# Interleukin-1β–Induced Alteration in a β-Cell Phenotype Can Reduce Cellular Sensitivity to Conditions That Cause Necrosis but Not to Cytokine-Induced Apoptosis

Zhidong Ling, Mark Van de Casteele, Décio L. Eizirik, and Daniel G. Pipeleers

Previous work has shown that interleukin-1β (IL-1β) alters protein expression in β-cells. This alteration is associated with cell death in isolated rat islets but not in isolated rat β-cells. We examined whether IL-1β pretreatment of isolated β-cells influences their sensitivity to toxic agents. After a 24-h culture with IL-1β (30 U/ml), β-cells exhibited a lower expression of the β-cell–specific protein transcription factor pancreatic and duodenal homeobox gene (PDX)-1, glucose transporter GLUT2, and proinsulin convertase PC2, with a marked reduction (60–70%) in glucose-induced insulin production and selective sensitivity to the toxins alloxan (ALX) and streptozotocin (STZ). On the other hand, the cells presented an increased expression of Mn-superoxide dismutase, heat shock protein 70, inducible heme oxygenase, and inducible nitrite oxide synthase. This IL-1β–induced alteration in β-cell phenotype resulted in a reduced cellular sensitivity to the β-cell–specific toxins ALX and STZ; the production of nontoxic conditions of nitric oxide (NO) also rendered the cells less susceptible to radical-induced damage. Exposure to IL-1β can thus protect β-cells against conditions that cause necrosis; however, it did not protect against apoptosis induced by the additional presence of interferon-γ or tumor necrosis factor-α. Release of IL-1β in the endocrine pancreas is thus not necessarily the cause of massive NO-dependent β-cell destruction. On the contrary, IL-1β may protect these cells against necrosis, though with a loss of their characteristic phenotype and homeostatic functions. *Diabetes* 49:340–345, 2000

From the Faculty of Medicine, Diabetes Research Center, Vrije Universiteit Brussel, Brussels, Belgium.

Address correspondence and reprint requests to Dr. Daniel G. Pipeleers, Department of Metabolism and Endocrinology, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium. E-mail: dpip@mebo.vub.ac.be.

Received for publication 31 December 1998 and accepted in revised form 11 November 1999.

ALX, alloxan; ARG, arginine; BSA, bovine serum albumin; GEA 3162, 1,2,3,4-oxatriazolium,5-amino-3-(3,4-dichlorophenyl)-chloride; HO-1, heme oxygenase; HSP70, heat shock protein 70; IBMX, 3-isobutyl-1-methylxanthine; IFN, interferon; IL-1β, interleukin-1β; iNOS, inducible nitrite oxide synthase; MnSOD, Mn-superoxide dismutase; NMA, $N^G$-methyl-L-arginine; NR, neutral red; OD, optical density; PDX, pancreatic and duodenal homeobox gene; STZ, streptozotocin; TNF, tumor necrosis factor.

Type 1 diabetes is caused by a marked reduction in the number of pancreatic β-cells (1). There is indirect evidence that the β-cell population actively participates in the process that can lead to β-cell death. Isolated rat β-cells can vary their sensitivity and/or defense capacity to cytotoxic conditions (2). It is still unknown whether this property is also operative during the development of diabetes. In rodents with type 1 diabetes, the destruction of β-cells has been associated with an increased local production of proinflammatory type 1 cytokines (3). Combinations of these cytokines are cytotoxic in isolated rodent and human islet preparations (3–5). If cytokines are released in islets with insulitis, it is conceivable that their composition and respective concentrations vary with the state of the infiltrate and its environmental conditions. Therefore, we consider it important to investigate also the effects of single cytokines on islet β-cells, preferably in the absence of other islet components that might influence the outcome and its interpretation (6). It was thus found that single purified rat β-cells are not destroyed during culture with interleukin-1β (IL-1β), whereas isolated islets are severely damaged (6). However, the cells underwent a profound change in their morphologic and functional characteristics (6). Their specific property as a glucose-regulated source for insulin was markedly reduced, but their overall rate of noninsulin protein synthesis was not affected (6,7). IL-1β is known to induce some noninsulin proteins that may influence the survival of the β-cells (8–15). Thus, IL-1β–induced nitric oxide synthase (iNOS) will lead to production of nitric oxide (NO), which can damage islet cells (8–10). On the other hand, the increased levels of superoxide dismutase, heat shock protein 70 (HSP70), and inducible heme oxygenase (HO-1) (11–15) may in theory reduce the cellular sensitivity to damaging radicals, as has been observed in a variety of cell types (16–21). A cytoprotective effect of IL-1β has not yet been observed in β-cells. It is difficult to investigate the possibility of a cytoprotective effect of IL-1β in isolated islet preparations, where the heterogeneous composition makes it difficult to analyze processes in one cell type, where local factors cause a preponderance of the cytotoxic effects, and where the compact tissue organization does not allow a precise quantification of cell death. These limitations are not encountered in purified rat β-cell preparations. We therefore used this model to examine whether IL-1β–induced changes in protein expression can lead to protection against damaging radicals.

