Beneficial effect of 1,25 dihydroxyvitamin D₃ on cytokine-treated human pancreatic islets

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Abstract

We examined whether 1,25 dihydroxyvitamin D₃ (1,25 D₃), the active form of vitamin D involved in the regulation of the immune system, may also protect human pancreatic islet cells from destruction induced by cytokines. In this study, we specifically investigated the effect of 1,25 D₃ on oxidative stress and major histocompatibility complex (MHC) induction, both implicated in cytokine-induced islet cell dysfunction and destruction. We also investigated the effects of 1,25 D₃ on interleukin (IL)-6, a pleiotropic cytokine implicated in the pathogenesis of immunoinflammatory disorders.

Human pancreatic islets, isolated from heart-beating donors, were treated with a combination of three cytokines, IL-1 β +tumor necrosis factor α +interferon γ , in the presence or absence of vitamin D, and compared with untreated control cells. Metabolic activity was assessed by cell viability and insulin content. Oxidative stress was estimated by heat shock protein 70 (hsp70) expression, cell manganese superoxide dismutase (MnSOD) activity and nitrite release, a reflexion of nitric

oxide (NO) synthesis. Variation of immunogenicity of islet preparations was determined by analysis of the MHC class I and class II transcripts. Inflammatory status was evaluated by IL-6 production. After 48 h of contact with cytokines, insulin content was significantly decreased by 40% but cell viability was not altered. MHC expression significantly increased six- to sevenfold as well as NO and IL-6 release (two- to threefold enhancement). MnSOD activity was not significantly induced and hsp70 expression was not affected by the combination of cytokines.

The addition of 1,25 D₃ significantly reduced nitrite release, IL-6 production and MHC class I expression which then became not significantly different from controls. These results suggest that the effect of 1,25 D₃ in human pancreatic islets cells may be a reduction of the vulnerability of cells to cytotoxic T lymphocytes and a reduction of cytotoxic challenge. Hence, 1,25 D₃ might play a role in the prevention of type 1 diabetes and islet allograft rejection.

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Introduction

Type 1 diabetes is a multifactorial autoimmune disease in which insulin-producing beta cells are selectively destroyed. Impaired function and destruction of beta cells may result from direct contact with islet-infiltrating macrophages and T lymphocytes and/or exposure to inflammatory products of the islet-infiltrating cells, such as free radicals and cytokines (Mandrup-Poulsen *et al.* 1990). The inflammatory cytokines, interleukin (IL)-1 β , tumor necrosis factor α (TNF α) and interferon γ (IFN γ), acting individually or more potently in combination, are cytotoxic to human islet beta cells *in vitro* by stimulating cytotoxic mediators such as other inflammatory cytokines, nitric oxide (NO), superoxide anion or major histo-

compatibility complex (MHC) (Iwahashi *et al.* 1998, Rabinovitch & Suarez-Pinzon 1998). This combination of cytokines may also activate pathways of gene regulation leading to induction of protective compounds such as stress proteins and antioxidant enzymes (Eizirik 1996). The modulation of such inductions may prove to be beneficial for beta cells and 1,25 dihydroxyvitamin D₃ (1,25 D₃), the active metabolite of vitamin D₃, may be a candidate able to fulfill some of these criteria.

Indeed, 1,25 D₃ has been shown to prevent autoimmune disease such as experimental encephalomyelitis (Lemire & Archer 1991), autoimmune thyroiditis (Fournier *et al.* 1990) or experimental rat nephritis (Hattori 1990). Other experiments carried out in the non-obese diabetic (NOD) mouse, a model for type 1 diabetes,

demonstrated that 1,25 D₃ also prevented insulinitis and diabetes (Mathieu *et al.* 1992, 1994, Casteels *et al.* 1998). In fact, the incidence of insulinitis was reduced by 1,25 D₃, a known powerful immunosuppressive compound which inhibits lymphocyte proliferation and cytokine production *in vitro* including IL-6 secretion (Müller *et al.* 1991, Lemire *et al.* 1995).

On the other hand, pancreatic beta cells taken from newly diagnosed patients with type 1 diabetes have been shown to express MHC class II molecules in addition to augmented levels of MHC class I molecules (Bottazzo *et al.* 1985, Foulis *et al.* 1987) and a previous report from Hahn *et al.* (1997) showed that, in islet cells of bio-breeding (BB) rats, MHC antigen expression stimulated by IFN γ was reduced by 1,25 D₃ and an analog ZXY 1106.

Besides immunomodulatory properties, 1,25 D₃ might also exhibit beta cell-protective properties since, in rat pancreatic islet cells, Sandler *et al.* (1994) demonstrated that 1,25 D₃ and some of its structural analogs counteracted the suppressive effect of IL-1 β on accumulated insulin release.

No demonstration of the potential protective action of 1,25 D₃ in human pancreatic islet cells has been achieved at this time. We therefore studied the effect of 1,25 D₃ on cytokine-induced damage to human pancreatic islets and particularly the effect on oxidative stress, IL-6 and MHC induction.

