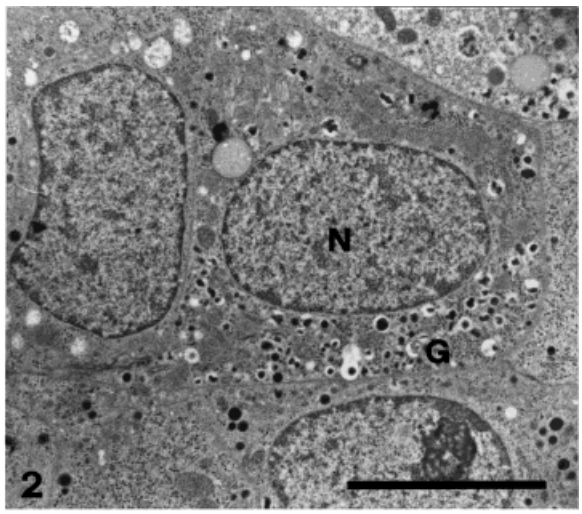
Fig. 1. Expression of Bcl-2, Bax, and iNOS mRNA in human pancreatic islets exposed to control medium (**A**) or a combination of IL-1beta, TNF-alpha, and IFN-gamma (**B**). Experiment was carried out three times with similar results.

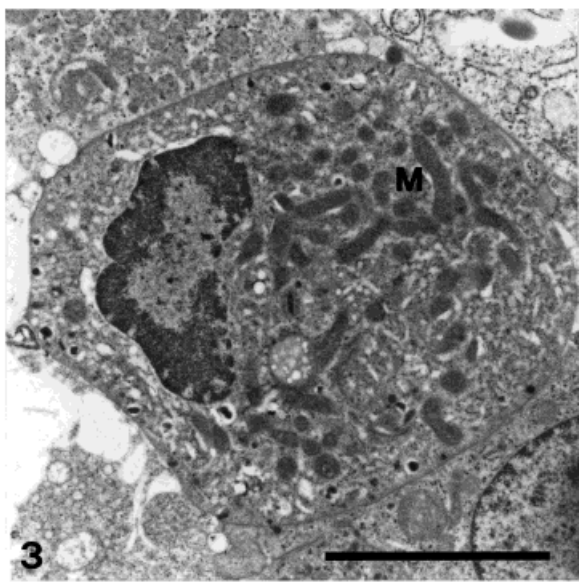
The experiments with islet membrane preparations confirmed that binding of labeled PK11195 was saturable and with high affinity; non-specific binding was less than 10%. Equilibrium binding parameters, dissociation constant (K_d) values and maximum numbers of binding sites (B_{max}), were obtained from linear Scatchard analysis of ^{3}H PK11195 saturation curves, as shown in Figure 7. As described in Table I, in untreated control islets, Scatchard analysis of ^{3}H PK11195 binding data suggested the presence of a homogeneous population of high-affinity binding sites with a K_d of 8.7 ± 1.16 nM. The incubation of the islets with a combination of 50 U/ml IL-1beta, 1,000 U/ml TNF-alpha and 1,000 U/ml IFN-gamma for 72 h induced a significant increase of B_{max} value of PBR binding sites from $3{,}421 \pm 336$ to $5{,}110 \pm 193$ fmol/mg of proteins. This 1.5-fold increase in binding was not accompanied by a significant change in the K_d value (9.45 ± 0.86 vs. 8.7 ± 1.15 nM in control islets). These data suggested that the increase in ^{3}H PK11195 binding, induced by cytokine treatment of pancreatic islets, was due to an upregulation in the number of the total binding sites and not a change in receptor affinity. The PBR binding to control islet membranes was not affected by either alone or combinated cytokines as demonstrated by competition binding assays (data not shown).

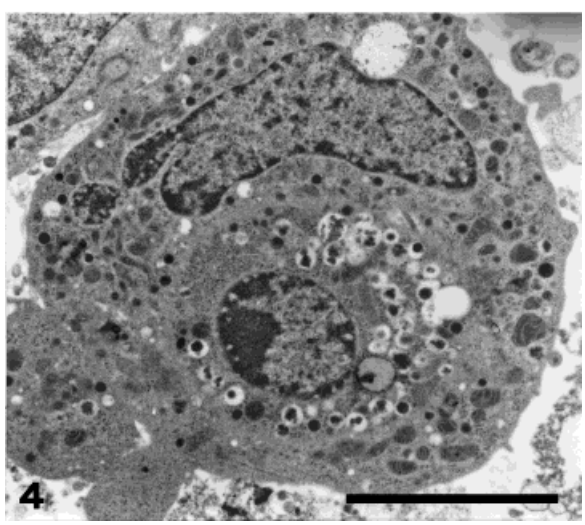
To evaluate whether the change in PBR density was determined either by an increase of mitochondrial membrane receptor availability or by an increased transcription of PBR coding gene, RT-PCR studies were performed as described in Materials and Methods. As shown in Figure 8, an increased expression of PBR mRNA was observed in the islets cultured for 72 h in the presence of IL-1beta, TNF-alpha, and IFN-gamma with respect to control islets.