## RESEARCH DESIGN AND METHODS

**Purification and culture of rat β-cells.** Pancreatic islets were isolated from adult male Wistar rats by collagenase digestion and dissociated into single cells in a calcium-free medium containing trypsin and DNase (22). Single β-cells were purified by autofluorescence-activated sorting by use of cellular light-scatter and flavin adenine dinucleotide–autofluorescence as discriminating parameters (22).

The cytotoxicity assay consists of counting the percentage of dead cells and therefore requires the use of single cells. Purified single rat β-cells ($3 \times 10^3$ cells/ condition) were distributed over polylysine-coated microtiter plates and precultured for 24 h in Ham's F10 medium containing 6 mmol/l glucose, supplemented with 0.075 mg/ml penicillin, 0.1 mg/ml streptomycin, 2 mmol/l L-glutamine, 1% (wt/vol) bovine serum albumin (BSA) pretreated with charcoal (fraction V; radioimmunoassay grade; Sigma, St. Louis, MO), and 50 µmol/l 3-isobutyl-1-methylxanthine (IBMX) (Janssen Chimica, Beerse, Belgium), with or without arginine (ARG) (1 mmol/l) (6). Addition of IBMX is needed for the survival of single purified β-cells in serum-free media (23). At this concentration, IBMX induces lower cAMP production than the locally released glucagon in cultured islet preparations (24) and does not affect IL-1β–induced NO formation from pure β-cells (data not shown). The preparations were then cultured for another 24 h in the absence or presence of recombinant human IL-1β (0.3–300 U/ml) (>95% pure, 280 U/ng; Genzyme, Cambridge, MA) with or without 1 mmol/l $N^G$-methyl-L-arginine (NMA) (Sigma), or tumor necrosis factor (TNF)-α (10–1,000 U/ml) (200 U/ng; Genzyme) with interferon (IFN)-γ (10–1,000 U/ml) (10 U/ng; Genzyme), or the NO donor 1,2,3,4-oxatriazolium,5-amino-3-(3,4-dichlorophenyl)-chloride (GEA 3162) (1–25 µmol/l) (Alexis, Nottingham, U.K.). Thereafter, cells were exposed for 30 min to different concentrations of alloxan (ALX) (0.5–2 mmol/l) or streptozotocin (STZ) (0.5–2 mmol/l), exposed for 60 min to GEA (50 µmol/l), and then cultured for 4 days without these agents before the percentage of dead cells was determined by vital staining with neutral red (NR) (2). A fluorescence assay was used to distinguish between necrosis and apoptosis (25). Under these culture conditions, dead cells did not disintegrate, and they remained detectable under the microscope. To analyze protein expression in conditions used in the cytotoxicity assay, purified β-cells were cultured in suspension after reaggregation of the cells during a shaking incubation (26). This method allows quantitative cell recovery from the wells in numbers that are sufficient for analysis. After the 24-h culture period with IL-1β or GEA, media were collected for measuring nitrite content (27), and cells were collected for protein analysis.

**Analysis of cellular proteins.** Newly synthesized proteins were labeled during a 2-h incubation at 37°C in 200 µl Ham's F10 medium containing 10 mmol/l glucose, 1% BSA, 2 mmol/l glutamine, 1 mmol/l ARG, and 50 µCi L-[3,5-$^3$H]-tyrosine (TRK200; Amersham, Bucks, U.K.) (6). At the end of incubation, the preparations were washed, extracted in 1 ml acetic acid (2 mmol/l containing 0.25% BSA), and assayed for $^3$H-protein, $^3$H-insulin, and total immunoreactive insulin content (6).

