High sensitivity of β -cell replication to the inhibitory effects of interleukin-1 β : modulation by adenoviral overexpression of IGF2 in rat islets

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Abstract

Interleukin-1 β (IL1B) is an important contributor to the autoimmune destruction of β -cells in type 1 diabetes, and it has been recently related to the development of type 2 diabetes. IGF2 stimulates β -cell proliferation and survival. We have determined the effect of IL1B on β -cell replication, and the potential modulation by IGF2 and glucose. Control-uninfected and adenovirus encoding for IGF2 (Ad-IGF2)-infected rat islets were cultured at 5.5 or 22.2 mmol/l glucose with or without 1, 10, 30, and 50 U/ml of IL1B. β -Cell replication was markedly reduced by 10 U/ml of IL1B and was almost nullified with 30 or 50 U/ml of IL1B. Higher concentrations of IL1B were required to increase β -cell apoptosis. Although IGF2 overexpression had a strong mitogenic effect on β -cells, IGF2 could preserve β -cell proliferation only in islets cultured with 10 U/ml IL1B, and

had no effect with 30 and 50 U/ml of IL1B. In contrast, IGF2 overexpression induced a clear protection against IL1B-induced apoptosis, and higher concentrations of the cytokine were needed to increase β -cell apoptosis in Ad-IGF2-infected islets. These results indicate that β -cell replication is highly sensitive to the deleterious effects of the IL1B as shown by the inhibition of replication by relatively low IL1B concentrations, and the almost complete suppression of β -cell replication with high IL1B concentrations. Likewise, the inhibitory effects of IL- β on β -cell replication were not modified by glucose, and were only modestly prevented by IGF2 overexpression, in contrast with the higher protection against IL1B-induced apoptosis afforded by glucose and by IGF2 overexpression.

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Introduction

β -Cell mass reduction has a central role in the development of type 1 and type 2 diabetes, and in both conditions the loss of β -cells has been largely attributed to increased β -cell death (Butler *et al.* 2003, Devendra *et al.* 2004). Recent reports have highlighted the fundamental contribution of β -cell replication to the physiological maintenance of β -cell mass (Montanya *et al.* 2000, Meier *et al.* 2008), and to β -cell mass regeneration in models with reduced β -cell mass (Dor *et al.* 2004, Nir *et al.* 2007). This may suggest that impaired β -cell replication could contribute to the reduction of β -cell mass in diabetes.

Interleukin-1 β (IL1B) is an important contributor to β -cell damage in type 1 diabetes (Mandrup-Poulsen 1996), and recently it has also been related to the development of type 2 diabetes (Larsen *et al.* 2007). It is well established that IL1B, alone or in combination with other pro-inflammatory cytokines interferon- γ (IFNG) and tumour necrosis factor- α (TNF), induces β -cell death in mouse, rat, and human islets

(Saldeen 2000, Eizirik & Mandrup-Poulsen 2001, Mathis *et al.* 2001). IL1B exerts also an inhibitory effect on β -cell replication (Eizirik *et al.* 1990, Southern *et al.* 1990, Sjöholm 1991, Maedler *et al.* 2001, Téllez *et al.* 2005), that has received less attention and is less well defined. In an islet transplantation model, we recently reported that islet overexpression of the IL-1 naturally occurring antagonist, IL-1 receptor antagonist protein, increased β -cell replication and mass (Téllez *et al.* 2007), suggesting that IL1B inhibition of β -replication was relevant in β -cell mass reduction. Thus, IL1B could play a dual role in β -cell mass reduction inducing β -cell death and inhibiting β -cell replication.