DISCUSSION

The present study showed that a combination of IL-1beta, TNF-alpha, and IFN-gamma



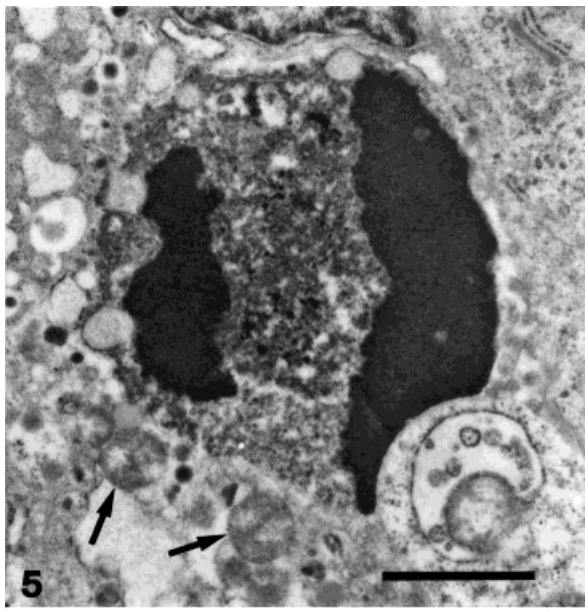


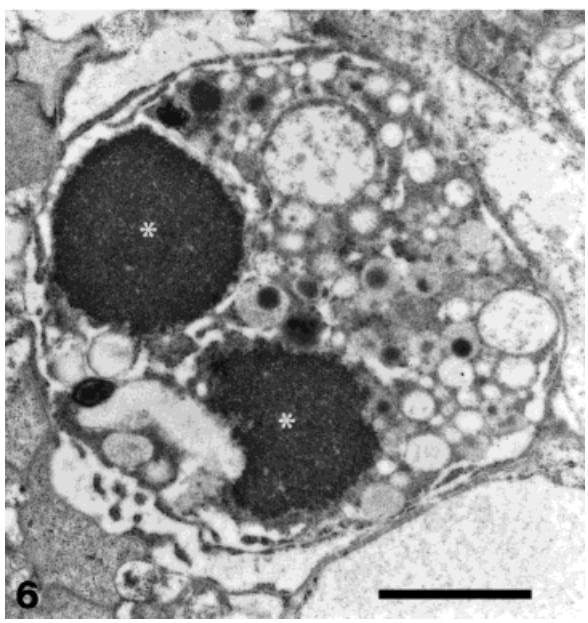


induces cell death in human islet cells, and that this effect was accompanied by an increased expression of iNOS mRNA. In turn, iNOS generates the free radical nitric oxide from L-arginine, and this molecule may exert a toxic effect on islet cells by inhibiting iron-dependent enzymes, thereby impairing cellular mithocondrial function and DNA synthesis [Adeghate and Parvez, 2000]. Ultrastructural analysis showed dying beta cells with typical morphological features of apoptosis after 48 h of cytokine treatment. Moreover, the presence of widespread typical apoptotic bodies and mitochondria swelling were remarkable findings after 72 h of cytokine treatment demonstrating a direct dangerous effect of cytokines on mitochondria integrity.