Materials and Methods

Human islet processing

Human pancreases (mean age 36 ± 15 years, $n=10$) were harvested from adult brain-dead donors in accordance with French regulations and with the local Institutional Ethical Committee. Pancreatic islets were isolated after ductal distension of the pancreas and digestion of the tissue with Liberase (Roche Diagnostics, Meylan, France) according to the automated method of Ricordi *et al.* (1988) with modifications (Kerr-Conte *et al.* 1994). Semi-purification was achieved with Euro-Ficoll discontinuous density gradient using a cell separator (Lake *et al.* 1989). Islet number was determined on samples of each preparation after dithizone staining and expressed as equivalent number of islets (IE) (Ricordi *et al.* 1990). Preparations used in this study exhibited an $85 \pm 7\%$ purity and an average yield of 2150 ± 545 IE/g pancreas. Semi-purified islets were cultured for 24 h in CMRL 1066 medium (Gibco BRL, Life Technologies, Cergy-Pontoise, France) with 2% Ultrosor G (Gibco BRL), then processed in batches of 500 IE in 4-well multidish plates (Nunc; Gibco BRL) or 40 IE in 96-well multiscreen plates (Millipore, Saint Quentin Yvelines, France) (Vandewalle *et al.* 1999). Islet processing consisted of a 48-h treatment at 37 °C in different media, including control basal medium (Ham's F10 containing 0.5% bovine serum albumin), cytokine-

treated medium (basal medium with IL-1 β (50 IU/ml)+TNF α (1000 IU/ml)+IFN γ (1000 IU/ml); cytokines from Biosource, Clinisciences, le Pont de Claix, France) and cytokine-treated medium containing 1,25 D₃ dissolved in ethanol (10^{-8} and 10^{-6} mol/l, final concentrations). Alcohol concentration did not exceed 0.01% in the culture media and was without measurable effect on cell cultures. 1,25 D₃ was a generous gift from Hoffmann la Roche AG, Basel, Switzerland.

Insulin content

Islets were pelleted by centrifugation and homogenized by sonication (20 kHz, 30 W) for 15 s in 200 μ l Tris-HCl, 1 mmol/l EDTA, pH 7.4 and stored frozen at -20 °C.

The islet insulin content was measured by radioimmunoassay (CIS bio International, Gif-sur Yvette, France) after overnight extraction at 4 °C of 50 μ l aliquots of homogenates with 125 μ l acid-ethanol (0.18 mol/l HCl in 95% ethanol).

Estimation of islet cell viability

We used the Alamar blue reagent (Alamar Blue TM; Biosource) which contains a non-toxic REDOX indicator. Reduction related to metabolic activity causes the REDOX indicator to change from an oxidized to a reduced fluorescent form (Ahmed *et al.* 1994). Alamar blue was added directly (10% v/v) to islet cells cultured in multiscreen wells containing 40 IE per condition in 200 μ l culture medium and incubated for 48 h and 144 h at 37 °C in an atmosphere of air:CO₂ (95:5). After transfer of the reaction mixture to a 96-well microtiter plate, the fluorescence was read at 544 nm (excitation wavelength) and 590 nm (emission wavelength) (Vandewalle *et al.* 1999).

Nitrite determination

The nitrite assays were performed in the culture medium from each well as previously described (Vandewalle *et al.* 1999). This assay was adapted from the study of Misko *et al.* (1993) where nitrite determination is based upon the reaction of nitrite with 2,3-diaminonaphthalene (Sigma-Aldrich Chemicals, Saint Quentin Fallavier, France) to form the fluorescent product 1-(H)-naphtotriazole.

Manganese superoxide dismutase activity

To measure superoxide dismutase (SOD) activity, batches of 500 IE were homogenized by sonication (20 kHz, 30 W) for 10 s in 250 μ l phosphate buffer, pH 7.8 (1/15 mol/l Na₂PO₄-KH₂PO₄-0.1 mmol/l EDTA). The activity of SOD was estimated as described by the manufacturer (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK). In this method superoxide radicals, generated by the action of xanthine oxidase on xanthine, reacted with

2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was thereafter measured at 505 nm by the degree of inhibition of this reaction. Estimation of manganese superoxide dismutase (MnSOD) activity was achieved after specific inhibition of copper–zinc superoxide dismutase with 5 mmol/l potassium cyanide (Flohé & Ötting 1984).