Insulin-like growth factors I and II (IGF1 and IGF2) are potent β -cell growth factors (Vasavada *et al.* 2006). *In vitro*, IGF1 and IGF2 promote DNA synthesis in β -cell lines, and in rat and human fetal β -cells (Hogg *et al.* 1993, Asfari *et al.* 1995, Huotari *et al.* 1998). *In vivo*, the mitogenic effect of IGF2 was shown in transgenic mice overexpressing *Igf2* gene (Petrik *et al.* 1999a). IGF1 and -2 can also increase β -cell survival. In early neonatal life there is a wave of apoptotic

β -cell death (Scaglia *et al.* 1997) that has been linked to the concomitant reduction in IGF2 expression (Petrik *et al.* 1998, Hill *et al.* 2000). In adult islets, IGF2 survival action has been shown in rats fed with a low protein diet (Petrik *et al.* 1999b), and in transplanted islets (Robitaille *et al.* 2003). Thus, IGF2 may play a dual beneficial role on β -cell mass, acting both as a mitogenic and as a survival factor for β -cells. Cytokines inhibit the expression of IGFs in several cell types (Ilvemark *et al.* 1993, Martin *et al.* 1993, Lin *et al.* 1994), and a reduction in IGF2 immunoreactivity has been found in islet cells undergoing insulinitis, suggesting that cytokines may inhibit the expression of IGF2 in islet cells (Hill *et al.* 1999). Although IGF2 may protect islet cells from cytokine-induced apoptosis (Hill *et al.* 1999), some studies have shown no effect of IGFs on IL1B-induced apoptosis (Raile *et al.* 2003), and it is not known whether IGF2 could modify IL1B-induced suppression of β -cell replication. In this study we aimed to investigate the effect of IL1B on β -cell replication, and the potential modulation by IGF2. Since the induction of β -cell proliferation by IGFs is dependent on ambient glucose concentration (Hügl *et al.* 1998), low and high glucose concentrations were used to better define the effects of IL1B and IGF2 on β -cell replication.

Material and Methods

Islet isolation

Islets from male LEW/SsNHsd rats (Harlan, Horst, The Netherlands; 6–8 weeks old, 175–200 g of body weight) were isolated by collagenase (Collagenase P; Roche Diagnostics) digestion of the entire pancreas as previously described (Nácher *et al.* 1996). Isolated islets were hand-picked under a stereomicroscope two or three times, until a population of pure islets was obtained. Islets were washed in serum-free RPMI 1640 11.1 mmol/l glucose (Sigma Immunochemicals) supplemented with 100 U/ml penicillin and 100 μ g/ml streptomycin before infection. Each experiment was performed using a pool of 1000–1200 islets obtained from four rats in a single isolation procedure. The islets of the pool were then randomly distributed among the different experimental conditions studied in each experiment. Each condition was studied in 3–11 different experiments, as indicated in figure legends.

Recombinant adenoviruses

E1–E3-deleted adenoviral vectors were used for islet transfection. Adenovirus encoding for green fluorescent protein (Ad-GFP) was used to assess the efficiency of infection, and adenovirus encoding for luciferase (Ad-Luc) as control of infection. Ad-GFP, Ad-Luc, and adenovirus encoding for IGF2 (Ad-IGF2) were generated by Pacific Northwest Research Institute (Seattle, USA). In all adenoviral vectors the transgene was driven by the cytomegalovirus (CMV) promoter.

Gene transfer

Groups of 200 islets were left uninfected (uninfected control group), or infected with Ad-Luc, Ad-GFP, or Ad-IGF2 at a plaque-forming unit of 7×10^7 in 400 μ l serum-free RPMI 1640 11.1 mmol/l glucose for 2 h at 37 °C and 5% CO₂. After infection, islets were washed three times in RPMI 1640 containing 10% heat-inactivated FCS, and incubated overnight in non-tissue culture-treated plasticware at 37 °C in serum containing medium at 11.1 mmol/l of D-glucose.

Islet culture

After overnight incubation, islets were cultured in RPMI 1640 10% FCS, 11.1 mmol/l glucose for 48 h to determine the efficiency of infection, islet viability, and islet function. To determine the effects of IL1B on β -cell apoptosis and replication, and the modulation by IGF2 overexpression and glucose, control-uninfected islets and Ad-IGF2-infected islets were cultured for 48 h in RPMI 1640 10% FCS at 5.5 and 22.2 mmol/l D-glucose with or without 1, 10, 30, and 50 U/ml recombinant human IL1B (BD Pharmingen, Heidelberg, Germany).