A reduction of Bcl-2 mRNA expression in cytokine exposed islets was also observed. Bcl-2 is a member of the growing family of apoptosis-regulator gene products which may either promote cell survival (Bcl-2, Bcl-XL, Bcl-w, and so on) or encourage cell demise (Bax, Bak, Bad, and so on). The relative amount of death agonists and antagonists from the Bcl-2 family is a regulatory rheostat which functions by selective protein–protein interaction. Bcl-2 and its homologous proteins inserted in intracellular membranes, including those of mithocondria, regulate mitochondrial changes preceding the activation of apoptogenic proteases and nucleases. In particular, Bcl-2 prevents all hallmarks of the early stages of apoptosis, such as the disruption of the inner mitochondrial transmembrane potential and the release of apoptogenic protease activators from mitochondria [Susin et al., 1996; Basu and Haldar, 1998]. As a matter of fact, Bcl-2 overexpression induces apoptosis-resistance in islet cell lines and can prevent the destructive effect of combined

Figs. 2–4. **2**: TEM micrograph of untreated beta cells showing a nucleus (N) with finely dispersed chromatin and a cytoplasm with randomly distributed organelles and granules (G). Bar, 5 μm. **3**: TEM micrograph of a cytokine treated beta cell (48 h cytokine incubation) at an initial step of apoptosis. Compact chromatin is arranged at the nuclear periphery and exhibits a granular appearance. Numerous dispersed and well-preserved mitochondria (M) and few granules are present in the cytoplasm. Bar, 5 μm. **4**: TEM micrograph of a cytokine treated beta cells (48 h cytokine incubation) in an advanced stage of apoptosis. Note the nuclear fragmentation and the condensation of the cytoplasm. Mitochondria are uniformly dispersed and still maintain their integrity. Bar, 5 μm.





Figs. 5–6. **5**: TEM micrograph of a cytokine treated beta cell (72 h cytokine incubation) in an advanced stage of apoptosis. The nucleus contains two prominent masses of condensed chromatin. In the degraded cytoplasm, large vacuoles and swollen mitochondria (arrow) showing cristolysis are evident. Bar, 2 μm. **6**: TEM micrograph of an apoptotic body representing the final stage of apoptotic process (72 h cytokine incubation). Note two micronuclei (*) and remnants of the degraded cytoplasm. Bar, 2 μm.

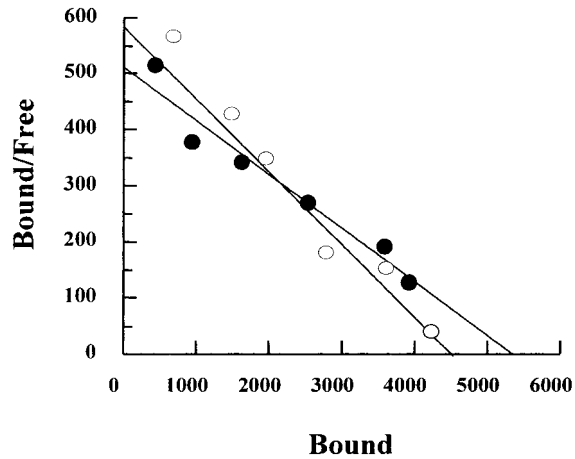


Fig. 7. Representative Scatchard plot of saturation binding data for ^{3}H PK 11195 to membranes from control untreated human islets (○) and from cytokine treated islets (●) of a single experiment performed twice with similar results. Membranes were incubated with increasing concentrations of ^{3}H PK 11195 ranging from 1 to 30 nM. Nonspecific binding was measured in the presence of 1 μM PK 11195. For K_d and B_{max} values, see Table I.

proinflammatory and Th1 cytokines in human islets [Rabinovitch et al., 1999].

Interestingly, Bcl-2 and Bax are particularly abundant exactly where the mitochondrial permeability transition pore is localized [Cai et al., 1998; Marzo et al., 1998; Ortiz et al., 2000]. MPT pore is a multiprotein complex which participates in the regulation of several mitochondrial key functions, including matrix Ca^{2+} concentration, pH, transmembrane proton gradient, and others [Gunter and Pfeiffer, 1990; Bernardi et al., 1994; Gunter et al., 1994; Zoratti and Szabo, 1995]. Among the proteins involved in the composition and function of MPT, we have focused on the PBRs and we have shown that, in our experimental conditions, the increased islet cell death and the decreased Bcl-2 expression were associated with an increase in the PBR binding density and mRNA expression levels. It has been previously

TABLE I. Effect of Human Islet Cytokine Treatment on ^{3}H PK 11195 Binding Parameters

	$K_d \pm$ SEM (nM)	$B_{max} \pm$ SEM (fmol/mg proteins)
Control islets	8.7 ± 1.159	3421 ± 336
Cytokine-treated islets	9.45 ± 0.869	5110 ± 193*

Human islets were incubated for 72 h with a control medium or a combination of 50 U/ml IL-1beta, 1,000 U/ml IFN-gamma, and 1,000 U/ml TNF-alpha. ^{3}H PK 11195 K_d and B_{max} values were obtained by Scatchard analysis as described in Materials and Methods. The values represent the mean ± SEM of three separate experiments performed on three islet preparations immediately used from three donors. The separate experiments were performed twice with similar results.
*$P < 0.05$ by unpaired Student's t-test.

