Insulin-like growth factors prevent cytokine-mediated cell death in isolated islets of Langerhans from pre-diabetic non-obese diabetic mice

D J Hill[1,3,4,8], J Petrik[1,3,8], E Arany[1,4], T J McDonald[1,4,5,6,9] and **T L Delovitch[2,4,7]**

[1]Lawson Research Institute, St Joseph's Health Centre, London, Ontario N6A 4V2, Canada

[2]The Autoimmunity and Diabetes Group, The John P Robarts Research Institute, 1400 Western Road, London, Ontario N6G 2V4, Canada

[3]Department of Physiology, University of Western Ontario, London, Ontario N6A 5A5, Canada

[4]Department of Medicine, University of Western Ontario, London, Ontario N6A 5A5, Canada

[5]Department of Biochemistry, University of Western Ontario, London, Ontario N6A 5A5, Canada

[6]Department of Pharmacology and Toxicology, University of Western Ontario, London, Ontario N6A 5A5, Canada

[7]Department of Microbiology and Immunology, University of Western Ontario, London, Ontario N6A 5A5, Canada

[8]Department of Paediatrics, University of Western Ontario, London, Ontario N6A 5A5, Canada

[9]London Health Sciences Centre, London, Ontario N6A 5A5, Canada

(Requests for offprints should be addressed to D J Hill, Lawson Research Institute, St Joseph's Health Centre, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada)

Abstract

Interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) contribute to the initial stages of the autoimmune destruction of pancreatic β cells. IL-1β is released by activated macrophages resident within islets, and its cytotoxic actions include a stimulation of nitric oxide (NO) production and the initiation of apoptosis. Insulin-like growth factors (IGFs)-I and -II prevent apoptosis in non-islet tissues. This study investigated whether IGFs are cytoprotective for isolated islets of Langerhans from non-obese diabetic mice (NOD) mice exposed to cytokines. Pancreatic islets isolated from 5–6-week-old, pre-diabetic female NOD mice were cultured for 48 h before exposure to IL-1β (1 ng/ml), TNF-α (5 ng/ml), IFN-γ (5 ng/ml) or IGF-I or -II (100 ng/ml) for a further 48 h. The incidence of islet cell apoptosis was increased in the presence of each cytokine, but this was significantly reversed in the presence of IGF-I or -II (IL-1β control 3·5 ± 1·6%, IL-1β 1 ng/ml 27·1 ± 5·8%, IL-1β+IGF-I 100 ng/ml 4·4 ± 2·3%, $P<0·05$). The majority of apoptotic cells demonstrated immunoreactive glucose transporter 2 (GLUT-2), suggesting that they were β cells. Islet cell viability was also assessed by trypan blue exclusion. Results suggested that apoptosis was the predominant cause of cell death following exposure to each of the cytokines. Co-incubation with either IGF-I or -II was protective against the cytotoxic effects of IL-1β and TNF-α, but less so against the effect of IFN-γ. Exposure to cytokines also reduced insulin release, and this was not reversed by incubation with IGFs. Immunohistochemistry showed that IGF-I was present *in vivo* in islets from pre-diabetic NOD mice which did not demonstrate insulitis, but not in islets with extensive immune infiltration. Similar results were seen for IGF-binding proteins (IGFBPs). These results suggest that IGFs protect pre-diabetic NOD mouse islets from the cytotoxic actions of IL-1β, TNF-α and IFN-γ by mechanisms which include a reduction in apoptosis.

Journal of Endocrinology (1999) **161,** 153–165

Introduction

In humans, type 1 diabetes is associated with a T lymphocyte and monocytic infiltration of the pancreas leading to insulitis, and T-cells directed against intrinsic β cell antigens have been isolated (Gepts & Lecompte 1981, Pankewycz *et al.* 1995). Using rodent models of spontaneous diabetes such as the non-obese diabetic (NOD) mouse, the crucial mediatory role of T-cells has been confirmed (Castano & Eisenbarth 1990, Elliott & Flavell 1994). However, the major population of infiltrating immune cells during the early stages of insulitis in animal models of diabetes are macrophages, which are capable of releasing cytokines such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) which can induce β cell damage. An inhibition of the cytotoxic actions of macrophage-derived cytokines may prevent initial β cell damage and limit autoantigen release.

0022–0795/99/0161–0153 *Printed in Great Britain*

Online version via http://www.endocrinology.org

The NOD mouse is a useful model of autoimmune diabetes in which an insulitis precedes β cell destruction in the majority of female animals, but not males. At 5–6 weeks, females show a heterogeneity of pancreatic islet morphology, with some islets demonstrating heavy infiltration of immune cells, and others being untouched.

Exposure of rat islets to exogenous IL-1β for 6 h or less induces a transient increase in glucose-stimulated insulin release, but a more prolonged exposure causes a decrease in β cell insulin synthesis and content, a decrease in DNA synthetic rate when using neonatal islets, and cell death (Mandrup-Poulsen 1986, Comens *et al.* 1987, Spinas *et al.* 1987). Human islets may be less sensitive to the detrimental effects of IL-1β (Rabinovitch *et al.* 1990, Mandrup-Poulsen 1996), and the mechanisms of cell death following cytokine exposure may also involve necrosis (Delaney *et al.* 1996). The initiation of β cell damage in rats by IL-1β requires signalling via the high affinity IL-1 receptor, mRNA transcription and *de novo* protein synthesis, and diminished mitochondrial function (Helqvist *et al.* 1989, Hammonds *et al.* 1990). IL-1-induced damage to β cell function and the loss of viability are both linked to the production of free radicals such as nitric oxide (NO), and specific inhibitors of NO formation will maintain β cell viability (Stamler *et al.* 1992, Kaneto *et al.* 1995). Corbett & McDaniel (1995) and Delaney *et al.* (1993) have demonstrated that an intra-islet release of IL-1 following passenger macrophage activation leads to the expression of inducible NO synthase (NOS) within the β cells, and consequent damage. Other cytokines, such as TNF-α and IFN-γ have also been shown to induce NO formation in β cells, and in potentially adjacent cells within the islets such as macrophages, endothelial cells and fibroblasts (Amber *et al.* 1988, Kilbourn & Belloni 1990). Ankarcrona *et al.* (1994) and Suarez-Pinzon *et al.* (1994) both showed that the ability of IL-1β, TNF-α and IFN-γ to induce NO synthesis causing cell death in RINm5F rat insulinoma cells involved the induction of programmed cell death, or apoptosis. Apoptosis is characterized by DNA fragmentation, leading to a typical laddering appearance, and has also been associated with an altered expression ratio of the genes Bcl-2 and Bax (Oltvai *et al.* 1993).

There is considerable evidence that insulin-like growth factors (IGFs) are major contributors to β cell growth, maturation and function, and are expressed by islet cells throughout life (Hill & Hogg 1992). However, IGFs may also act as survival factors by limiting apoptosis in many cell types (Geier *et al.* 1992, Rodriguez-Tarduchy *et al.* 1992, D'Mello *et al.* 1993, Muta & Krantz 1993, Chun *et al.* 1994, Harrington *et al.* 1994, Galli *et al.* 1995, Stewart & Rotwein 1996). The mechanisms by which IGFs are able to protect cells from induced apoptosis are not known, and could be varied. However, IGF-I has been shown to inhibit the induction of NOS in some tissue types, such as vascular smooth muscle (Schini *et al.* 1994), and could potentially interfere with cytokine-stimulated NO synthesis.

Circumstantial evidence exists to link the IGF axis to the regulation of apoptosis *in vivo* within islets of Langerhans. Recently, it has been suggested that a transient wave of apoptosis is responsible for a reduction in β cell number after 1–2 weeks of postnatal life in the rat (Scaglia *et al.* 1997). This would coincide temporarily with our demonstration of a falling pancreatic expression of IGF-II at this time in the rat, when pancreatic expression of IGF-I has not yet achieved adult values (Hogg *et al.* 1994). A hyperplasia of β cells occurs during pregnancy in the rat as part of a progressive rise in the percent endocrine tissue in the pancreas, and in peripheral blood insulin. Following parturition, β cell mass and insulin release quickly return to that in the non-pregnant state, and this is more rapid in lactating than in non-lactating rats (Marynissen *et al.* 1983). The reduction in β cell number has been shown to result from apoptosis (Scaglia *et al.* 1995). Circulating levels of IGF-I are greatly elevated in the rat during pregnancy, and fall rapidly following delivery (Daughaday & Kapadia 1978). Treatment of the pre-diabetic NOD mouse with IGF-I prevents the onset of autoimmune diabetes (Bergerot *et al.* 1995, Kaino *et al.* 1996). IGF-I is also able to reduce the incidence of diabetes following the adoptive transfer of autoreactive T-cells from diabetic to pre-diabetic mice (Bergerot *et al.* 1996). This was associated with a reduced insulitis, and also altered T-cell trafficking to the spleen and thymus, which suggests a possible mechanism of action of IGF-I in lymphoid tissues in addition to direct effects on islet survival.