Efficiency of infection

The efficiency of infection was determined by flow cytometry, confocal microscopy, and immunohistochemical confirmation of IGF2 overexpression 48 h after adenoviral infection.

Flow cytometry Forty-eight hours after infection, Ad-GFP-infected islets were dispersed into single cells and analyzed on a FACS calibur cytometer (Beckton Dickinson Instruments, Heidelberg, Germany) using 488 nm excitation and a 530 ± 15 nm band-pass filter, as previously described (Téllez *et al.* 2005).

Confocal microscopy *In vivo* observation of whole Ad-GFP-infected islets with a confocal microscope (Leica TC6-SL Spectral confocal; Mannheim, Germany) was used to determine the distribution of infected islet cells.

IGF2 expression Cultured islets were fixed overnight in 4% paraformaldehyde at 4 °C, embedded in paraffin, sectioned and immunostained after deparaffinization and rehydration. Sections were incubated overnight at 4 °C with a rabbit anti-human IGF2 antibody (final dilution 1:100; Novozymes GroPep, Adelaide, Australia). Visualization was performed with LSAB+System-HRP (DakoCytomation, Carpinteria, CA, USA).

Islet cell viability

To assess islet cell viability after adenovirus infection, fluorescein diacetate (FDA; Sigma) assay was used (Persidsky & Baillie 1977). After 48 h culture, Ad-Luc-infected islets

were dispersed into single cells, incubated with FDA (4 $\mu\text{g}/\mu\text{l}$) at 37 °C, 5% CO₂ for 10 min, and analyzed on the flow cytometer at 488 nm excitation and a 530 $\pm$ 15 nm band-pass filter for FDA detection (green).

Propidium iodide (PI) was used to determine islet cell late apoptosis and necrosis. After 48 h culture, Ad-GFP-infected islets were dispersed into single cells and immediately before cytometric analysis, 0.05 $\mu\text{g}/\text{ml}$ PI (Sigma) was added. PI fluorescence emission was collected at 620 nm (red).

Insulin secretion

Glucose-stimulated insulin secretion was used as a functional assay to determine the effects of adenoviral infection. Control-uninfected islets and Ad-Luc-infected islets were cultured for 48 h with RPMI 1640 medium supplemented with 11.1 mmol/l glucose and 10% FCS. Control-uninfected islets and Ad-IGF2-infected islets were also cultured for 48 h in RPMI 1640 10% FCS at 11.1 mmol/l D-glucose with or without 50 U/ml recombinant human IL1B to determine the effects of IL1B and IGF2 overexpression on β -cell function. Cultured islets were then washed twice with Krebs–Ringer bicarbonate buffer supplemented with HEPES and BSA (KRBH buffer: 115 mmol/l NaCl; 24 mmol/l NaHCO₃; 5 mmol/l KCl; 1 mmol/l MgCl₂; 2.5 mmol/l CaCl₂; 10 mmol/l HEPES; and 0.5% BSA, pH 7.4) with 2.8 mmol/l glucose, and were pre-incubated 1 h at 37 °C in triplicate groups of 10 islets in 1 ml fresh KRBH buffer containing 2.8 mmol/l glucose. The medium was removed and islets were incubated with 1 ml of KRBH buffer containing 2.8 or 16.7 mmol/l glucose for an additional hour with continuous shaking. Supernatants were removed and stored at –80 °C until assayed for insulin content. Insulin was measured by ELISA (Mercodia Rat insulin ELISA, Mercodia AB, Uppsala, Sweden).

DNA content

After the insulin secretion assay, islets were rinsed three times with phosphate buffer (2 M NaCl; 40 mmol/l Na₂HPO₄·H₂O; 2 mmol/l EDTA) to avoid the interfering effect of BSA in the DNA test and were disrupted by sonication. DNA was determined by a fluorimetric assay using Hoechst 33258 (Sigma; excitation wave length 356 nm, and emission wave length 448 nm) on a fluorescence spectrophotometer (F-2000, Hitachi Ltd, Tokyo, Japan).