Fig. 8. Expression of 18 kDa PBR mRNA in human pancreatic islets exposed to control medium (**A**) or a combination of IL-1 β, TNF-α, and IFN-γ (**B**). Experiment was carried out three times with similar results.

shown that murine thymocytes incubated with peripheral type benzodiazepines underwent apoptosis, and that this phenomenon (not observed after incubation with central type benzodiazepines) was correlated with perturbations of the mitochondrial transmembrane potential [Hirsch et al., 1998]. In addition, the PBR agonist PK-11195 was found to facilitate the induction of apoptosis by several conditions on different cell lines, and to reverse the protection induced by Bcl-2 [Alenfall et al., 1998; Tanimoto et al., 1999]. Moreover, it has been demonstrated that peripheral benzodiazepine receptor agonists that induce MPT, potentiate TNF-induced cell death that is correlated with MPT occurrence [Pastorino et al., 1996]. These findings could suggest a synergic pathway of cytokines and PBR agonists in the regulation of MTP pore opening, that is a self-amplifier process involved in the "decision to die" of the cell. However, in lymphoblastoid cells [Bono et al., 1999] and in Leydig cells [Rey et al., 2000], an anti-apoptotic effect of PBR and PBR agonists has been demonstrated. In particular, Rey et al. [2000] hypothesized that the TNF-alpha-induced upregulation of PBR expression may prevent the opening of the MPT pore and was responsible for the cell resistance to TNF-alpha cytotoxicity.

We obtained evidences that, in isolated human pancreatic islets, the exposure to a combination of cytotoxic cytokines causes an increase of PBR density and mRNA expression, the latter suggesting an increased transcription from the coding gene. In vitro and in vivo studies suggest that certain proinflammatory cytokines, such as IL-1beta and TNF-alpha, may be involved in the upregulation of PBR within the injured central nervous system [Bourdiol et al., 1991; Oh et al., 1992]. Although it is not known how these cytokines upregulate PBR in pathological diseases, it is possible that a receptor-mediated transduction pathway regulating the intracellular gene trascription may be activated. Cytokines bind to specific receptors of beta-cells and elicit a number of proreceptor events by activating phospholipase, proteases, nitric oxide synthase, and by increasing oxygen-free radical production and intracellular ceramide levels. Potential targets of ceramide in the control of cell fate are different MAP kinase pathways involved in the control of cell survival/death, cytoskeletal reorganization and nuclear gene expression [Blazquez et al., 2000]. In this view, the increase of mRNA PBR expression observed following cytokine islet treatment might be the result of a nuclear gene transcription regulation elicited by cytokines through the MAP kinase cascade. As previously described in rat cortex and striatum [Bourdiol et al., 1991], cytotoxic cytokines increase the availability of PBRs in human pancreatic islet cells, and this effect might favor the cytokine-induced islet cell death. The cytokine-mediated increase of PBR density might enhance the susceptibility of cells to the induction of apoptosis by a synergic mechanism as previously described [Hirsch et al., 1998]. In fact, proapoptotic signal transduction pathways, activated also by cytokines, provoke MPT, activating a self-amplifying process that culminates in the apoptogenic factor release from mitochondria [Susin et al., 1998]. PBR, increased by cytokine production, may participate as amplifier of the apoptosis mitochondrial phase contributing to the decision of the cell to die. This raises the possibility that in certain clinical conditions, such as Type I diabetes, modulation of PBRs activity might affect cell survival and might suggest PBR as a marker of the disease. On the contrary, it is also possible that a PBR expression increase, induced by cytokines, represents a defense mechanism of the cell to counteract the reduction of anti-apoptotic protein Bcl-2. Studies are in progress to shed light on the role of PBR in the control of human islet death induced by proinflammatory cytokines.

REFERENCES

Adams JM, Cory S. 1998. The Bcl-2 protein family: arbiters of cell survival. Science 281:1322–1326.

Adeghate E, Parvez SH. 2000. Nitric oxide and neuronal and pancreatic beta cell death. Toxicology 153:143–156.

Agnello D, Carvelli L, Muzio V, Villa P, Bottazzi B, Polentarutti N, Pennini T, Mantovani A, Grezzi P. 2000. Increased peripheral benzodiazepine binding sites and pentraxin 3 expression in the spinal cord durino

EAE: relation to inflammatory cytokines and modulation by dexamethasone and rolipram. J Neuroimmunol 109:105–111.