The purpose of the present study was to examine the ability of IGFs-I and -II to alter the survival of islets isolated from pre-diabetic NOD mice, following exposure to deleterious cytokines, and to examine the presence of IGF-I in the pancreata of such animals.

Materials and Methods

Isolation of islets

Female NOD mice of 5–6 weeks age were provided from the breeding colony at the J P Robarts Research Institute, London, Ontario, and were allowed free access to food and water. All procedures were performed with ethical approval of the Animal Care Committee of the University of Western Ontario. Pancreatic islets were isolated by a modification of the method of Lacy & Kostianovsky (1967). Briefly, mice were anaesthetized by intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and Rompun xylazine (15 mg/kg); the pancreas was exposed and injected at multiple sites with a total of 5 ml sterile, ice-cold Hank's buffered salts solution, pH 7·5 (HBSS; Gibco BRL, Burlington, ON, Canada) containing 2% (v/v) fetal calf serum (FCS; Gibco BRL). The animals

were killed by a surgical pneumothorax and the distended pancreas was removed, finely-chopped with scissors in a Petri dish containing 20 ml ice-cold HBSS+2% (v/v) FCS, and the fragments transferred to glass centrifuge tubes for digestion with collagenase type V (14 mg/pancreas; Sigma Chemical Co., St Louis, MO, USA) from *Clostridium histoliticum*. Collagenase was solubilized in HBSS (5 ml) and the tissue digestion performed initially for 3 min at 38 °C in a shaking water bath (200 cycles/min), followed by 2–3 min at 120 cycles/min. The digestion was terminated by the addition of 10 ml ice-cold HBSS, the contents dispersed by pipette, and tissue collected by centrifugation at 1000 ***g*** for 10 min. Two washes with ice-cold HBSS were performed, with collection by centrifugation at 450 and 250 ***g*** respectively.

Ficoll gradient centrifugation was used to separate islets of Langerhans from the digested tissue (McDaniel *et al.* 1984). Ficoll 400DL (Sigma) was prepared in 0·5 M Hepes buffer (Sigma) containing HBSS, 10 000 U/ml penicillin and 10 mg/ml streptomycin, pH 7·4. Solutions were sterilized by autoclave prior to addition of antibiotics. Each tissue digest was dispersed within 4 ml 25% (w/v) Ficoll, and 2 ml each of 23, 20·5 and 11% Ficoll layered sequentially above this as discontinuous gradients. The glass tubes were then centrifuged at 700 ***g*** for 10 min at 22 °C, and islet tissue harvested by pipette from the 20·5–11% Ficoll interface and placed in 10 ml HBSS+2% (v/v) FCS. The islets were dispersed by pipette and collected by centrifugation at 1000 ***g*** for 20 s. Islets were washed twice in fresh HBSS buffer with collection by centrifugation at 450 and 200 ***g*** respectively, and the final yield suspended in 15 ml HBSS+2% (v/v) FCS in a plastic Petri dish.

Islets were counted and harvested manually with a pipette under a dissecting microscope, and dispersed into non-tissue culture grade plastic culture dishes (10 cm diameter; Falcon, Lincoln Park, NJ, USA) containing 20 ml RPMI 1640 medium (Gibco) supplemented with 10% (v/v) FCS, 25 mM Hepes, 2·06 M L-glutamine (Sigma), 10 000 U/ml penicillin, 50 μg/ml streptomycin, and 11·1 mM glucose. Islets were incubated for 48 h at 37 °C in a humidified atmosphere of 95% O_2, 5% CO_2. A yield of islets from a single pancreas was between approximately 80 and 150, and the islets obtained from the pancreata of between four and six animals were pooled to generate sufficient islets for a single experiment.

Islet culture

For studies of apoptosis, islets were plated into eight-well chamber slides (Lab-Tek, Nalge-Nunc Int., Naperville, IL, USA) (20 islets per well) and allowed to attach to the glass slides overnight in a humidified incubator at 37 °C. Islets were plated at a density of approximately 20 islets per chamber, and the culture medium consisted of glucose-free Dulbecco's Modified Eagle Medium (DMEM; Imperial Laboratories, Andover, Hants, UK) pH 7·4, containing antibiotics and fungizone (as above), supplemented with 2 mM glutamine (Gibco BRL) and 8·7 mM glucose. Medium was further supplemented as required with recombinant human IGF-I or-II (10–100 ng/ml) (GroPep Ltd, Adelaide, SA, Australia), recombinant human IL-1β (0·5–10 ng/ml), TNF-α (2–20 ng/ml), or IFN-γ (1–10 ng/ml) (R&D Labs, Minneapolis, MN, USA), alone or in combination. Where IGF-I or -II and cytokines were present together, the IGF was added 5 min prior to the cytokine and allowed to equilibrate with the islets at 37 °C. After 48 h the culture medium was removed, and the islets washed in PBS. Islets attached during culture to chamber slides were dehydrated through ascending ethanol concentrations (50, 70, 90 and 100%), and air-dried. Slides were stored with desiccant until histological processing.

For studies of islet viability, the islets were distributed in equal batches (50–60 islets per plate) onto non-tissue culture grade Petri dishes (50 mm, Falcon) containing 1 ml tissue culture medium supplemented as described above. Islets were incubated for 48 h. At the end of the treatment period, conditioned medium was removed following centrifugation of the contents of each culture plate at 1000 ***g*** for 10 min, and stored at − 20 °C for subsequent analysis of insulin content. The islets were washed in phosphate-buffered saline (PBS; Gibco BRL) and assessed for viability as described below.

Visualization of apoptosis

Immunocytochemistry was performed to localize apoptotic nuclei within either tissue sections or isolated islets by molecular histochemistry (Wijsman *et al.* 1993) using the Apoptag *in situ* apoptosis detection kit (Oncor Inc., Gaithersburg, MD, USA). Islets attached during culture to chamber slides were rehydrated through descending ethanol concentrations (100, 90 and 70%), and incubated in PBS for 5 min. Staining was performed according to the manufacturer's protocol following incubation with proteinase K (20 μg/ml; Boehringer-Mannheim, Dorval, Québec, Canada) for 15 min, washing in distilled water, and quenching of endogenous peroxidase by incubation in 2% (v/v) hydrogen peroxide in PBS for 5 min. Colour was generated with diaminobenzidine (1·89 mM activated with 0·03% (v/v) hydrogen peroxide for 2 min) and the tissue counterstained with methyl green or Carazzi's haematoxylin for 1 min. Sections were dehydrated in alcohol, cleared in xylene and mounted with Permount (Eukitt, Newmarket, Ontario, Canada) under glass coverslips. Dual staining for insulin or glucose transporter 2 (GLUT-2) was performed by immunocytochemistry after detection of apoptosis and prior to counterstaining and dehydration. For the localization of insulin in islets cultured within chamber slides, a primary guinea-pig anti-insulin antibody (1:1000 dilution) (provided by Dr T J McDonald) was used with an alkaline phosphatase (blue)

chromagen. Alkaline phosphatase substrate kit III was obtained from Vector Labs Inc., Burlingame, CA, USA. Anti-mouse alkaline phosphatase conjugate (Sigma) was applied to each section for 1 h at room temperature, sections washed, and alkaline phosphatase substrate applied for 20 min. Sections were washed and counterstained with Mayer's haemalum and mounted with aqueous mounting solution. GLUT-2 was similarly localized with a rabbit anti-rat GLUT-2 antiserum (1:500 dilution) (Biogenesis Inc., Sandown, NH, USA) and an anti-rabbit alkaline phosphatase conjugate.

Assessment of islet viability

To assess islet cell viability following incubation, the islets from each plate were resuspended in 0·5 ml PBS containing 5 mg/ml trypan blue (Sigma). Trypan blue solution was filtered through an ultramembrane (0·2 μm, Gelman Science, Ann Arbor, IL, USA) to remove particulates before use. All islets were examined immediately under a dissecting microscope and islets containing cells which had taken up trypan blue were considered non-viable. This analysis was based on a photographic assessment of at least 300 cells from each of 20 islets per variable. At the time of viability assessment, the recovery of islets was 94 ± 3% (mean ± S.D.) of those initially added to each culture dish, and did not differ between control cultures or those which had contained cytokines. The coefficient of variation of viability assessment for repeated measures was 4%. To determine if islets were dispersed in the presence of cytokines, and therefore lost from collection and analysis, the DNA content of the recovered islets was measured. Islets were suspended in PBS (500 μl) and solubilized by ultrasonication. DNA was precipitated with 1 ml ice-cold 10% trichloroacetic acid (TCA) and solubilized by overnight incubation with 1 M sodium hydroxide. Following neutralization with 1 mol HCl, the DNA content of islets was measured by fluorimetry using Hoechst fluorochrome 33258 (Aldrich Chemical Co., Inc., Milwaukee, WI, USA) with an excitation wavelength of 375 nm and an emission wavelength of 458 nm. Calf thymus DNA (1·5–24 μg/ml; Sigma) was used for calibration.