Immunocytochemical quantification of β -cell apoptosis and replication

Cultured islets were fixed overnight in 4% paraformaldehyde at 4 °C, embedded in paraffin, sectioned and immunostained after deparaffinization and rehydration.

β -Cell apoptosis Sections were double stained by immunoperoxidase for apoptotic nuclei with the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling

(TUNEL) technique (*In Situ* Cell Death Detection Kit, ApopTag, Intergene, Oxford, UK) and by alkaline phosphatase for the endocrine non- β -cells of the islets. A cocktail of antibodies (Dako) rabbit anti-swine glucagon (final dilution 1:1000), rabbit anti-human somatostatin (final dilution 1:1000), and rabbit anti-human pancreatic polypeptide (final dilution 1:500) were used as previously described (Biarnés *et al.* 2002). After immunoperoxidase staining, β -cells and TUNEL positive β -cells were counted using an Olympus BX microscope connected to a digital camera Olympus DP70 with a color monitor (Téllez *et al.* 2005). When assessing apoptotic nuclei we excluded necrotic regions. β -Cell apoptosis was expressed as percentage of TUNEL-positive β -cells. A minimum of 1200 cells per sample were counted; the sections were systematically sampled, all endocrine nuclei were counted, and when needed a second section was included.

β -Cell replication The thymidine analog 5-bromo-2'-deoxyuridine (BrdU, Amersham) was added to the islet culture for the last 24 h of culture prior to fixation. Sections were double stained with immunoperoxidase for BrdU using a Cell Proliferation Kit (Amersham) with a modified protocol (Biarnés *et al.* 2002), and for endocrine non- β -cells of the islets using the cocktail of antibodies described above. After immunoperoxidase staining, β -cells and BrdU-positive β -cells were counted as described for β -cell apoptosis. β -Cell replication was expressed as percentage of BrdU-positive β -cells, and at least 1200 cells were counted.

Nitrite determination

Nitric oxide (NO) production by islets was measured as nitrite accumulation in culture media (nitrite is a stable product of NO oxidation). Groups of 100 control-uninfected and Ad-IGF2-infected islets were cultured for 48 h in RPMI 1640 medium without phenol red supplemented with 11.1 mmol/l glucose and 5% FCS with or without 10 U/ml of recombinant human IL1B. Samples of the conditioned media were collected and nitrite was measured by nitrate/nitrite fluorometric assay kit (Cayman Chemical, Ann Arbor, MI, USA).

Statistical analysis

Results were expressed as means $\pm$ S.E.M. Statistics were performed using SPSS 12.0 (Chicago, IL, USA) for windows, and differences among means were evaluated by the Kruskal–Wallis test, followed by the Mann–Whitney test. A *P* value of <0.05 was considered significant.

Results

Efficiency of infection

Forty-eight hours after gene transfer 100% of Ad-GFP-infected islets expressed GFP when observed by confocal microscopy (Fig. 1A). The majority of infected cells were in

Figure 1 Efficiency of adenovirus islet infection analyzed by confocal microscopy and immunocytochemistry 48 h after infection. Ad-GFP-infected islets visualized by confocal microscopy showing GFP positive cells (green). The image is a 2D average projection of a series along fixed axis (A). Infected cells were predominantly found in the periphery of the islets, as visualized by confocal microscopy in Ad-GFP-infected islets (green) (B), and in Ad-IGF2-infected islets stained with an anti-IGF2 antibody (brown) (C).

the periphery of the islets (Fig. 1B). The predominantly peripheral expression of IGF2 protein by Ad-IGF2-infected islets was confirmed by immunocytochemical staining with an anti-IGF2 antibody (Fig. 1C). When the efficiency of infection was determined at the level of individual islet cells by flow cytometry of dispersed islet cells, it was found that 29% of islet cells were infected (Fig. 2).