Alenfall J, Kant R, Batra S. 1998. Cytotoxic effects of ^{125}I-labeled PBZr ligand PK 11195 in prostatic tumor cells: therapeutic implications. Cancer Lett 134:187–192.

Ashkenazi A, Dixit VM. 1998. Death receptors: signalling and modulation. Science 281:1305–1308.

Basu A, Haldar S. 1998. The relationship between Bcl2, Bax and p53: consequences for cell cycle progression and cell death. Mol Hum Reprod 4:1099–1112.

Bernardi P, Broekemeier KM, Pfeiffer DR. 1994. Recent progress on regulation of the mitochondrial permeability transition pore; a cyclosporin-sensitive pore in the inner mitochondrial membrane. J Bioenerg Biomembr 26:509–517.

Blazquez C, Galve-Roperh I, Guzman M. 2000. De novo-synthesized ceramide signals apoptosis in astrocytes via extracellular signal-regulated kinase. FASEB J 14: 2315–2322.

Bono F, Lamarche I, Prabonnaud V, LeFur G, Herbert JM. 1999. Peripheral benzodiazepine receptor agonists exhibit potent antiapoptotic activities. Biochem Biophys Res Commun 265:457–461.

Bourdiol F, Toulmond S, Serrano A, Benavides J, Scatton B. 1991. Increase in omega 3 (peripheral type benzodiazepine) binding sites in the rat cortex and striatum after local injection of interleukin-1, tumor necrosis factor-alpha and lypopolysaccharide. Brain Res 543:194–200.

Cai J, Yang J, Jones DP. 1998. Mitochondrial control of apoptosis: the role of cytochrome c. Biochim Biophys Acta 1366:139–149.

Delaney CA, Pavlovic D, Hoorens A, Pipeleers DG, Eizirik DL. 1997. Cytokines induce deoxyribonucleic acid strand breaks and apoptosis in human pancreatic islet cells. Endocrinology 138:2610–2614.

Eizirik DL, Sandler S, Welsh N, Cetkovic-Cvrlje M, Nieman A, Geller DA, Pipeleers DG, Bendtzen K, Hellerstrom C. 1994. Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. J Clin Invest 93:1968–1974.

Gavish M, Backman I, Shoukrun R, Katz Y, Veenman L, Weisinger G, Weizman A. 1999. Enigma of the peripheral benzodiazepine receptor. Pharmacol Rev 51:629–650.

Giusti L, Marchetti P, Trincavelli L, Lupi R, Martini C, Lucacchini A, Del Guerra S, Tellini C, Carmellini M, Navalesi R. 1997. Peripheral benzodiazepine receptors in isolated human pancreatic islets. J Cell Biochem 64:273–277.

Green D, Kroemer G. 1998. The central executioners of apoptosis: caspases or mithocondria? Trends Cell Biol 8:267–271.

Gunter TE, Pfeiffer DR. 1990. Mechanisms by which mitochondria transport calcium. Am J Physiol 258: C755–786.

Gunter TE, Gunter KK, Sheu SS, Gavin CE. 1994. Mitochondrial calcium transport: physiological and pathological relevance. Am J Physiol 267:C313–C339.

Hirsch T, Decaudin D, Susin SA, Marchetti P, Larochette N, Resche-Rigon M, Kroemer G. 1998. PK11195, a ligand of the mitochondrial benzodiazepine receptor, facilitates the induction of apoptosis and reverses Bcl-2-mediated cytoprotection. Exp Cell Res 241:426–434.

Kroemer G, Dallaporta B, Resche-Rigon M. 1998. The mitochondrial death/life regulator in apoptosis and necrosis. Annu Rev Physiol 60:619–642.

Kukreja A, Maclaren NK. 1999. Autoimmunity and diabetes. J Clin Endocrinol Metab 84:4371–4378.

Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, Cascio WE, Bradham CA, Brenner DA, Herman B. 1998. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. Biochim Biophys Acta 1366:177–196.

Mandrup-Poulsen T. 1996. The role of interleukin-1 in the pathogenesis of IDDM. Diabetologia 39:1005–1029.

Marchetti P, Dotta F, Ling Z, Lupi R, Del Guerra S, Santangelo C, Realacci M, Marselli L, Di Mario U, Navalesi R. 2000. The function of pancreatic islets isolated from Type I diabetic patients. Diabetes Care 23:701–703.