Immunohistochemical localization of IGFs and IGF-binding proteins

Male or female pre-diabetic NOD mice of 5–6 weeks of age were killed by inhalation of CO_2 and the pancreas quickly removed and placed in ice-cold fixative (0·2% glutaraldehyde, 4% paraformaldehyde buffered with 70 mM phosphate buffer) for 16 h at 4 °C, followed by

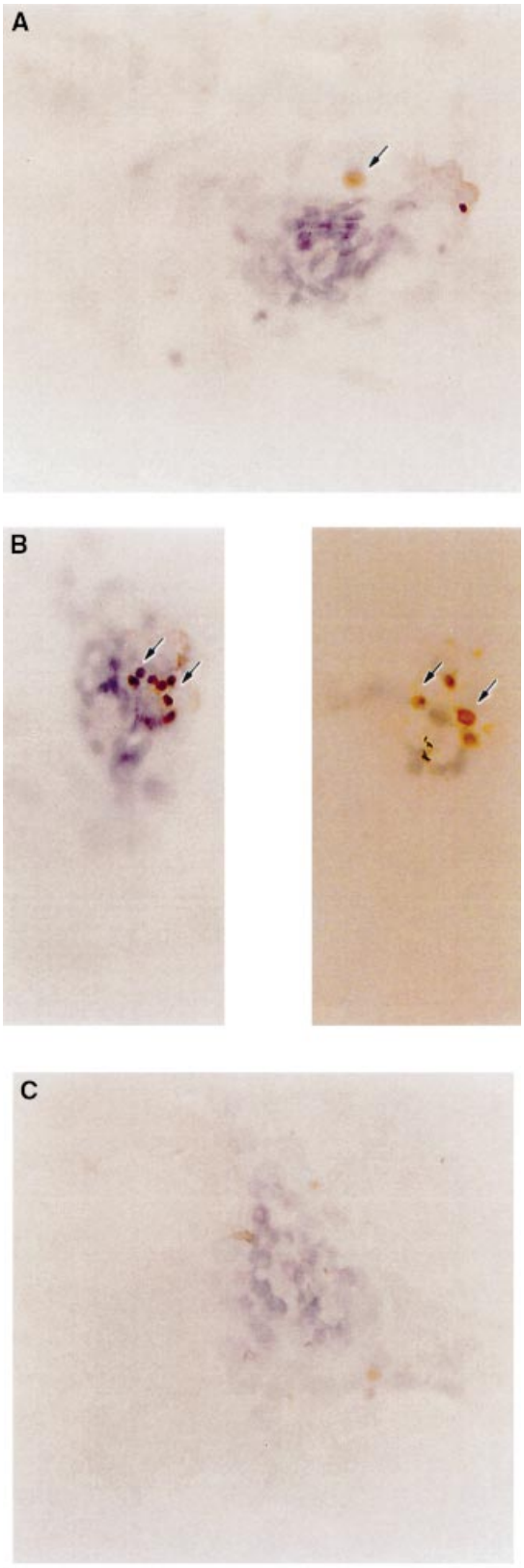

Figure 1 Endocrine cell apoptosis (arrows) detected using molecular histochemistry in islets isolated from a pre-diabetic female mouse in control medium (A), after incubation with IL-1β (1 ng/ml) (B), or with IL-1β and IGF-I (100 ng/ml) (C).

Table 1 Percentage of islets containing apoptotic nuclei (%) in isolated pancreatic islets from NOD mice following incubation with IL-1β (1 ng/ml), TNF-α (5 ng/ml) or IFN-γ (5 ng/ml), without or with IGF-I or -II (100 ng/ml). Values are means ± S.E.M. (n=3–4)

	Control	IGF-I	IGF-II
Control	3·5 ± 1·6	3·1 ± 1·8	4·3 ± 1·5
IL-1β	27·1 ± 5·8*	4·4 ± 2·3†	6·5 ± 2·0
Control	5·8 ± 2·1	5·0 ± 1·8	4·8 ± 1·3
TNF-α	54·2 ± 6·9*	29·3 ± 4·4*†	22·6 ± 4·6*†
Control	6·9 ± 2·6	5·7 ± 4·1	5·3 ± 2·2
IFN-γ	61·3 ± 4·5*	43·8 ± 2·5*†	44·3 ± 3·1*†

*P<0·01 vs no cytokine; †P<0·05 vs incubation with cytokine but without IGF-I or -II.

four washes at 4 °C in PBS over a 48 h period. Fixed tissues were dehydrated in 70% (v/v) ethanol and embedded in paraffin. Histological sections of pancreas (5 μm) were cut from paraffin blocks with a rotary microtome and mounted on Superfrost plus glass microscope slides (VWL Scientific, Mississauga, Ontario, Canada). Immunohistochemistry was performed to localize IGF-I or -II within islets by a modified avidin–biotin peroxidase method (Hsu *et al.* 1981) as described by us previously (Hill & Clemmons 1992). The primary antisera were rabbit anti-human IGF-I or -II (1:2000 dilution) (GroPep), rabbit anti-bovine IGF-binding protein-2 (IGFBP-2), rabbit anti-human IGFBP-3, or rabbit anti-human IGFBP-4 (1:1000 dilution) (UBI, Lake Placid, NY, USA). Biotinylated goat anti-rabbit IgG (Sigma) diluted 1:500 was used as a secondary antiserum. Tissue sections were counterstained with Carazzi's haematoxylin, dehydrated in an ascending ethanol series and then cleared with xylene and mounted under glass coverslips. To establish specificity of staining, the primary antisera to IGF-I or -II, or IGFBPs, were pre-absorbed overnight at 4 °C with 100 nM homologous ligand prior to application to the sections. In each case, staining was abolished. IGF staining within islets was not abolished when the primary antisera were pre-absorbed with up to 1 μM insulin. Further controls included substitution of primary antisera with non-immune serum and omission of the secondary antiserum.

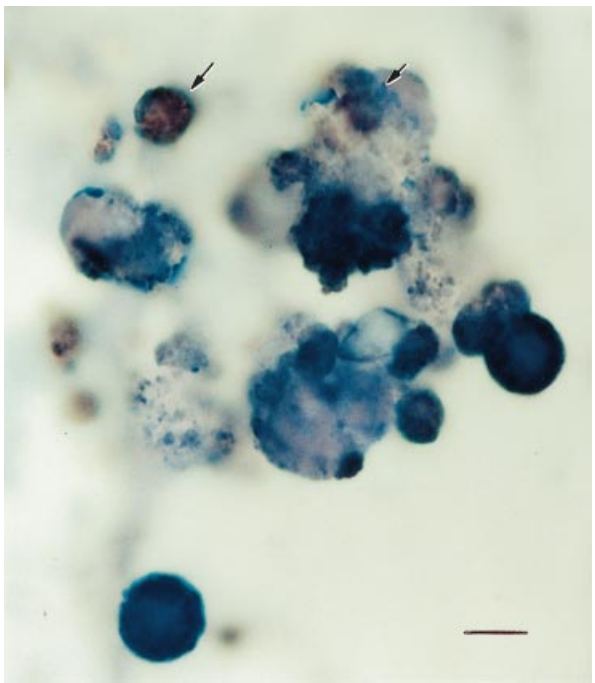

Figure 2 Endocrine cell apoptosis (arrows) detected using molecular histochemistry (brown) in islets from a pre-diabetic female mouse following tissue culture in medium containing IL-1β. The preparations were also subjected to immunocytochemistry for GLUT-2 (blue). Islet β cells, but not other endocrine cell types, exhibit GLUT-2 localization on the plasma membranes. The majority of condensed, apoptotic cells exhibit GLUT-2 immunoreactivity on the plasma membranes (arrows). Scale bar represents 10 μm.