Extraction of RNA

Total cellular RNAs were obtained by lysis of 500 IE per condition in 1 ml RNAzol® (Bioprobe Systems, Montreuil, France) and quantified spectrophotometrically (260 nm). The cDNAs were synthesized from 2 µg DNA-free total RNA with 1000 ng oligo(dT) 12–18 primer and 4 U RNAsin (Gibco BRL) first incubated for 10 min at 70 °C. Reverse transcription was then performed at 42 °C for 1 h by the addition of 200 U reverse transcriptase (M-MLV; Life Technologies) plus 2 mmol/l of each dNTP (Pharmacia, Saint Quentin Yvelines, France), 4 U RNAsin and 1 µg tRNA (Boehringer Mannheim, Meylan, France). Products were further incubated for 1 h at 42 °C using 100 U reverse transcriptase then the final reaction mixture was heated at 95 °C for 5 min and stored at –20 °C until use.

Heat shock protein 70 analysis by quantitative RT-PCR

Heat shock protein 70 (hsp70) mRNA was monitored in 96-well plates by quantitative RT-PCR of the target fragment and an 18S housekeeping gene sequence as an internal standard. Amplification was achieved with specific primers of the target sequence hsp70 (5'-GACTT TGACAACAGGCTGGTGA-3') and (5'-ACAGGGTC CTCTTGGCCC-3') and 18S (5'-TCCCAGTAAGTGC GGGTCA-3') and (5'-GATCCGAGGGCCTCACTA AAC-3') (GENSET SA, Paris, France), resulting in 129 bp and 104 bp products respectively.

The template concentration per reaction represented one-tenth of the cDNA reaction performed on 2 µg total RNA. The amplification was achieved in 25 µl reaction mixture containing 3 µl cDNA, 15 pmol of each oligonucleotide primer and 2 × Sybr Green Master Mix (4309155; Perkin-Elmer Biosystems, Courtaboeuf, France).

After denaturation for 10 min at 95 °C, 40 cycles of amplification were performed in an ABI Prism 7700 Sequence Detection System (Perkin-Elmer Biosystems), amplification parameters included 30-s denaturation at 94 °C and 1-min annealing step at 60 °C. Direct detection of PCR products was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green to double-stranded DNA; all quantitations were achieved with the comparative C_T method and normalized to the endogenous control 18S rRNA as described by the manufacturer (Perkin-Elmer Biosystems).

Analysis of MHC I and MHC II transcripts by semi-quantitative RT-PCR

Semi-quantitative, non-competitive RT-PCR was performed with AmpliTaqGold™ (Perkin-Elmer) which allowed simultaneous co-amplification of the housekeeping gene (β-actin) and each of our target genes.

Primer sets (Perkin-Elmer Biosystems) included the β-actin primers (position 405–722): sense 5'-ATCATGT TTGAGACCTCCAA-3', antisense 5'-CATCTCTTGC TCGAAGTCCA-3' resulting in a 314 bp product; MHC I primers (locus A.B.C): sense 5'-CTCCCCAGACG CCGAGA-3', antisense 5'-GCGCTGCAGCGTCTC CTT-3' resulting in a 632 bp product; MHC II primers (locus DR β): sense (position 508–528 in exon 1) 5'-TC TCCAGCATGGTGTGTCTGA-3', antisense (position 1146–1166 in exon 2) 5'-CTCGCCGCTGCACTGTG AAGC-3' resulting in a 390 bp product. PCR was performed with 1 µl cDNA from each sample in the presence of 200 µmol/l dNTP, 1.5 mmol/l MgCl₂, 5 U AmpliTaq DNA polymerase and 25 pmol/l of each couple of primers. PCR (32 cycles) was carried out in a thermal cycler (2400; Perkin-Elmer Biosystems) with each cycle consisting of denaturation at 94 °C for 30 s, annealing at 54 °C for 1 min and polymerization at 75 °C for 30 s. The last PCR step was a final extension at 75 °C for 5 min. The PCR products were electrophoresed in 2% agarose gel and bands were scanned with an integration camera CDD (COHU 4912; Clara Vision, Orsay, France) and analyzed with GelAnalysts™ 3.01 FR software (© GreyStone-Iconix; MEB Electronique, Paris, France). Band intensity was expressed in arbitrary units and MHC I or MHC II mRNA expression indexed to β-actin mRNA expression.

IL-6 determination

Concentrations of IL-6 released in culture medium were determined with commercial enzyme-linked immunosorbent assay kits (Immunotech, Marseille, France). Assays were performed as described by the manufacturer using monoclonal antibodies and acetylcholinesterase as the tracer enzyme (Brailly *et al.* 1994).

Presentation of results

Data analyses were performed using Statview (Abacus Concepts Inc., Berkeley, CA, USA). Given protocols were tested in islet cells from five to ten preparations from different donors. The statistical significance between means was assessed by Student's *t*-test for paired values.

Results

After 48 h of contact of human islets with cytokines, insulin content was significantly decreased (Fig. 1), cell

Figure 1 The insulin content of 48-h treated cells was assessed by immunoassay after extraction as described in Materials and Methods. Values are expressed as percentage of control cells (2.7 ng/IE). Data are means ± S.E.M. (n=7). *P<0.05 (Student's t-test for paired values).

viability was not altered at that time but became significantly lower than the control after 6-day exposure (Fig. 2). In both cases, 1,25 D₃ addition resulted in a tendency to revert values to those of controls.