Adenoviral infection did not modify islet viability and function

Islet viability was similar in control-uninfected and in Ad-Luc-infected islets. In both cases, FDA staining showed 90–95% viability after 48 h in culture (data not shown). PI staining yielded similar results, and only $5.61 \pm 0.54\%$ of control-uninfected and $5.57 \pm 0.18\%$ of Ad-GFP-infected islets cells were found to be stained by PI (Fig. 2). Glucose-stimulated insulin secretion was also similar in Ad-Luc-infected and control-uninfected islets (Supplementary Fig. 1, available in the online version of the Journal of Endocrinology at <http://joe.endocrinology-journals.org/cgi/content/full/JOE-09-0047/DC1>), indicating that adenoviral infection had no effect on β -cell function.

Effects of IGF2 overexpression on IL1B-induced inhibition of β -cell-function

The well-established inhibitory effects of IL1B on β -cell function were confirmed in control-uninfected islets incubated with IL1B that showed a profoundly impaired glucose stimulated insulin secretion. IGF2 overexpression partly preserved β -cell function in islets exposed to 50 U/ml of IL1B that showed an increased insulin secretion in response to 16.7 mmol/l glucose of borderline statistical significance ($P=0.077$), and an insulin stimulation index that doubled that of control-uninfected islets (2.24 ± 1.69 vs 5.45 ± 0.92 ; Supplementary Fig. 2, available in the online version of the Journal of Endocrinology at <http://joe.endocrinology-journals.org/cgi/content/full/JOE-09-0047/DC1>).

Effects of IGF2 overexpression on IL1B-induced β -cell apoptosis

IL1B increased β -cell apoptosis in a dose-dependent manner in islets cultured at low (5.5 mmol/l) and at high (22.2 mmol/l) glucose concentration (Fig. 3). β -Cell apoptosis was higher in

Figure 2 Efficiency of adenovirus islet infection and islet cell viability analyzed by flow cytometry 48 h after infection. Dispersed islet cells of control-uninfected islets (left dot-plot) and Ad-GFP-infected islets (right dot-plot) were analyzed by flow cytometry for GFP production and propidium iodide (PI) staining. 29% of individual cells from Ad-GFP-infected islets showed GFP expression, and only 5.6% of both control-uninfected and Ad-GFP-infected islets were stained by PI.

Figure 3 Effect of IL1B and IGF2 overexpression on β -cell apoptosis. Control-uninfected and Ad-IGF2-infected islets were exposed to 0, 1, 10, 30 and 50 U/ml of IL1B, at 5.5 (A) or 22.2 (B) mmol/l glucose for 48 h to study the effect of IL1B and of IGF2 overexpression on β -cell apoptosis. Values are means $\pm$ S.E.M. ($n=9-11$ for control-uninfected and Ad-IGF2-infected islets not exposed to IL1B, and $n=5$ for all other groups). Kruskal–Wallis, $P<0.001$ among all groups cultured at 5.5 mmol/l glucose (A), or among all groups cultured at 22.2 mmol/l glucose (B). Mann–Whitney * $P<0.01$ versus control-uninfected group not exposed to IL1B; # $P<0.05$ between control-uninfected islets and IGF2-infected islets cultured with 50 U/ml IL1B.

islets cultured at low glucose concentration that showed increased apoptosis when exposed to 10 U/ml or higher concentrations of IL1B. In islets cultured at 22.2 mmol/l glucose, higher concentrations of IL1B (30 and 50 U/ml) were required to increase β -cell apoptosis that was not modified by 10 U/ml of IL1B.

IGF2 overexpression protected β -cells against IL1B-induced apoptosis. In islets cultured at 22.2 mmol/l glucose and exposed to 50 U/ml of IL1B, β -cell apoptosis was lower in Ad-IGF2 islets than in control-uninfected islets (1.62 ± 0.19 vs $0.99 \pm 0.13\%$, $P<0.03$). Moreover, a higher concentration of IL1B was required to increase β -cell apoptosis in Ad-IGF2-infected islets compared to control-uninfected islets, both in islets incubated at low (Fig. 3A) and at high glucose (Fig. 3B). In islets cultured at 5.5 mmol/l glucose, 10 U/ml of IL1B were sufficient to increase β -cell

apoptosis in control islets but not in Ad-IGF2 islets that had to be exposed to 30 U/ml IL1B. Similarly, in islets cultured at 22.2 mmol/l glucose, incubation with 30 U/ml IL1B increased β -cell apoptosis in uninfected islets but not in Ad-IGF2 islets that had to be exposed to 50 U/ml IL1B. Thus, the IL1B pro-apoptotic effect on β -cells was higher at low glucose concentrations, and was reduced in islets overexpressing IGF2.