Insulin release

The insulin content of conditioned culture medium was measured by radioimmunoassay (RIA) using the Wright antiserum in a modification of the method of Hales & Randle (1963) as modified by Herbert *et al.* (1965), and described by us previously (Schnuerer *et al.* 1990). Rat insulin (Novo Nordisk, Mississauga, ON, Canada) was used for the standard curve. The within assay coefficient of variation (CV) was 6·5% and the between assay CV was 9%. The minimum level of detection was 2 fmol/ml. There was no detectable cross-reactivity in the insulin assay with IGF-I or -II.

Statistical evaluation

Islet cultures were performed with three or four replicate culture plates or chamber slides for each variable within individual experiments derived from a pool of islets. Each experiment was repeated three to five times using pools of

Table 2 Percentage cell viability in isolated islets from pre-diabetic female NOD mice incubated for 48 h with or without IL-1β (1 ng/ml), TNF-α (2 ng/ml), or IFN-γ (5 ng/ml), alone or in the presence of IGF-I or -II (100 ng/ml. Values are means ± S.E.M. (n=3–4)

	No cytokine	IL-1β	TNF-α	IFN-γ
Control	90 ± 3	57 ± 2**	65 ± 6*	47 ± 6**
IGF-I	91 ± 4	87 ± 7†	80 ± 1†	58 ± 9
IGF-II	88 ± 3	91 ± 2††	84 ± 2†	82 ± 3†

*P<0·05, **P<0·005 vs no cytokine; †P<0·05, ††P<0·005 vs incubation without IGF-I or -II.

islets derived from separate animals. Representative experiments are shown as the mean values ± S.E.M. Differences between mean values for variables within individual experiments were compared for statistical difference by analysis of variance. For the analysis of islet cells undergoing apoptosis when grown in chamber slides, the number of islets which contained apoptotic nuclei was considered. For histological analyses of IGF-I or -II presence, at least five separate pancreata were examined and representative views are shown.

Results

Islets isolated from pre-diabetic, female NOD mice were allowed to attach to chamber slides before exposure for 48 h to IL-1β (1 ng/ml), TNF-α (5 ng/ml) or IFN-γ (5 ng/ml), with or without IGF-I or -II (100 ng/ml). Apoptotic nuclei were visualized using molecular histochemistry. Incubation with IL-1β caused an eight-fold increase in the presence of apoptotic nuclei which was significantly reduced in the presence of IGF-I (Fig. 1 and Table 1). Both TNF-α and IFN-γ also caused an eight-fold increase in apoptosis in islet cells, which was partly reversed by co-incubation with IGF-I or -II. These results show that cytokines induced apoptosis in islet cells, and that the incidence of apoptosis was reduced in the presence of IGFs.

Attempts to demonstrate that the islet cells undergoing apoptosis were β cells by co-staining for insulin were unsuccessful. The compacted cells with apoptotic nuclei contained little cytoplasm and insulin immunoreactivity could not be convincingly seen. However, the central clusters of such cells within isolated islets is compatible with them once having been functional β cells. Since β cells preferentially express GLUT-2 on the plasma membrane, compared with other pancreatic endocrine cell types (Thorens 1992, Pang *et al.* 1994), we examined the presence of immunoreactive GLUT-2 on islet cells from NOD mice which were allowed to attach to chamber slides and were subsequently cultured for 48 h in the presence of IL-1β (2·5 ng/ml) (Fig. 2). Islets were co-stained for apoptotic nuclei by molecular histochemistry. Although, under these culture conditions, islets had begun to dissociate and spread along the culture slide, clusters of cells were seen within islets which exhibited strong staining for GLUT-2, while other islet cells were immunonegative. In the presence of IL-1β, condensed, apoptotic islet cells were seen which also contained GLUT-2 on the plasma membrane. This sometimes had an irregular distribution suggestive of blebbing of the plasma membrane, which is a feature of the apoptotic process. These results suggest that the majority of cells in which cytokine exposure induced apoptosis were β cells.

To determine if a substantial amount of necrosis occurred in addition to apoptosis when NOD mouse islets were exposed to cytokines, a simple viability assay was used, based on trypan blue exclusion. In the presence of IL-1β, TNF-α or IFN-γ, each cytokine alone caused a significant reduction in islet cell viability after 48 h (Table 2). Co-incubation with either IGF-I or -II significantly increased cell viability in the presence of IL-1β or TNF-α. Co-incubation with IGF-II significantly increased viability in the presence of IFN-γ, but IGF-I did not. When islets were exposed to IL-1β (2·5 ng/ml), TNF-α (10 ng/ml) and IFN-γ (5 ng/ml) in the same incubations, viability was substantially reduced (control 94 ± 2%, cytokine addition 6 ± 2%; mean ± S.E.M., n=4). Viability was significantly increased if islets were co-cultured with the three cytokines in the presence of IGF-I (100 ng/ml) (51 ± 8%, P<0·05). The islet cell DNA content per culture was not altered significantly by the presence of each cytokine (control 2·61 ± 0·14 μg; IL-1β 1·98 ± 0·35 μg; TNF-α 2·27 ± 0·44 μg; IFN-γ 1·76 ± 0·50 μg (mean ± S.E.M.)), suggesting that islets were not disaggregating and being lost from analysis. The release of insulin into conditioned culture medium was substantially reduced in response to each of the cytokines alone, and this was not reversed by the presence of IGF-I or -II (Table 3).

To investigate the effects of cytokine concentration on the ability of IGFs to decrease cytokine-induced cell death, islets were incubated for 48 h with increasing concentrations of IL-1β, TNF-α or IFN-γ with or without IGF-I (100 ng/ml). The ability of IL-1β to decrease islet cell viability was dose-dependent and maximally effective at a concentration of 1 ng/ml (Table 4). Co-incubation with IGF-I significantly decreased IL-1β-induced cell death at all concentrations of cytokine tested from 0·5 to 10 ng/ml. TNF-α was maximally effective in reducing islet cell viability at 5 ng/ml and this was significantly

Table 3 Release of insulin (μUnits/ml) by isolated pancreatic islets from NOD mice following exposure to IL-1β (1 ng/ml), TNF-α (2 ng/ml) or IFN-γ (5 ng/ml) with or without IGF-I or -II (100 ng/ml). Values are means ± S.E.M. (n=3–4)

	No cytokine	**IL-1β**	**TNF-α**	**IFN-γ**
Control	212 ± 24	146 ± 11*	41 ± 9**	33 ± 5**
IGF-I	129 ± 14†	83 ± 12	42 ± 10**	28 ± 8**
IGF-II	136 ± 9†	99 ± 24	56 ± 13**	40 ± 17**

*P<0·05, **P<0·01 vs no cytokine; †P<0·05 vs incubation without IGF-I or -II.

Table 4 Percentage cell viability in isolated islets from pre-diabetic female NOD mice incubated for 48 h with increasing concentrations of IL-1β, TNF-α, or IFN-γ, alone or in the presence of IGF-I (100 ng/ml). Values are means ± S.E.M.. (n=3–4)

	Cytokine (ng/ml)							
	0	0·5	1	2	2·5	5	10	20
IL-1β	96 ± 2	60 ± 2*	22 ± 4*	—	20 ± 2*	—	19 ± 3*	—
IL-1β+IGF-I	94 ± 1	81 ± 3†	78 ± 4††	—	66 ± 2††	—	57 ± 7††	—
TNF-α	96 ± 1	—	—	76 ± 2*	—	43 ± 1*	40 ± 5*	35 ± 3*
TNF-α+IGF-I	93 ± 2	—	—	81 ± 2	—	63 ± 2†	56 ± 3†	58 ± 2†
IFN-γ	91 ± 3	—	28 ± 5*	13 ± 2*	—	18 ± 1*	16 ± 4*	—
IFN-γ+IGF-I	88 ± 6	—	33 ± 6	31 ± 3†	—	28 ± 3†	15 ± 4	—

*P<0·05 vs no cytokine; †P<0·05, ††P<0·005 vs incubation without IGF-I.

Table 5 Percentage cell viability in isolated islets from pre-diabetic female NOD mice incubated for 48 h with IL-1β (10 ng/ml) with or without increasing concentrations of IGF-I. Values are means ± S.E.M.. (n=3–4)

	IGF-I (ng/ml)				
	0	10	25	50	100
No cytokine	92 ± 3	86 ± 5	89 ± 2	85 ± 7	87 ± 3
IL-1β	23 ± 3†	36 ± 4†	42 ± 2†	55 ± 3	65 ± 5

†P<0·05 vs no cytokine.

reversed by co-incubation with IGF-I together with 5–20 ng/ml TNF-α (Table 4). IFN-γ was already maximally effective at inducing islet cell death at the lowest concentration examined, 1 ng/ml. A modest cytoprotective action of IGF-I was seen but was limited to concentrations of 2 and 5 ng/ml IFN-γ, and was absent at 10 ng/ml (Table 4). Since the cytoprotective effects of IGF-I were most apparent in the presence of IL-1β, this cytokine was used to investigate the dose-dependency of IGF-I. A maximally-effective concentration of IL-1β was utilized, 10 ng/ml, co-incubated with decreasing concentrations of IGF-I from 100 to 10 ng/ml. The ability of IGF-I to decrease IL-1β-induced cell death was clearly dose-related, with only slight protection present at 10 ng/ml but a 70% reversal of IL-1β action at 100 ng/ml (Table 5).