The effect of 1,25 D₃ on the oxidative stress induced by cytokines was first estimated by the measurement of nitrite accumulation in the culture medium. Cytokines significantly enhanced nitrite levels (two- to threefold increase).

Figure 2 The metabolic activity of 48-h (solid bars) and 6-day (open bars)-treated cells was assessed using the REDOX indicator Alamar blue as described in Materials and Methods. Values are expressed as percentage of control cells. Data are means ± S.E.M. (n=6). *P<0.05 (Student's t-test for paired values).

Figure 3 Nitrite released by cells in the culture medium during 48 h of treatment was assessed by fluorometric assay as described in Materials and Methods. Values are expressed as percentage of control cells (1.7 pmol NaNO₂/IE). Data are means ± S.E.M. (n=7). **P<0.01 (Student's t-test for paired values).

The addition of 1,25 D₃ resulted in a significant reduction of values that were no longer significantly different from controls (Fig. 3).

We also observed a stimulation of MnSOD by cytokines that did not, however, reach statistical significance (Fig. 4), while the expression of hsp70 was not modified at all by cytokines (data not shown). The addition of 1,25 D₃ did not induce further significant modifications in these two latter results.

Figure 4 MnSOD activity in 48-h treated cells was assessed by a photometric assay as described in Materials and Methods. Values are expressed as percentage of control cells (74 μIU/IE). Data are means ± S.E.M. (n=5).

Figure 5 MHC was determined by RT-PCR analyses performed on total RNA extracted from 48-h treated cells as described in Materials and Methods. Values are expressed as percentage of control cells. Data are means $\pm$ S.E.M. ($n=7$). * $P<0.05$, ** $P<0.01$ (Student's t -test for paired values).

Analysis of MHC expression in cytokine-treated islet cells highlighted a strong stimulation of both class I and II complexes (six- to sevenfold increase); 1,25 D₃ significantly decreased MHC class I but not class II expression (Fig. 5).

Finally, cytokines significantly enhanced IL-6 release (three- to fourfold increase) and a significant reduction of values by 1,25 D₃ was noticed (Fig. 6).

Discussion

In this study we have tried to assess, in human pancreatic islet cells, the effect of 1,25 D₃ on cytokine-mediated inhibition of beta cell function in association with the expression of MHC antigens and the production of oxygen species. Indeed, important factors in cytokine-induced beta cell death may be NO-mediated cell damage and the toxic effects of oxygen-free radicals. Other factors are cytokine-stimulated class I and/or class II molecules which, when associated with antigens and recognized

Figure 6 IL-6 production by 48-h treated cells was determined in the culture media by immunoassay as described in Materials and Methods. Values are expressed as percentage of control cells (4 pg/IE). Data are means $\pm$ S.E.M. ($n=6$). * $P<0.05$ (Student's t -test for paired values).

by T-Helper cells, enhance the targeting of cytotoxic T-lymphocytes against islet cells. In addition, in pancreatic beta cells, as in a variety of cell types including fibroblasts, endothelial cells and monocytes, IL-1 β and TNF α also stimulate the production of other cytokines such as IL-6 which may act as a co-stimulator for autoreactive B and T lymphocytes in autoimmune diabetes (Campbell *et al.* 1989).

Simultaneously, potentially important defence and repair responses such as formation of heat shock proteins and superoxide dismutase are induced by cytokines. Human pancreatic islet cells have been shown to possess more defence molecules than murine islet cells (Welsh *et al.* 1995, Eizirik 1996) so we used a combination of three cytokines at the concentrations known to be required to induce damage in human islet cells (Rabinovitch *et al.* 1990, Eizirik *et al.* 1994).

We noticed, as described by others, that cytokines induce a significant decrease in insulin content. The addition of 1,25 D₃ showed a clear tendency to revert values to control levels. After 48 h of contact with cytokines, the cell viability was not affected. Longer time-periods (5–6 days) have been reported to be necessary for the destruction of human islet cells (Eizirik *et al.* 1994, Delanay *et al.* 1997). In our experiments, a significant reduction in cell viability was also observed after 6 days of exposure; the addition of 1,25 D₃ also reverted values to those of the control.

NO, induced by cytokines and assessed by nitrite accumulation in the culture medium, was significantly enhanced after 48 h of culture compared with control values. The addition of 1,25 D₃ significantly reduced nitrite levels. NO is known to mediate physiological

processes via a reaction with superoxide or by complexing with a metal (e.g. iron/sulfur complexes) at the active sites of enzymes. In pancreatic beta cells, these NO-induced complexes result in an inhibitory effect on the Krebs cycle and, particularly, in an inhibition of the activity of aconitase, an enzyme which converts citrate to isocitrate, thus leading to a severe decrease in glucose metabolism and ATP production (Welsh *et al.* 1991). IL-1 β -induced NO production has been reported to be mediated by the nuclear transcription factor NF- κ B (Flodstrom *et al.* 1996). The action of 1,25 D₃ on cytokine-induced NO production might lie at the level of NF- κ B activation, since, in other systems, a modulation of NF- κ B protein levels by 1,25 D₃ has been reported (Yu *et al.* 1995, Larsen *et al.* 1998). Other transcription factors of potential relevance for inducible nitric oxide synthase (iNOS) activation are c-Jun N-terminal kinase (Bonny *et al.* 2000) and activator protein-1 (Xie *et al.* 1993) which might also represent potential targets for 1,25 D₃.