Effects of IGF2 overexpression IL1B-induced inhibition of β -cell replication

IL1B reduced β -cell replication in islets exposed to 10, 30, and 50 U/ml, both at low and high glucose concentrations (Fig. 4). β -Cell replication showed a high sensitivity to IL1B as indicated by the dramatic reduction in islets exposed to 10 U/ml IL1B and the almost complete suppression with 30 and 50 U/ml of IL1B.

At 5.5 mmol/l glucose, 1 U/ml IL1B increased β -cell replication compared with uninfected islets not exposed to IL1B. No effect of 1 U/ml IL1B was detected in islets cultured at 22 mmol/l glucose. Glucose increased β -cell replication in uninfected and in Ad-IGF2 islets in the absence of IL1B and in islets exposed to low (1 and 10 U/ml) IL1B concentrations.

IGF2 overexpression doubled β -cell replication in islets cultured at 5.5 and at 22.2 mmol/l glucose compared with control-uninfected islets, and showed a protective effect on β -cell replication in islets exposed to 10 U/ml IL1B that maintained β -cell replication similar to that of control-uninfected islets not exposed to IL1B. At higher IL1B concentrations (30 and 50 U/ml), IGF2 overexpression had minimal impact on β -cell replication that remained almost completely suppressed both in islets cultured at 5.5 and 22.2 mmol/l glucose.

Nitrite production

Nitrite production was measured to determine whether IGF2 overexpression modified IL1B-induced production of NO. Nitrite accumulation in cell culture supernatants was increased in control-uninfected islets exposed to 10 U/ml of IL1B for 48 h (Fig. 5). In contrast, no changes in nitrite accumulation were detected when islets overexpressing IGF2 were exposed to IL1B, indicating that IGF2 prevented the formation of NO in islets exposed to IL1B.

Discussion

In this study, we show that IL1B has a profound inhibitory effect on β -cell replication that was partly prevented by adenoviral overexpression of IGF2 on islet cells. The high sensitivity of β -cell replication to IL1B was established based on the almost complete suppression of β -cell replication, the absence of modulation by glucose, and the relatively

Figure 4 Effect of IL1B and IGF2 overexpression on β -cell replication. Control-uninfected and Ad-IGF2-infected islets were exposed to 0, 1, 10, 30, and 50 U/ml of IL1B, at 5.5 (A) or 22.2 (B) mmol/l glucose for 48 h to study the effect of IL1B and of IGF2 overexpression on β -cell and replication. Values are means $\pm$ S.E.M. ($n=9-11$ for control-uninfected and IGF2-infected islets not exposed to IL1B, and $n=5$ for all other groups). Kruskal-Wallis, $P<0.001$ among all groups cultured at 5.5 mmol/l glucose (A) or among all groups cultured at 22.2 mmol/l glucose (B). Mann-Whitney, $*P<0.05$ versus control-uninfected group not exposed to IL1B.

modest prevention achieved by IGF2 overexpression compared with the higher protection afforded by glucose and IGF2 on IL1B-induced apoptosis.

β -Cell replication was increased in islets exposed to very low IL1B (1 U/ml) concentration and was severely reduced with higher amounts of IL1B. The mitogenic effect of very low IL1B concentrations is in agreement with the results reported in human islets, a beneficial action that could reflect a physiological role of low cytokine concentrations on islet cells (Maedler *et al.* 2006). In contrast, higher IL1B concentrations had a clear deleterious effect, and induced a strong inhibition of β -cell replication. The suppression of β -cell replication was detected with IL1B concentrations that were insufficient to induce β -cell apoptosis, indicating a high sensibility of β -cell replication to IL1B. β -Cell replication was substantially reduced in rat islets exposed to 10 U/ml IL1B, and was almost completely suppressed in

islets exposed to 30 and 50 U/ml of IL1B. In contrast, 30 and 50 U/ml of IL1B was required to consistently increase β -cell apoptosis.