Marzo I, Brenner C, Zanzami N, Susin SA, Beutner G, Brdiczka D, Remy R, Xie ZH, Reed JC, Kroemer G. 1998. The permeability transition pore complex: a target for apoptosis regulation by caspases and bcl2 related proteins. J Exp Med 187:1261–1271.

Oh YJ, Francis JW, Markeloins GJ, Oh TH. 1992. Interleukin-1- and tumor necrosis factor-increase peripheral benzodiazepine binding sites in cultured polygonal astrocytes. J Neurochem 58:2131–2138.

Ortiz A, Lorz C, Catalan MP, Justo P, Egido J. 2000. Role and regulation of apoptotic cell death in the kidney. Y2K update. Front Biosci 5:D735–D749

Pastorino JG, Simbula G, Yamamoto K, Glascott PA. 1996. The cytotoxicity of tumor necrosis factor depends on induction of the mitochondrial permeability transition. J Biol Chem 47:29792–29798.

Peterson GL. 1977. A simplification of the protein assay method which is more generally applicable. Anal Biochem 84:346–356.

Piro S, Lupi R, Dotta F, Patane G, Rabuazzo MA, Marselli L, Santangelo C, Realacci M, Del Guerra S, Purrello F, Marchetti P. 2001. Bovine islets are less susceptible than human islets to damage by human cytokines. Transplantation 71:21–26.

Rabinovitch A, Suarez-Pinzon W, Strynadka K, Ju Q, Edelstein D, Brownlee M, Korbutt GS, Rajotte RV. 1999. Transfection of human pancreatic islets with an anti-apoptotic gene (bcl2) protects beta-cells from cytokine-induced destruction. Diabetes 48:1223–1229.

Rey C, Mauduit C, Naureils O, Benahmed M, Louisot P, Gasnier F. 2000. Upregulation of mitochondrial peripheral benzodiazepine receptor expression by tumor necrosis factor alpha in testicular leydig cells. Biochem Pharmacol 60:1639–1646.

Riond J, Mattei MG, Kaghad M, Dumont X, Guillemot JC, Le Fur G, Caput D, Ferrara P. 1991. Molecular cloning and chromosomal localization of a human peripheral-type benzodiazepine receptor. Eur J Biochem 195:305–311.

Rosati B, Marchetti P, Crociari O, Lecchi M, Lupi R, Arcangeli A, Olivotto M, Wanke E. 2000. Glucose-and arginine induced insulin secretion by human pancreatic beta-cells: the role of HERG K+ channels is firing and release. FASEB J 14:2601–2610.

Saldeen J. 2000. Cytokines induce both necrosis and apoptosis via a common Bcl-2 inhibitable pathway in rat insulin-producing cells. Endocrinology 141:2003–2010.

Salituro FG, Germann UA, Wilson KP, Bemis GW, Fox T, Su MS-S. 1999. Inhibitors of p38 MAP kinase: therapeutic intervention in cytokine-mediated diseases. Curr Med Chem 6:807–823.

Sjoholm A. 1998. Aspects of the involvement of interleukin-1 and nitric oxide in the pathogenesis of insulin-dependent diabetes mellitus. Cell Death Differ 5:461–468.

Susin SA, Zanzami N, Castedo M, Hirsch T, Marchetti P, Macho A, Daugas E, Geuskens M, Kroemer G. 1996. Bcl-2 inhibits the mitochondrial release of an apoptogenic proteases. J Exp Med 184:1331–1342.

Susin SA, Zanzami N, Kroemer G. 1998. Mitochondria as regulators of apoptosis: doubt no more. Biochim Biophys Acta 1366:151–165.

Tanimoto Y, Onishi Y, Sato Y, Kizaki H. 1999. Benzodiazepine receptor agonists modulate thymocyte apoptosis through reduction of the mitochondrial transmembrane potential. Jpn J Pharmacol 79:177–183.

Verma A, Facchina SL, Hirsch DJ, Song S-Y, Dillahey LF, Williams JR, Snyder SH. 1996. Photodynamic tumor therapy: mitochondrial benzodiazepine receptors as a therapeutic target. Mol Med 4:40–45.

Yoon JW, Jun HS, Santamaria P. 1998. Cellular and molecular mechanisms for the initiation and progression of beta cell destruction resulting from the collaboration between macrophages and T cells. Autoimmunity 27:109–122.

Zoratti M, Szabo I. 1995. The mitochondrial permeability transition. Biochim Biophys Acta 1241:139–176.