Cytokine-induced iNOS mRNA expression and NO production is paralleled by increased MnSOD expression (Bigdeli *et al.* 1994) and NF- κ B has also been shown to be required for cytokine-induced MnSOD expression in insulin-producing cells (Darville *et al.* 2000). A combination of three cytokines was also reported to be necessary to induce MnSOD protein expression in human pancreatic islets (Welsh *et al.* 1995). In our study, islets exposed to cytokines for 48 h showed a mean 2.8-fold greater activity of MnSOD than in control islets; however, this was not significant ($P=0.08$) due to the great dispersion of values; moreover, the addition of 1,25 D₃ did not significantly modify this enhancement. We concluded that 1,25 D₃ prevents the induction of NO by cytokines in human islets, without affecting cytokine-induced MnSOD activity.

We also found that the combination of cytokines did not significantly increase the hsp70 expression. The high level of human hsp70 in basal conditions may explain this absence of a stimulatory effect of cytokines (Welsh *et al.* 1995, Eizirik 1996); in addition, 1,25 D₃ did not modify hsp70 expression. However, an emerging body of evidence suggests that hsp70 participates in cytokine signal transduction (Moseley 1998) and that its protective effect on islet metabolic function may be associated with an inhibition of IL-1 β -stimulated iNOS expression (Scarim *et al.* 1998). In our experiments, such a mechanism was not likely to be effective since hsp70 expression was unrelated to NO modifications.

In the pancreatic islets of rodents, BB rats and humans with autoimmune diabetes, over-expression of class I MHC (Allison *et al.* 1988, Kay *et al.* 1991) is observed. Class I MHC expression is up-regulated by cytokines, and especially IFN γ , and is induced in beta cells during destructive insulinitis (Campbell *et al.* 1986, Lafferty 1997, Thomas *et al.* 1998). IFN γ also induces MHC class II expression in many cell types, including fibroblasts and

endothelial cells (Pujol-Borell *et al.* 1987). In human pancreatic islet cells, MHC II has been demonstrated in 5–10% of ductal cells; this percentage increases in the presence of IFN γ (Pavlovic *et al.* 1997). No expression, however, has been found in human endocrine beta and non-beta cells and conflicting results regarding the possible induction by IFN γ have been reported (Campbell *et al.* 1986, Pujol-Borell *et al.* 1987, Pavlovic *et al.* 1997).

In cytokine-stimulated human pancreatic islet cells, we demonstrated a significant increase in both MHC I and MHC II expression and a significant inhibition of MHC I induction by 1,25 D₃ contrary to MHC II expression which remained statistically unchanged by 1,25 D₃. Hyperexpression of MHC molecules, in response to certain cytokines, could be a primary event leading directly to beta cell destruction (Okamoto 1999) and suppression of MHC class I antigen hyperexpression has been shown to prevent insulinitis and diabetes (Mashima *et al.* 1999). The counteracting effect of 1,25 D₃ on MHC induction has also been described by Hahn *et al.* (1997) in rat pancreatic islet cells; nevertheless, the mechanisms implicated in these processes remain to be established.

The toxic effects of cytokines such as IL-1 β , TNF α and IFN γ may also be linked to the production of other cytokines such as IL-6. Indeed, this inflammatory cytokine, which is mainly secreted by monocytes, may also be produced by isolated mouse islets or rat insulinoma cells after exposure to IFN γ and TNF α (Müller *et al.* 1991). This IL-6 production is of pathogenic importance since in NOD mice, animal models of insulin-dependent diabetes mellitus, the administration of antibodies directed against IL-6 reduces the incidence of diabetes (Campbell *et al.* 1991). In human islets, we found a significant threefold increase of IL-6 release after 48 h of contact with cytokines and a significant reduction of values by the addition of 1,25 D₃. Likewise, in other tissues such as mice thymocytes (Müller *et al.* 1991) and human fibroblast cell lines (Srivastava *et al.* 1994), such modifications of the effects of IL-6 by 1,25 D₃ have also been described. Our results demonstrate that production of IL-6 by the islets, which may enhance the activity of local autoreactive lymphocytes and thus play a role in the development of autoimmune disease, may be efficiently counteracted by 1,25 D₃.