To establish that apoptosis was also a feature of islet cell death in the pre-diabetic NOD mouse pancreas *in vivo*, sections of pancreas from female animals of 5–6 weeks age were subjected to TUNEL immunohistochemistry. Insulitis was evident in approximately 40% of islets. In islets without immune cell infiltration, apoptotic nuclei were rare (Fig. 3A). Isolated apoptotic nuclei could be seen in islets with no evidence of insulitis (Fig. 3B), and were often seen in islets with heavy immune cell infiltration (Fig. 3D). While some apoptotic bodies may have been T-cells or macrophages, others were clearly located in the endocrine cell populations at the centre of islets. In a minority of islets, extensive apoptosis was seen with only limited insulitis (Fig. 3C). We examined the presence of both IGFs-I and -II in pancreata from pre-diabetic, female NOD mice and from male mice. In islets from females with no or little evidence of immune infiltration, most endocrine cells showed specific staining for IGF-I (Fig. 4A), but not for IGF-II. Within the same pancreata, islets with heavy immune infiltration showed negligible staining

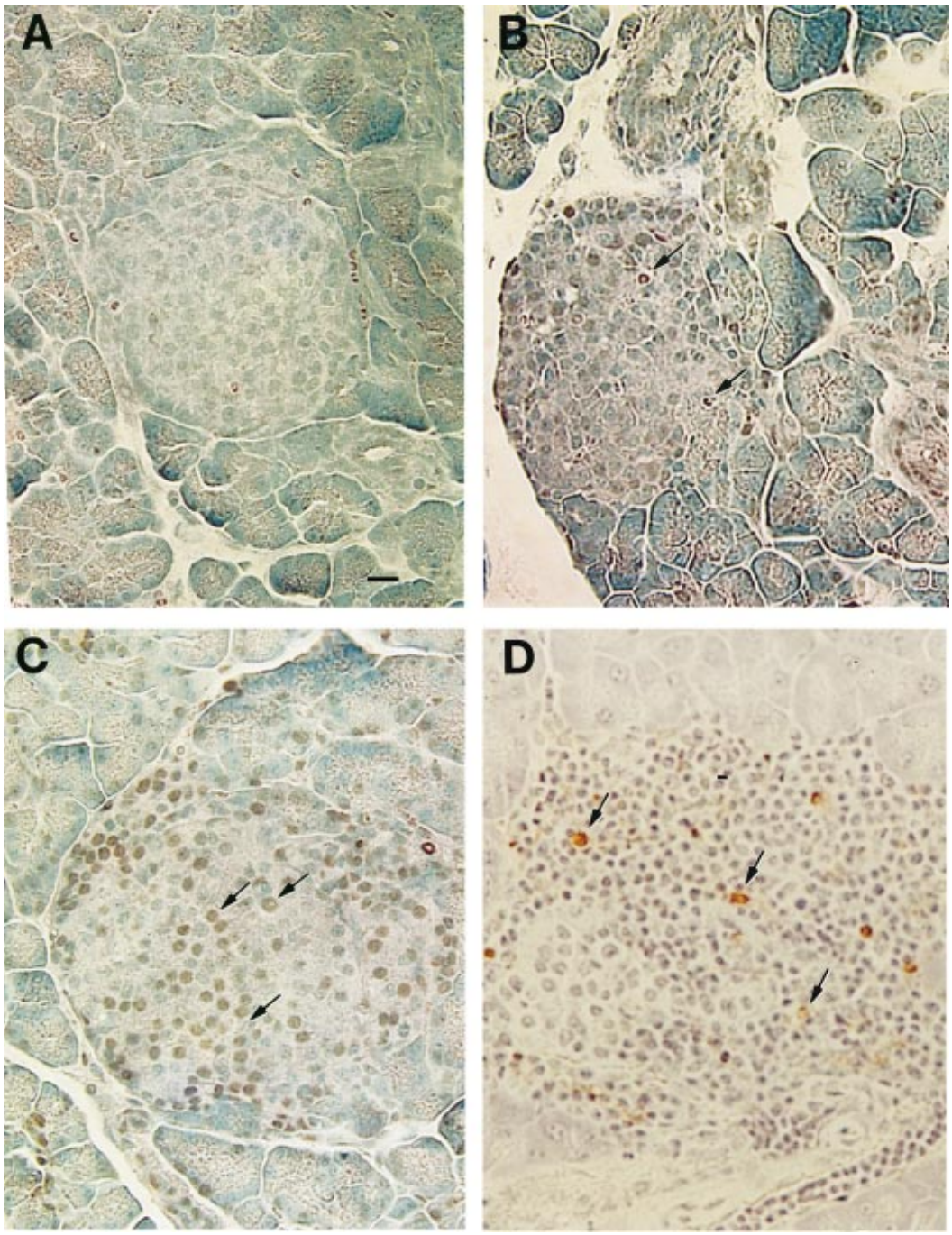

Figure 3 Molecular histochemical localization of cell apoptosis (arrows) in islets from a pre- diabetic female NOD mouse. Islets are shown without insulitis and no evidence of apoptosis (A), no insulitis and isolated apoptotic nuclei (B), peripheral insulitis, and multiple apoptosing cells (C), or heavy insulitis engulfing the endocrine cells (D). During heavy insulitis, apoptotic cells with large nuclei characteristic of the endocrine cells are surrounded by infiltrating immune cells. Scale bar represents 10 μm.

for either IGF-I or -II (Fig. 4B). Little IGF immunoreactivity was present in the acinar cell population. Pancreata from male NOD mice of the same age showed no evidence of immune cell infiltration of islets, and all islets were immunopositive for IGF-I. To determine whether the IGF-I immunoreactivity seen in