The strong suppression of β -cell replication by IL1B was also found in islets overexpressing IGF2. In the absence of IL1B, IGF2 overexpression increased β -cell replication in islets cultured at low and high glucose concentration in agreement with the well-known mitogenic effects of IGF2 (Hogg *et al.* 1993, Asfari *et al.* 1995, Petrik *et al.* 1999a). In islets exposed to IL1B, IGF2 overexpression had a clear anti-apoptotic action, but resulted in a more modest preservation of β -cell replication. IGF2 overexpression reduced β -cell apoptosis in all groups showing IL1B-induced β -cell apoptosis, but preserved β -cell replication only in islets exposed to 10 U/ml IL1B, and had no effects on the suppressed β -cell replication of islets exposed to 30 or 50 U/ml IL1B. Overall, the results indicate that β -cell replication is more sensitive to the deleterious effects of IL1B than β -cell survival.

The effects of glucose on β -cell survival are modified by glucose concentration, duration of exposure, and genetic background. *In vitro*, glucose has been found to promote the survival of rat single β -cells (Hoorens *et al.* 1996), but also to induce apoptosis in islets of diabetic-prone animals (Donath *et al.* 1999), and in human islets (Maedler *et al.* 2001). *In vivo*, we have reported increased β -cell apoptosis in transplanted mice islets exposed to chronic hyperglycemia (Biarnés *et al.* 2002). The effects of glucose on IL1B-induced apoptosis are not well established, and although high glucose concentrations amplified cell damage in islets exposed to IL1B (Spinus *et al.* 1988), other studies reported no effects of glucose on IL1B-induced β -cell apoptosis (Raile *et al.* 2003, Téllez *et al.* 2005). In the current study, the use of several IL1B concentrations allowed us to identify the protective action of glucose on IL1B-induced apoptosis. In the absence of IL1B, β -cell apoptosis was similar in islets incubated at low

Figure 5 Effects of IL1B and IGF2 overexpression on islet cell production of nitric oxide. Control-uninfected islets and Ad-IGF2-infected islets were incubated for 48 h in the presence or absence of 10 U/ml of IL1B. Nitrite production is expressed as pmol of nitrite/islet every 48 h. Values are means $\pm$ S.E.M. ($n=4$) $*P<0.05$ between control islets exposed to 10 U/ml IL1B and Ad-IGF2 islets exposed to 10 U/ml IL1B.

and high glucose concentration. However, when islets were exposed to 10 U/ml IL1B, β -cell apoptosis was increased in islets cultured at low (5.5 mmol/l), but not at high (22.2 mmol/l) glucose, suggesting that glucose had a pro-survival effect. This protective effect of glucose was confirmed in islets overexpressing IGF2 and exposed to 30 U/ml IL1B that showed increased β -cell apoptosis only when cultured at low glucose. In contrast, despite the strong and well established proliferative effect of glucose on β -cells (Svenne 1982, Lingohr *et al.* 2006) glucose did not modulate the inhibitory effect of IL1B on β -cell replication, an additional indication of the high sensitivity of β -cell replication to the deleterious effects of IL1B.

Several of the deleterious effects of IL1B on rodent islets are mediated by NO (Darville & Eizirik 1998) which is produced by the inducible form of NO synthase (iNOS). We found that in islets exposed to IL1B, NO production was lower in islets overexpressing IGF2 than in control-uninfected islets, suggesting that the protection afforded by IGF2 was mediated in part by the inhibition of iNOS expression. These results are in agreement with the observation that the neonatal peak of islet apoptosis coincides with low IGF2 and with increased iNOS levels, suggesting that IGF2 has an inhibitory effect on NO formation (Petrik *et al.* 1998). Furthermore, signaling by IGF1 involves the activation of IGF1 receptor, and IGF1 has been shown to decrease IL1B-mediated NO formation by inhibition of iNOS expression and synthesis in rodent islets (Mabley *et al.* 1997, Castrillo *et al.* 2000), and to prevent IL1B-mediated NO production in human islets, as well as β -cell dysfunction and apoptosis (Giannoukakis *et al.* 2000).