Identification of mediators of beta cell destruction and of mechanisms involved in beta cell repair and protection may help develop strategies favoring beta cell survival in the early stages of the disease where there is a negative balance between beta cell damage and beta cell repair. The ability of immune cells to synthesize 1,25 D₃ indicates a possible paracrine/autocrine mode of action for this hormone which may influence a wide range of immune functions, including modulation of cytotoxic macrophage activity and antigen presentation by MHC molecules in immune as well as in somatic cells.

Suppression of NO production or inhibition of NO activity, in endocrine as well as in ductal cells (Pavlovic

et al. 1999), is also a potential strategy to prevent islet cell dysfunction and killing and to increase the early function and engraftment of transplanted islets in the clinical setting. Indeed, pancreatic islet preparations intended for allografting contain various types of cells which may contribute to triggering beta cell destruction. Although variable based on the purity of the preparations ($85 \pm 7\%$ in this study), pancreatic islets are contaminated with 10–30% of exocrine cells, other minor contaminants being passenger leucocytes (1%). Purified islet fractions consist of ductal cells (20–30%) plus endocrine cells (40–50%) (Kerr-Conte *et al.* 1996) including beta cells accounting for only about 25% of total cells (Shapiro *et al.* 2000). With the exception of insulin, the parameters which were modified by cytokines may involve all cell types, in particular MHC I which is expressed on all nucleated cells. The beneficial effect exerted by 1,25 D₃ may therefore affect the whole inflammatory status of the graft and thereby be beneficial to insulin-secreting cells.

To summarize, the overall effect of 1,25 D₃ in human pancreatic islet cells may be a reduction of vulnerability of cells to allogenic cytotoxic T lymphocytes and a reduction of cytotoxic challenge. Hence 1,25 D₃ and its analogs might become useful both in the prevention or treatment of type I diabetes as well as in the management of transplant rejection.

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References

- Ahmed SA, Gogal RM Jr & Walsh JE 1994 A new rapid and simple non radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [³H] thymidine incorporation assay. *Journal of Immunological Methods* **170** 211–224.
- Allison J, Campbell IL, Morahan G, Mandel TE, Harrison LC & Miller JF 1988 Diabetes in transgenic mice resulting from over-expression of class I histocompatibility molecules in pancreatic beta cells. *Nature* **333** 529–533.
- Bigdeli N, Niemann A, Sandler S & Eizirik D 1994 Dissociation between interleukin-1 β -induced expression of mRNA for superoxide dismutase and nitric oxide synthase in insulin-producing cells. *Biochemical and Biophysical Research Communications* **203** 1542–1547.
- Bonny C, Oberson A, Steinmann M, Schorderet DF, Nicod P & Waeber G 2000 IB1 reduces cytokine-induced apoptosis of insulin-secreting cells. *Journal of Biological Chemistry* **275** 16466–16472.
- Bottazzo G, Dean B, McNally J, MacKay E, Swift P & Gamble D 1985 *In situ* characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulinitis. *New England Journal of Medicine* **60** 313–353.
- Brailey H, Montero-Julian FA, Zuber C, Flavetta S, Grassi J, Houssiau F & vanSnick J 1994 Total interleukin-6 in plasma measured by immunoassay. *Clinical Chemistry* **40** 116–123.
- Campbell IL, Bizilj K, Colman P, Tuch B & Harrison L 1986 Interferon-gamma induces the expression of HLA-A,B,C but not HLA-DR on human pancreatic beta-cells. *Journal of Clinical Endocrinology and Metabolism* **62** 1101–1109.
- Campbell IL, Cutri A, Wilson A & Harrison L 1989 Evidence for IL-6 production by and effects on the pancreatic beta-cells. *Journal of Immunology* **143** 1188–1191.
- Campbell IL, Kay T, Oxbrow L & Harrison L 1991 Essential role for interferon-gamma and interleukin-6 in autoimmune insulin-dependent diabetes in NOD/Wehi mice. *Journal of Clinical Investigation* **87** 739–742.
- Casteels KM, Mathieu C, Waer M, Valckx D, Overbergh L, Laureys JM & Bouillon R 1998 Prevention of type I diabetes in nonobese diabetic mice by late intervention with nonhypercalcemic analogs of 1,25-dihydroxyvitamin D₃ in combination with a short induction course of cyclosporin A. *Endocrinology* **139** 95–102.
- Darville M, Ho Y & Eizirik D 2000 NF κ B is required for cytokine-induced manganese superoxide dismutase expression in insulin-producing cells. *Endocrinology* **141** 153–162.
- 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 1996 Beta-cell defence and repair mechanisms in human pancreatic islets. *Hormone and Metabolic Research* **28** 302–305.
- Eizirik DL, Sandler S, Welsh N, Cetkovic M, Nieman A, Geller D, Pipeleers D, Bendtzen K & Hellerström C 1994 Cytokines suppress human islet function irrespective of their effects on nitric oxide generation. *Journal of Clinical Investigation* **93** 1968–1974.
- Flodstrom M, Welsh N & Eizirik DL 1996 Cytokines activate the nuclear factor kappa B (NF-kappa B) and induce nitric oxide production in human pancreatic islets. *FEBS Letters* **385** 4–6.
- Flohé L & Ötting F 1984 Superoxide dismutase assays. *Methods in Enzymology* **105** 93–115.
- Foulis A, Farquharson M & Hardman R 1987 Aberrant expression of class II major histocompatibility complex molecules by B cells and hyperexpression of class I major histocompatibility complex molecules by insulin containing islets in type I (insulin dependent) diabetes mellitus. *Diabetologia* **30** 333–343.
- Fournier C, Gepner P, Sadouk M & Charriere J 1990 *In vivo* beneficial effects of cyclosporine A and 1,25 dihydroxyvitamin D₃ on the induction of experimental autoimmune thyroiditis. *Clinical Immunology and Immunopathology* **54** 53–63.
- Hahn HJ, Kuttler B, Mathieu C & Bouillon R 1997 1,25-Dihydroxyvitamin D₃ reduces MHC antigen expression on pancreatic beta-cells *in vitro*. *Transplantation Proceedings* **29** 2156–2157.
- Hattori M 1990 Effect of 1,25(OH)₂ D₃ on experimental rat nephrotoxic serum nephritis. *Nippon Jinzo Gakkai Shi* **32** 147–159.
- Iwahashi H, Itoh N, Yamagata K, Imagawa A, Nakajima H, Tomita K, Moriawaki M, Waguri M, Yamamoto K, Miyagawa J, Namba M, Hanafusa T & Matsuzawa Y 1998 Molecular mechanisms of pancreatic beta-cell destruction in autoimmune diabetes: potential targets for preventive therapy. *Cytokines, Cellular and Molecular therapy* **4** 45–51.
- Kay TW, Campbell IL, Oxbrow L & Harrison LC 1991 Overexpression of class I major histocompatibility complex accompanies insulinitis in the non-obese diabetic mouse and is prevented by anti-interferon-gamma antibody. *Diabetologia* **34** 779–785.
- Kerr-Conte J, Pattou F, Xia Y, Proye C & Lefebvre J 1994 Simple dithizone-stained multilayer test for selection of density gradient before human islet mass purification. *Transplantation Proceedings* **26** 4013–4015.