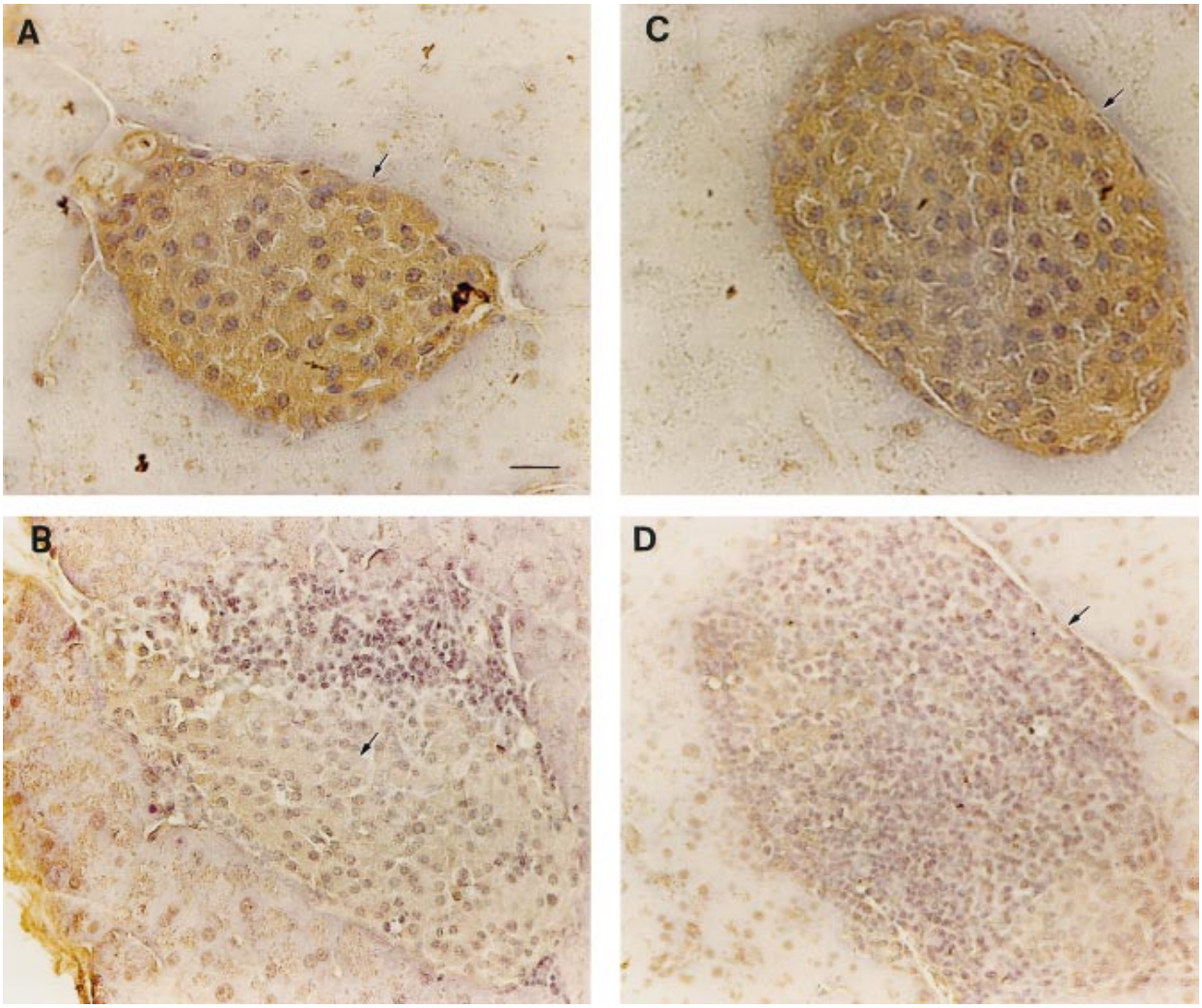

Figure 4 Immunohistochemical localization of IGF-I (A and B), or IGFBP-2 (C and D) in pancreatic islets (arrow) of pre-diabetic female NOD mice without insulitis (A and C), and in islets of the same pancreas demonstrating insulitis (B and D). Immunoreactivity for both IGF-I and IGFBP-2 is diminished during insulitis. Scale bar represents 10 μm.

non-infiltrated islets was due to an association with IGFBPs, immunohistochemistry was performed for IGFBPs-2, -3 and -4. Immunoreactivity for IGFBP-2 was seen in islets not undergoing obvious infiltration (Fig. 4C), but was absent from islets with heavy immune infiltration (Fig. 4D). Similar findings were seen for the distribution of IGFBPs-3 and -4 (not shown). The co-distribution of IGF-I with IGFBPs suggests that they exist in complex on or in islet cells.

Discussion

The autoimmune process in the NOD mouse is known to involve cytokine-mediated destruction of the pancreatic β cells in a process analogous to that seen in human type 1 diabetes (Castano & Eisenbarth 1990). The ability of cytokines such as IL-1β, TNF-α and IFN-γ to induce β cell death is known to be at least partly mediated by the induction of apoptosis (Delaney *et al.* 1993). This may involve the up-regulation of iNOS and increased intracellular levels of NO (Corbett & McDaniel 1995), although other mechanisms may also operate including the generation of oxygen free radicals and a Fas-dependent pathway (Rabinovitch *et al.* 1992, Yamada *et al.* 1996). The present experiments show that an induction of apoptosis is a major contributor to cytokine-induced cell death in isolated islets from pre-diabetic NOD mice, and that IGFs-I and -II, which have been shown previously to limit induced apoptosis in a variety of cell types (Geier *et al.* 1992, Muta & Krantz 1993, Chun *et al.* 1994), will also protect such islets against cytokine-mediated cell

death, at least partly by reducing cytokine-induced apoptosis. IGF-I may also limit apoptosis by suppression of an IL-1β-converting enzyme (Jung *et al.* 1996). The cells which underwent apoptosis in isolated islets exposed to cytokines demonstrated immunoreactive GLUT-2, a marker of β cells (Thorens 1992, Pang *et al.* 1994), although immunoreactive insulin was no longer detectable in such compacted cells with little cytoplasm. This may reflect the terminal nature of the detection system for apoptosis used: the breakage of DNA, which occurs after considerable cell compaction and autolysis has taken place. IGF-I has recently been shown to limit IL-1β-induced apoptosis in adult rat islets of Langerhans by a mechanism which involved a suppression of iNOS (Mabley *et al.* 1997). Our study extends these observations to other cytokines, and to islets during the process of autoimmune diabetes.

Some islets did not show evidence of cell death or apoptosis in response to cytokines at the doses and incubation time used. This may reflect the heterogeneous nature of the islets obtained from the pre-diabetic NOD mice, where some will already have evidence of insulitis *in vivo*. Those islets may well retain macrophages and/or T-cells once isolated, although the high viability of islets in control cultures suggests that these did not exert a noticeable cytotoxic effect. However, they may represent the islets that have the greatest sensitivity to exogenous cytokines *in vitro*. The sensitivity of islets from the NOD mouse to exogenous cytokines was broadly similar to that reported by us recently for isolated islets from weanling rats (Petrik *et al.* 1998).

IGF-I (100 ng/ml) was able to significantly increase islet cell survival in the presence of up to 10 ng/ml IL-1β, or 20 ng/ml TNF-α. It was less effective in preventing β cell death in response to IFN-γ, with partial protection being seen at concentrations of 2 and 5 ng/ml cytokine only. This may reflect differences in the mechanisms of IFN-γ-induced cell death compared with the other cytokines. For instance, IFN-γ induces the increased expression of IFN-regulatory factor-1 in pancreatic β cells, leading to a greater increase in iNOS mRNA than achieved with other cytokines alone (Flodstrom & Eizirik 1997). IFN-γ is able to induce cell death in the INS-1β cell line independently of NO production (Laffranchi & Spinas 1997), and this may be mediated by the induction of Fas (Yamada *et al.* 1996). However, in our studies, IGF-I was able to significantly reduce the rapid loss of islet cell viability seen when all three cytokines were present together. The cytoprotective effects of IGF-I in the presence of IL-1β were dose-related between 10 and 100 ng/ml, and did not reach a maximum. It is possible that greater concentrations of IGF-I would induce increased cell viability. Exposure of pre-diabetic NOD islets to cytokines also caused a rapid decrease in insulin release, and this was not reversed by incubation with IGFs. Rather, incubation with IGFs tended to further decrease insulin release. Our estimation of insulin release will include that released from apoptosing cells, so that the direct inhibitory effects of cytokines on insulin release are probably under-estimated. These findings differ from those reported for rat islets, where IGF-I was able to reverse the inhibitory effects of IL-1β on insulin release (Mabley *et al.* 1997). Both IGFs-I and -II have previously been reported to inhibit the release of insulin from isolated adult rat islets when continuously present (Van Schravendijk *et al.* 1990, Hill *et al.* 1997, Petrik *et al.* 1998) in line with our findings in the NOD mouse. Other growth factors have also been found to promote the survival of β cells following exposure to proinflammatory cytokines. Transforming growth factor β was able to reverse the effects of IL-1β on insulin secretion and NO formation in rat islets and RINm5F cells (Cunningham & Green 1994, Cunningham *et al.* 1994).

The pancreata of juvenile and adult rodents express IGF-I, both in the islets of Langerhans and in the ductal epithelial cells (Romanus *et al.* 1985, Swenne & Hill 1989, Hogg *et al.* 1993, 1994). In postnatal rat islets, the source of IGF-I may primarily be glucagon cells (Maake & Reinecke 1993). IGF-I inhibits pro-glucagon gene expression and glucagon release from an α cell line (Fehmann *et al.* 1996). All endocrine pancreas cell types express both types-1 and -2 IGF receptors (Van Schravendijk *et al.* 1987, Fehmann *et al.* 1996). However, it is not known if IGF-I is present in the pancreas of the pre-diabetic NOD mouse. Using immunohistochemistry, we found that IGF-I was associated with most cells within islets without insulitis, but that immune infiltration was associated with a loss of IGF-I immunoreactivity within the same pancreas. Little IGF-I was associated with exocrine tissues. The widespread distribution of IGF-I throughout the cells of some islets may reflect sequestration to IGFBPs which are known to be expressed within the rodent pancreas (Hill & Hogg 1992, Hogg *et al.* 1994), and may not necessarily indicate *de novo* synthesis. This was supported by the co-localization in islets of IGF-I and IGFBPs-2, -3 and -4, all of which showed a much reduced immunoreactivity in islets undergoing insulitis. Cytokines known to be involved in the early stages of pancreatic β cell destruction may inhibit either the local expression or action of IGF-I or -II, or alter IGFBP release, in non-islet tissues. Both TNF-α and IFN-γ were found to inhibit IGF-II gene expression in human fetal adrenal cell cultures (Ilvesmaki *et al.* 1993), while the latter decreased both DNA synthetic rate and IGF-II expression in human neuroblastoma cells (Martin *et al.* 1993). IL-1β and TNF-α were both shown to reduce IGF-I expression by rat Leydig cells (Lin *et al.* 1992, 1994). IGF-I is also expressed by macrophages, but this is suppressed by IFN-γ (Arkins *et al.* 1995) but not by TNF-α (Noble *et al.* 1993). The loss of IGF-I immunoreactivity in islets undergoing insulitis may also reflect the presence of proteases which can selectively modify IGFBPs, and reduce their affinity

for the ligands (McCusker & Clemmons 1992). Exposure of rodent islets to exogenous IL-1β causes an increased activation of proteases, while the short-term effects of IL-β, such as decreased insulin release, could be reversed in the presence of serine protease inhibitors (Eizirik *et al.* 1991, Welsh *et al.* 1991). In summary, these experiments show that exogenous IGFs-I and -II are able to improve islet cell viability in isolated islets from the pre-diabetic NOD mouse following exposure to proinflammatory cytokines, by a process that includes suppression of apoptosis. This suggests new strategies for the use of naturally occurring growth factors to retard islet cell destruction in type 1 diabetes.