Protein expression was analyzed by Western blot as previously described (7). Briefly, samples of $10^5$ cells were sonicated in 50 µl SDS-gel sample buffer (5% SDS, 5% β-mercaptoethanol, 80 mol/l TrisCl (pH 6.8), 5 mol/l EDTA, 10% glycerol, and 1 mmol/l phenylmethylsulfonyl fluoride) and then run on 10% SDS-polyacrylamide gels, electrically transferred to nitrocellulose filters, and incubated with rabbit anti-rat iNOS antibody (1:10,000) (Transduction Laboratories, Lexington, KY), mouse anti-human inducible form of heat shock protein 70 (HSP70) (1:1,000) (Stressgen, Victoria, BC, Canada), rabbit anti-rat HO-1 antibody (1:1,000) (Stressgen), rabbit anti-rat Mn–superoxide dismutase (MnSOD) antibody (1:3,000) (provided by Dr. Kohtaro Asayama, Yamanashi Medical University, Yamanashi, Japan) (28), rabbit anti-rat GLUT2 antibody (1:1,000) (East Acres, Southbridge, MA), rabbit anti-rat PC2 (1:1,000) (provided by Dr. C.J. Rhodes, University of Texas Southwestern Medical Center, Dallas, TX) (29), rabbit anti-rat pancreatic and duodenal homeobox gene (PDX)-1 (1:1,000) (provided by Dr. Ole D. Madsen, Hagedorn Research Institute, Gentofte, Denmark) (30), or goat anti–β-actin (1:1,000) (Santa Cruz Biotechnology, Santa Cruz, CA). Horseradish peroxidase–linked Ig was used as second antibodies and peroxidase activity was detected by enhanced chemiluminiscence (Amersham, Bucks, U.K.). The intensity of the bands was quantified in an Ultrascan XL Enhanced Laser Densitometer (LKB, Bromma, Sweden) and expressed in arbitrary units of optical density (OD).

**Data analysis.** The toxicity of an agent $x$ was calculated from the percentage of $NR^+$ cells counted in the presence of $x$ and the corresponding control $c$ (2):

$$\text{Cytotoxicity } x = ([\%NR^+c - \%NR^+x]/\%NR^+c) \times 100$$

Data are shown as means ± SE. The statistical significance of differences was calculated by use of the Mann-Whitney $U$ or Wilcoxon's matched-pairs signed-rank tests. Multiple comparisons were examined by use of the Kruskal-Wallis test.

## RESULTS

**Effect of IL-1β on expression of β-cell proteins.** After a 24-h culture with IL-1β (30 U/ml), β-cells exhibited an increased expression of MnSOD, HSP70, and HO-1 but not of actin (Fig. 1). These effects were associated with an induction of iNOS expression (Fig. 1) and elevated nitrite levels in the culture medium (19 ± 3 vs. 1 ± 1 pmol · $10^3$ cells$^{-1}$ · 24 h$^{-1}$; $P < 0.01$; $n = 5$). On the other hand, the expression of GLUT2, PC2, and PDX1 was decreased (Fig. 1). The cellular content in insulin was not altered, but the rate of insulin synthesis was suppressed by 60%; no difference was measured in the rate of noninsulin protein synthesis (Table 1). The ratio of insulin synthesis to noninsulin protein synthesis was twofold lower than in the control preparations (Table 1).