- Kerr-Conte J, Pattou F, Lecomte-Houcke M, Xia Y, Boilly B, Proye C & Lefebvre J 1996 Ductal cyst formation in collagen-embedded adult human islet preparations. *Diabetes* **45** 1108–1114.
- Lafferty KJ 1997 Immunobiology of autoimmune diabetes. *Research in Immunology* **148** 313–319.
- Lake S, Bassett P, Larkins A, Revell J, Walczak K, Chamberlain J, Rumford G, London N, Veitch P, Bell P & James R 1989 Large-scale purification of human islets utilizing discontinuous albumin gradient on IBM 2991 cell separator. *Diabetes* **38** 143–145.
- Larsen CM, Wadt KAW, Juhl LF, Andersen HU, Karlsen AE, Su MSS, Seedorf K, Shapiro L, Dinarello CA & Mandrup-Poulsen T 1998 Interleukin-1 beta-induced rat pancreatic islet nitric oxide synthesis requires both the p38 and extracellular signal-regulated kinase 1/2 mitogen-activated protein kinases. *Journal of Biological Chemistry* **273** 15294–15300.
- Lemire JM & Archer DC 1991 1,25-Dihydroxyvitamin D₃ prevents the *in vivo* induction of murine experimental autoimmune encephalo-myelitis. *Journal of Clinical Investigation* **87** 1103–1107.
- Lemire JM, Archer DC, Beck L & Spiegelberg HL 1995 Immunosuppressive actions of 1,25-dihydroxyvitamin D₃: preferential inhibition of Th-1 functions. *Journal of Nutrition* **125** 1704S–1708S.
- Mandrup-Poulsen T, Helquist S & Wogensen L 1990 Cytokines and free radicals as effector molecules in the destruction of pancreatic beta-cells. *Current Topics in Microbiology and Immunology* **164** 169–193.
- Mashima H, Yamada S, Tajima T, Seno M, Yamada H, Takeda J & Kojima I 1999 Genes expressed during differentiation of pancreatic AR42J cells into insulin-secreting cells. *Diabetes* **48** 304–309.
- Mathieu C, Laureys J, Sobis H, Vandeputte M, Waer M & Bouillon R 1992 1,25-Dihydroxyvitamin D₃ prevents insulinitis in NOD mice. *Diabetes* **41** 1491–1495.
- Mathieu C, Waer M, Laureys J, Rutgeerts O & Bouillon R 1994 Prevention of type I diabetes in NOD mice by 1,25 dihydroxyvitamin D₃. *Diabetologia* **37** 552–558.
- Misko T, Salvemini D, Moore W & Currie M 1993 A fluorometric assay for the measurement of nitrite in biological samples. *Analytical Biochemistry* **214** 11–16.
- Moseley P 1998 Heat shock proteins and the inflammatory response. *Annals of the New York Academy of Sciences* **856** 206–213.
- Müller K, Diamant M & Bendtzen K 1991 Inhibition of production and function of interleukin-6 by 1,25-dihydroxyvitamin D₃. *Immunology Letters* **28** 115–120.
- Okamoto H 1999 The Reg gene family and Reg proteins: with special attention to the regeneration of pancreatic B-cells. *Journal of Hepato-Biliary-Pancreatic Surgery* **6** 254–262.
- Pavlovic D, Vandewinkel M, Vanderauwera B, Chen MC, Schuit F, Bouwens L & Pipeleers D 1997 Effect of interferon-gamma and glucose on major histocompatibility complex class I and class II expression by pancreatic beta- and non-beta-cells. *Journal of Clinical Endocrinology and Metabolism* **82** 2329–2336.
- Pavlovic D, Chen MC, Bouwens L, Eizirik DL & Pipeleers D 1999 Contribution of ductal cells to cytokine responses by human pancreatic islets. *Diabetes* **48** 29–33.