Acknowledgements

We are grateful to the Juvenile Diabetes Foundation, the Canadian Diabetes Association and the Medical Research Council of Canada for financial support to D J Hill and T L Delovitch.

References

Amber IJ, Hibbs Jr JB, Taintor RR & Vavrin Z 1988 Cytokines induce an L-arginine-dependent effector system in non-macrophage cells. *Journal of Leukocyte Biology* **44** 58–65.

Ankarcrona M, Dypbukt JM, Brune B & Nicotera P 1994 Interleukin-1 beta-induced nitric oxide production activates apoptosis in pancreatic RINm5F cells. *Experimental Cell Research* **213** 172–177.

Arkins S, Rebeiz N, Brunkereese DL, Biragyn A & Kelley KW 1995 Interferon-gamma inhibits macrophage insulin-like growth factor-I synthesis at the transcriptional level. *Molecular Endocrinology* **9** 350–360.

Bergerot I, Fabien N, Maguer V & Thivolet C 1995 Insulin-like growth factor-I (IGF-I) protects NOD mice from insulitis and diabetes. *Clinical and Experimental Immunology* **102** 335–340.

Bergerot I, Fabien N & Thivolet C 1996 Effects of insulin-like growth factor-I and insulin on effector T cells generating autoimmune diabetes. *Diabetes and Metabolism* **22** 235–239.

Castano L & Eisenbarth GS 1990 Type 1 diabetes: a chronic autoimmune disease of human, mouse and rat. *Annual Reviews of Immunology* **8** 647–679.

Chun SY, Billig H, Tilly JL, Furuta I, Tsafriri A & Hsueh AJW 1994 Gonadotropin suppression of apoptosis in cultured preovulatory follicles: mediatory role of endogenous insulin-like growth factor I. *Endocrinology* **135** 1845–1853.

Comens PG, Wolf BA, Unanue ER, Lacy PE & McDaniel ML 1987 Interleukin 1 is potent modulator of insulin secretion from isolated rat islets of Langerhans. *Diabetes* **36** 963–970.

Corbett JA & McDaniel ML 1995 Intra-islet release of interleukin-1 inhibits β cell function by inducing β cell expression of inducible nitric oxide synthase. *Journal of Experimental Medicine* **181** 559–568.

Cunningham JM & Green IC 1994 Cytokines, nitric oxide and insulin secreting cells. *Growth Regulation* **4** 173–180.

Cunningham JM, Mabley JG, Mwebe B, Kovani E & Green IC 1994 Suppression of interleukin-1β-induced nitric oxide synthase activity by transforming growth factor-β in the insulin-secreting cell line RINm5F. *Journal of Endocrinology* **140** (Suppl) P37.

Daughaday WH & Kapadia M 1978 Maintenance of serum somatomedin activity in hypophysectomized pregnant rats. *Endocrinology* **102** 1317–1320.

Delaney CA, Green MHL, Lowe JE & Green IC 1993 Endogenous nitric oxide induced by interleukin-1β in rat islets of Langerhans and HIT–T15 cells causes significant DNA damage as measured by the 'comet' assay. *FEBS Letters* **333** 291–295.

Delaney CA, Tyrberg B, Bouwens L, Vaghef H, Hellman B & Eizirik DL 1996 Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death. *FEBS Letters* **394** 300–306.

D'Mello SR, Galli C, Ciotti T & Calissano P 1993 Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor-I and cAMP. *Proceedings of the National Academy of Sciences of the USA* **90** 10989–10993.

Eizirik DL, Bendtzen K & Sandler S 1991 Short exposure of rat pancreatic islets to interleukin-1 beta induces a sustained but reversible impairment in beta cell function: influence of protease activation, gene transcription, and protein synthesis. *Endocrinology* **128** 1611–1616.

Elliott EA & Flavell RA 1994 Transgenic mice expressing constitutive levels in islet β cells develop diabetes. *International Immunology* **6** 1629–1637.

Fehmann HC, Jehle P, Markus U & Goke B 1996 Functional active receptors for insulin-like growth factors-I (IGF-I) and IGF-II on insulin-, glucagon-, and somatostatin-producing cells. *Metabolism* **45** 759–766.

Flodstrom M & Eizirik DL 1997 Interferon-γ-induced interferon regulatory factor-1 (IRF-1) expression in rodent and human islet cells precedes nitric oxide production. *Endocrinology* **138** 2747–2753.

Galli C, Meucci O, Scorziello A, Werge TM, Calissano P & Schettini G 1995 Apoptosis in cerebellar granule cells is blocked by high KCl, forskolin, and IGF-I through distinct mechanisms of action: the involvement of intracellular calcium and RNA synthesis. *Journal of Neuroscience* **15** 1172–1179.

Geier A, Haimshon M, Beery R & Lunenfeld B 1992 Insulin-like growth factor-I inhibits cell death induced by cycloheximide in MCF-7 cells – a model system for analyzing control of cell death. *In Vitro Cellular and Developmental Biology* **28A** 725–729.

Gepts W & Lecompte PM 1981 The pancreatic islets in diabetes. *American Journal of Medicine* **70** 105–115.

Hales CN & Randle PJ 1963 Immunoassay of insulin with insulin antibody precipitate. *Biochemical Journal* **88** 137–146.

Hammonds P, Beggs M, Beresford G, Espinol J, Clarke J & Mertz RJ 1990 Insulin-secreting β cells possess specific receptors for interleukin-1β. *FEBS Letters* **261** 97–100.

Harrington EA, Bennett MR, Fanidi A & Evan GI 1994 C-myc induced apoptosis in fibroblasts is inhibited by specific cytokines. *EMBO Journal* **13** 3286–3295.

Helqvist S, Hansen BS, Johanneson J, Anderson HU, Nielsen JH & Nerup J 1989 Interleukin 1 induces new protein formation in isolated rat islets of Langerhans. *Acta Endocrinologica* **121** 136–140.

Herbert V, Lau K, Gottlieb CW & Bleicher SJ 1965 Coated charcoal immunoassay of insulin. *Journal of Clinical Endocrinology* **25** 1375–1384.

Hill DJ & Clemmons DR 1992 Similar distribution of insulin-like growth factor-binding proteins -1, -2 and -3 in human fetal tissues. *Growth Factors* **6** 315–326.

Hill DJ & Hogg J 1992 Expression of insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) during pancreatic development in rat, and modulation of IGF actions on rat islet DNA synthesis by IGFBPs. In *Pancreatic Islet Cell Regeneration and Growth,* pp 113–120. Ed A Vinik. New York: Plenum.

Hill DJ, Sedran RJ, Brenner SL & McDonald TJ 1997 Insulin-like growth factor-I (IGF-I) has a dual effect on insulin release from isolated, perifused adult rat islets of Langerhans. *Journal of Endocrinology* **153** 15–25.

Hogg J, Han VKM, Clemmons DR & Hill DJ 1993 Interactions of glucose, insulin-like growth factors (IGFs) and IGF binding proteins in the regulation of DNA synthesis by isolated fetal rat islets of Langerhans. *Journal of Endocrinology* **138** 401–412.

Hogg J, Hill DJ & Han VKM 1994 The ontogeny of insulin-like growth factor (IGF) and IGF binding protein gene expression in the rat pancreas. *Journal of Molecular Endocrinology* **13** 49–58.

Hsu SM, Raine L & Fanger H 1981 Use of avidin–biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. *Journal of Histochemistry and Cytochemistry* **29** 577–580.

Ilvesmaki V, Jaattela M, Saksela E & Voutilainen R 1993 Tumor necrosis factor-alpha and interferon-gamma inhibit insulin-like growth factor-II gene expression in human fetal adrenal cell cultures. *Molecular and Cellular Endocrinology* **91** 59–65.