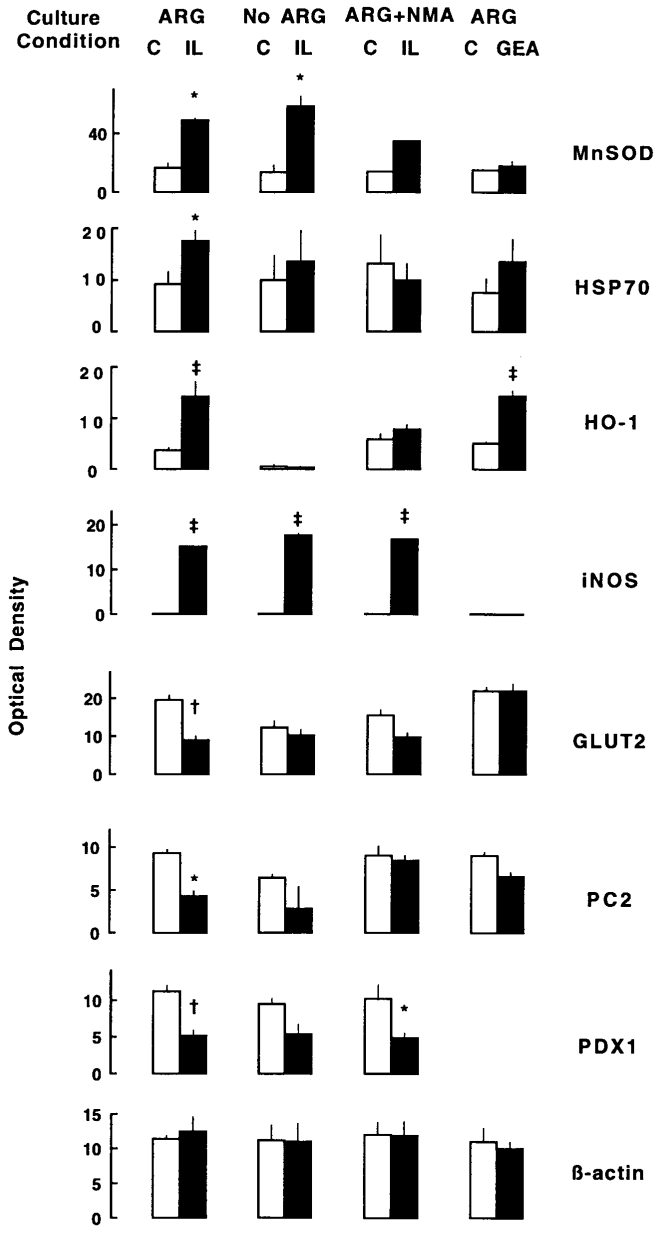


FIG. 1. Protein expression in β-cell aggregates cultured for 24 h with or without IL-1β (0.1 ng/ml) or GEA (10 µmol/l) in the presence or absence of 1 mmol/l ARG or 1 mmol/l NMA. Data are mean OD ± SE from three to nine independent experiments. C, control. *$P < 0.05$ between test and control conditions; †$P < 0.01$ between test and control conditions; ‡$P < 0.001$ between test and control conditions.

Exposure to IL-1β in an ARG-free culture medium prevented nitrite production (2 ± 1 vs. 0.3 ± 0.3 pmol · $10^3$ cells$^{-1}$ · 24 h$^{-1}$;

TABLE 1
Effects of IL-1β on insulin content and protein synthesis in β-cell aggregates

| Pretreatment | *n* | Insulin content (ng/$10^3$ cells) | Protein synthesis: Insulin ($10^3$ dpm · $10^3$ cells$^{-1}$ · 2 h$^{-1}$) | Protein synthesis: Noninsulin protein ($10^3$ dpm · $10^3$ cells$^{-1}$ · 2 h$^{-1}$) | Protein synthesis: Insulin/noninsulin protein (dpm/dpm) |
|---|---|---|---|---|---|
| $ARG^+$ $NMA^-$ control | 12 | 17.9 ± 1.3 | 17.6 ± 1.0 | 6.6 ± 1.0 | 3.9 ± 0.8 |
| $ARG^+$ $NMA^-$ IL-1β | 12 | 16.1 ± 1.3 | 7.0 ± 0.8* | 4.4 ± 0.6 | 1.8 ± 0.8† |
| $ARG^-$ $NMA^-$ control | 4 | 12.8 ± 3.5 | 15.0 ± 0.5 | 4.8 ± 0.3 | 3.2 ± 0.3 |
| $ARG^-$ $NMA^-$ IL-1β | 4 | 10.5 ± 2.2 | 11.3 ± 0.9 | 5.9 ± 0.5 | 1.9 ± 0.2 |
| $ARG^+$ $NMA^+$ control | 6 | 16.9 ± 0.6 | 18.9 ± 1.4 | 5.4 ± 1.3 | 4.6 ± 1.1 |
| $ARG^+$ $NMA^+$ IL-1β | 6 | 15.9 ± 1.5 | 17.4 ± 1.3 | 7.2 ± 1.8 | 3.5 ± 1.0 |

Data are means ± SE of *n* independent experiments. β-Cell aggregates were cultured for 24 h with or without IL-1β (30 U/ml). *$P$ < 0.001 between conditions with and without IL-1β; †$P$ < 0.05 between conditions with and without IL-1β.