- Pujol-Borrell R, Todd I, Doshi M, Bottazzo G, Sutton R, Gray D, Adolf G & Feldmann M 1987 HLA class II induction in human islet cells by interferon-gamma plus tumor necrosis factor or lymphotoxin. *Nature* **326** 304–306.
- Rabinovitch A & Suarez-Pinzon WL 1998 Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. *Biochemical Pharmacology* **55** 1139–1149.
- Rabinovitch A, Sumoski W, Rajotte R & Warnock G 1990 Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. *Journal of Clinical Endocrinology and Metabolism* **71** 152–156.
- Ricordi C, Lacy P, Finke E, Olack B & Scharp D 1988 Automated method for isolation of human pancreatic islets. *Diabetes* **37** 413–420.
- Ricordi C, Gray D, Hering B, Kaufman D, Warnock G, Kneteman N, Lake S, London N, Soccia C, Alejandro R, Zeng Y, Scharp D, Viviani G, Falqui L, Tzakis A, Bretzel R, Federlin K, Pozza G, James R, Rajotte R, Di Carlo V, Morris P, Sutherland D, Starzl T, Mintz D & Lacy P 1990 Islet isolation assessment in man and large animals. *Acta Diabetologica* **27** 185–195.
- Sandler S, Buschard K & Bendtzen K 1994 Effects of 1,25-dihydroxyvitamin D₃ and the analogues MC903 and KH1060 on interleukin-1 beta-induced inhibition of rat pancreatic islet beta-cell function *in vitro*. *Immunology Letters* **41** 73–77.
- Scarim AL, Heitmeier MR & Corbett JA 1998 Heat shock inhibits cytokine-induced nitric oxide synthase expression by rat and human islets. *Endocrinology* **139** 5050–5057.
- Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM & Rajotte RV 2000 Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *New England Journal of Medicine* **343** 230–238.
- Srivastava MD, Deluca H & Ambrus JL 1994 Inhibition of IL-6 and IL-8 production in human fibroblast cell lines by 1,25(OH)(2) vitamin D₃ and two of its analogs with lower calcemic activity. *Research Communications in Chemical Pathology and Pharmacology* **83** 145–150.
- Thomas HE, Parker JL, Schreiber RD & Kay TWH 1998 IFN-gamma action on pancreatic beta cells causes class I MHC upregulation but not diabetes. *Journal of Clinical Investigation* **102** 1249–1257.
- Vandewalle B, Douillard C, Kerr-Conte J, Gmyr V, Riachi R, d'Herbomez M, Pattou F & Lefebvre J 1999 Human pancreatic islet quality control: easy assessment of metabolic functions. *Experimental and Clinical Endocrinology and Diabetes* **107** 214–219.
- Welsh N, Eizirik DL, Bendtzen K & Sandler S 1991 Interleukin 1-beta-induced nitric oxide production in isolated pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitase. *Endocrinology* **129** 3167–3173.
- Welsh N, Margulis B, Borg LAH, Wiklund HJ, Saldeen J, Flodström M, Mello MA, Andersson A, Pipeleers DG, Hellerström C & Eizirik DL 1995 Differences in the expression of heat-shock proteins and antioxidant enzymes between human and rodent pancreatic islets: Implications for the pathogenesis of insulin-dependent diabetes mellitus. *Molecular Medicine* **7** 806–820.
- Xie Q, Whisnant R & Nathan C 1993 Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon gamma and bacterial lipopolysaccharide. *Journal of Experimental Medicine* **177** 1779–1784.
- Yu XP, Bellido T & Manolagas SC 1995 Down-regulation of NF-kappa B protein levels in activated human lymphocytes by 1,25-dihydroxyvitamin D₃. *PNAS* **92** 10990–10994.

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