Jung Y, Miura M & Yuan J 1996 Suppression of IL-1 beta converting enzyme-mediated cell death by insulin-like growth factor. *Journal of Biological Chemistry* **271** 5112–5117.

Kaino Y, Hirai H, Ito T & Kida K 1996 Insulin-like growth factor I (IGF-I) delays the onset of diabetes in non-obese diabetic (NOD) mice. *Diabetes Research and Clinical Practice* **34** 7–11.

Kaneto H, Fujii J, Seo HG, Suzuki K, Matsuoka T, Nakamura M, Tatsumi H, Yamasaki Y, Kamada T & Taniguchi N 1995 Apoptotic cell death triggered by nitric oxide in pancreatic cells. *Diabetes* **44** 733–738.

Kilbourn RG & Belloni P 1990 Endothelial cell production of nitrogen oxides in response to interferon gamma in combination with tumor necrosis factor, interleukin-1 and endotoxin. *Journal of the National Cancer Institute* **82** 772–776.

Lacy P & Kostianovsky M 1967 Method for isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **34** 35–39.

Laffranchi R & Spinas GA 1997 Interferon-gamma inhibits insulin release and induces cell death in the pancreatic beta-cell line INS-1 independently of nitric oxide production. *Experimental Cell Research* **237** 217–222.

Lin T, Wang DL, Nagpal ML, Chang WW & Calkins JH 1992 Down-regulation of Leydig cell insulin-like growth factor-I expression by interleukin-1. *Endocrinology* **130** 1217–1224.

Lin T, Wang DL, Nagpal ML & Cahang W 1994 Recombinant murine tumor necrosis factor-alpha inhibits cholesterol side-chain cleavage cytochrome P450 and insulin-like growth factor-I gene expression in rat Leydig cells. *Molecular and Cellular Endocrinology* **101** 111–119.

Maake C & Reinecke M 1993 Immunohistochemical localization of insulin-like growth factor I and II in the endocrine pancreas of rat, dog, and man, and their coexistence with classical islet hormones. *Cell and Tissue Research* **273** 249–259.

Mabley JG, Belin V, John N & Green IC 1997 Insulin-like growth factor I reverses interleukin-1 beta inhibition of insulin secretion, induction of nitric oxide synthase and cytokine-mediated apoptosis in rat islets of Langerhans. *FEBS Letters* **417** 235–238.

McCusker RH & Clemmons DR 1992 The insulin-like growth factor binding proteins: structure and biological functions. In *The Insulin-Like Growth Factors, Structure and Biological Functions*, pp 110–150. Ed PN Schofield. Oxford: Oxford University Press.

McDaniel ML, Colca JR & Kitagal M 1984 Islet cell isolation and characterization. In *Methods in Diabetes Research, Vol. 1 Laboratory Methods,* pp 153–166. Eds J Larner & S Pohl. New York: John Wiley.

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

Martin DM, Carlson R & Feldman EL 1993 Interferon-gamma inhibits DNA synthesis and insulin-like growth factor-II expression in human neuroblastoma cells. *Journal of Neuroscience Research* **34** 489–501.

Marynissen G, Aerts L & Van Assche FA 1983 The endocrine pancreas during pregnancy and lactation in the rat. *Journal of Developmental Physiology* **5** 373–381.

Muta K & Krantz S 1993 Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin. *Journal of Cellular Physiology* **156** 264–271.

Noble PW, Lake FR, Henson PM & Riches DWH 1993 Hyaluronate activation of CD44 induces insulin-like growth factor-I expression by a tumor necrosis factor-alpha-dependent mechanism in murine macrophages. *Journal of Clinical Investigation* **91** 2368–2377.

Oltvai ZN, Milliman CL & Korsemeyer SJ 1993 Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74** 609–619.

Pang K, Mukonoweshuro C & Wong GG 1994 Beta cells arise from glucose transporter type 2 (Glut2)-expressing epithelial cells of the developing rat pancreas. *Proceedings of the National Academy of Sciences of the USA* **91** 9559–9563.

Pankewycz OG, Guan J-X & Benedict JF 1995 Cytokines as mediators of autoimmune diabetes and diabetic complications. *Endocrine Reviews* **16** 164–176.

Petrik J, Arany E, McDonald TJ & Hill DJ 1998 Apoptosis in the pancreatic islet cells of the neonatal rat is associated with a reduced expression of insulin-like growth factor II that may act as a survival factor. *Endocrinology* **139** 2994–3004.

Rabinovitch A, Sumoski W, Rajotte RV & Warnock GL 1990 Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer cultures. *Journal of Clinical Endocrinology and Metabolism* **71** 152–156.

Rabinovitch A, Suarez WL, Thomas PD, Strynadka K & Simpson I 1992 Cytotoxic effects of cytokines on rat islets: evidence for involvement of free radicals and lipid peroxidation. *Diabetologia* **35** 409–413.

Rodriguez-Tarduchy G, Collins MKL, Garcia I & Lopez-Rivas A 1992 Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. *Journal of Immunology* **149** 535–540.

Romanus JA, Rabinovitch A & Rechler MM 1985 Neonatal rat islet cell cultures synthesize insulin-like growth factor I. *Diabetes* **34** 696–702.

Scaglia L, Smith FE & Bonner-Weir S 1995 Apoptosis contributes to the involution of beta cell mass in the postpartum rat pancreas. *Endocrinology* **136** 5461–5468.

Scaglia L, Cahill CJ, Finegood DT & Bonner-Weir S 1997 Apoptosis participates in the remodeling of the endocrine pancreas in the neonatal rat. *Endocrinology* **138** 1736–1741.

Schini VB, Catovsky S, Schray-Utz B, Busse R & Vanhoutte PM 1994 Insulin-like growth factor I inhibits induction of nitric oxide synthase in vascular smooth muscle cells. *Circulation Research* **74** 24–32.

Schnuerer EM, Rökaeus Å, Carlquist M, Bergman T, Dupré J & McDonald TJ 1990 Rat and porcine galanin are equipotent in inhibiting insulin responses to glucose in the anesthetized rat. *Pancreas* **5** 70–74.

Spinas GA, Hansen BS, Linde S, Kastern W, Molvig J, Mandrup-Poulsen T, Dinarello CA, Nielsen JH & Nerup J 1987 Interleukin 1 dose-dependently affects the biosynthesis of (pro)insulin in isolated rat islets of Langerhans. *Diabetologia* **30** 474–480.

Stamler JS, Singel DJ & Loscalzo J 1992 Biochemistry of nitric oxide and its redox-activated forms. *Science* **258** 1898–1902.

Stewart CE & Rotwein P 1996 Insulin-like growth factor-II is an autocrine survival factor for differentiating myoblasts. *Journal of Biological Chemistry* **271** 11330–11338.

Suarez-Pinzon WL, Strynadka K, Schulz R & Rabinovitch A 1994 Mechanisms of cytokine-induced destruction of rat insulinoma cells: the role of nitric oxide. *Endocrinology* **13** 1006–1010.

Swenne I & Hill DJ 1989 Growth hormone regulation of DNA replication, but not insulin production is mediated by somatomedin-C/insulin-like growth factor I in isolated pancreatic islets from adult rats. *Diabetologia* **32** 191–197.

Thorens B 1992 Molecular and cellular physiology of GLUT-2, a high-Km facilitated diffusion glucose transporter. *International Reviews in Cytology* **137** 209–238.

Van Schravendijk CF, Foriers A, Van Den Brande JL & Pipeleers DG 1987 Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. *Endocrinology* **121** 1784–1788.

Van Schravendijk CFH, Heylen L, Van Den Brande JL & Pipeleers DG 1990 Direct effect of insulin and insulin-like growth factor-I on the secretory activity of rat pancreatic beta cells. *Diabetologia* **33** 649–653.

Welsh N, Bendtzen K & Sandler S 1991 Influence of protease on inhibitory and stimulatory effects of interleukin 1 beta on beta-cell function. *Diabetes* **40** 290–294.

Wijsman JH, Jonker RR, Keijzer R, Van de Velde CJ, Cornelisse CJ & Van Dierendonck JH 1993 A new method to detect apoptosis in paraffin sections: *in situ* end labelling of fragmented DNA. *Journal of Histochemistry and Cytochemistry* **41** 7–12.

Yamada K, Takane-Gyotuku N, Yuan X, Ichikawa F, Inada C & Nonaka K 1996 Mouse islet cell lysis mediated by interleukin-1-induced Fas. *Diabetologia* **39** 1306–1312.

Received 9 July 1998
Accepted 4 November 1998