The inhibitory effect of IL1B on β -cell secretion are well established and have been studied in detail (Eizirik *et al.* 1988, Scarim *et al.* 1997). Our experiments have confirmed the profound impairment of β -cell function induced by IL1B, and we found a limited protection in islets infected with Ad-IGF2. Some studies have shown a role for insulin in adult β -cell replication and β -cell mass maintenance (Okada *et al.* 2007). In our experiments, although IGF2 overexpression partly preserved insulin secretion in islets exposed to high IL1B, β -cell replication remained fully suppressed, indicating that the secreted insulin was not sufficient to increase β -cell proliferation.

The high sensitivity of β -cell replication to IL1B may be relevant for the reduction of β -cell mass which takes place in diabetes. In normal conditions, β -cell mass is maintained by a balance between cell regeneration and death, and recent data have shown that β -cells have a strong potential for regeneration that can compensate severe reductions in β -cell mass (Dor *et al.* 2004, Nir *et al.* 2007), even though β -cell growth potential could be more limited in human islets (Parnaud *et al.* 2008). IL1B-induced β -cell damage has been implicated in the pathogenesis of type 1 diabetes and more recently in type 2 diabetes, essentially based on the suppressive effects of IL1B on β -cell function and on the induction of β -cell death. The strong inhibitory effect of IL1B on β -cell

replication that we show suggests that impaired β -cell replication may be important in the demise of β -cells in diabetes. The suppression of β -cell replication could abrogate the replicative response needed to compensate the reduction of β -cell mass induced by increased β -cell death. The recent indication that β -cell replication is not increased in recent-onset type 1 diabetic patients could support this hypothesis (Butler *et al.* 2007). By suppressing β -cell replication IL1B could prevent the renewal of β -cells and reduce β -cell mass even in the absence of increased β -cell apoptosis.

Adenoviral overexpression is a useful technique to assess the effects achieved by the local and transient administration of proteins with potential therapeutic action. This is of particular interest in islet transplantation, where the expression in the graft of proteins with therapeutic action could obtain the beneficial effect and avoid the unwanted toxicity associated with systemic administration. The beneficial effects of adenoviral overexpression of IGF2 that we have found *in vitro* provide the bases to tests these effects in *in vivo* in experimental islet transplantation, a condition where increased levels of IL1B in the graft in the initial days after transplantation are thought to play a deleterious role in the survival of transplanted β -cells (Montolio *et al.* 2007). The predominantly peripheral expression of IGF2 in islet cells suggests that the majority of infected cells were endocrine non- β cells, most of them α -cells. However, since IGF2 is secreted, it was active on more cells than just the islet cells infected by the adenovirus, and exerted a paracrine effect on neighboring cells as indicated by the substantial effects found on β -cells. The different distribution of endocrine cell types in rodent and human islets, where α -cells and β -cells are scattered throughout the islet, could increase the number of infected β -cells in human islets. However, the paracrine effect of secreted IGF2 reduces the significance of the specific endocrine cell type that becomes infected by the adenovirus.

In summary, our results indicate that β -cell replication is highly sensitive to the deleterious effect of IL1B. The strong suppressive effect of IL1B on β -cell replication may be relevant for the process leading to the loss of β -cell mass in diabetes. Adenoviral transfer of IGF2 to islets protected against IL1B inhibition of β -cell replication and IL1B-induced β -cell apoptosis, and prevented IL1B-induced NO production, suggesting that IGF2 could have a role in strategies to induce the regeneration of β -cell mass in diabetes or in islet transplantation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this scientific work.

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