$P$ > 0.05; $n$ = 5). In this condition, the cells also exhibited an increased expression of MnSOD and iNOS, but not of HSP70 and HO-1; their expression of GLUT2, PC2, and PDX1 was no longer significantly reduced and neither was their rate of insulin synthesis (Fig. 1 and Table 1). We had previously excluded the possibility that culture in an ARG-free medium would be cytotoxic for the β-cells; after 3 days, cell viability was comparable (79 ± 2% living cells) to that in control medium with 1 mmol/l ARG (84 ± 2% living cells; $n$ = 4; $P$ > 0.05) as judged by the cytotoxicity assay. When IL-1β was examined in an ARG-containing medium supplemented with an inhibitor of iNOS (1 mmol/l NMA), it also failed to affect the expression of HSP70, HO-1, or GLUT2 and to reduce the rate of insulin synthesis; however, under this condition, the reduction on PDX-1 was significant, and the induction of MnSOD remained (Fig. 1).

These effects of IL-1β were not reproduced by TNF-α or IFN-γ. Exposure for 24 h to TNF-α (100 U/ml) or IFN-γ (100 U/ml) did not affect the expression of iNOS, HO-1, MnSOD, or GLUT2 (Fig. 2) or the rate of insulin synthesis (18.7 ± 2.3 and 16.2 ± 1.8 dpm/cell after TNF-α and IFN-γ treatment, respectively, vs. 19.6 ± 1.7 in control preparations; $n$ = 4; $P$ > 0.05).

**Effect of NO donor GEA on expression of β-cell proteins.** A low concentration of the NO donor GEA (10 µmol/l) did not induce death of β-cells during an exposure period of 24 h. When β-cell aggregates pretreated with 10 µmol/l GEA were analyzed for their proteins, an increased expression of HO-1 was observed, whereas no significant differences were noticed for any of the other tested proteins (Fig. 1). Insulin content was not affected, and the rate of insulin biosynthesis was similar to that in control preparations (data not shown).

**Sensitivity of IL-1β–pretreated β-cells to the toxic effects of ALX, STZ, and the NO-donor GEA.** The toxic effect of ALX on single β-cells was assessed at the end of a 4-day culture period that followed a 30- or 60-min exposure to this agent. Without ALX exposure, viability of the cells was 85 ± 2% in control cells and 80–85% in cells pretreated with IL-1β (0.3–300 U/ml for 1 or 4 days; $P$ > 0.05 vs. control preparations), confirming that IL-1β is not cytotoxic under these conditions.

ALX (0.5–2 mmol/l) induced a dose-dependent toxicity in the control cells (Fig. 3). IL-1β–pretreated cells (30 U/ml, 24 h) were less sensitive to the ALX toxicity: no cell death after 1 mmol/l ALX and <20% dead cells after 2 mmol/l vs. 55% in control preparations (Fig. 3). This lower sensitivity was observed for a wide IL-1β concentration range (3–300 U/ml, 24 h) (Fig. 4); it occurred irrespective of the presence of ARG (Fig. 3 and Table 2) or a blocker of iNOS activity (42 ± 2% dead cells after ALX in IL-1β– and NMA-pretreated cells vs. 64 ± 5% in NMA-pretreated cells; $P$ < 0.05; $n$ = 5). A lower sensitivity to ALX was also measured when the IL-1β pretreatment was prolonged to 4 days (Fig. 4); however, the effect was less pronounced, in particular at the highest IL-1β concentration (300 U/ml).

The effect of IL-1β was not reproduced by TNF-α or IFN-γ. Preculture for 24 h with TNF-α (100 U/ml) or IFN-γ (100 U/ml) did not affect the survival of β-cells (80–85% living cells, as in control preparations). In contrast to the IL-1β pretreatment, it did not reduce the cellular sensitivity to ALX (Table 3). Similar results were obtained with lower (10 U/ml) and higher (1,000 U/ml) concentrations of TNF-α or IFN-γ (data not shown). Furthermore, addition of TNF-α (100 U/ml) with IFN-γ (100 U/ml) to IL-1β (30 U/ml) did not prevent IL-1β–induced protection during a 24-h pretreatment (Table 3). A 4-day exposure to the cytokine combination was in itself toxic and consequently led to apoptosis (25).

It was then tested whether IL-1β pretreatment also protected against a subsequent exposure to STZ (0.5–2 mmol/l for 30 min) or to toxic concentrations of the NO donor GEA (50 µmol/l for 60 min). As with ALX, the cells were subsequently cultured for 4 days before calculating the percentage of dead cells. In control preparations, STZ (0.5–2 mmol/l)

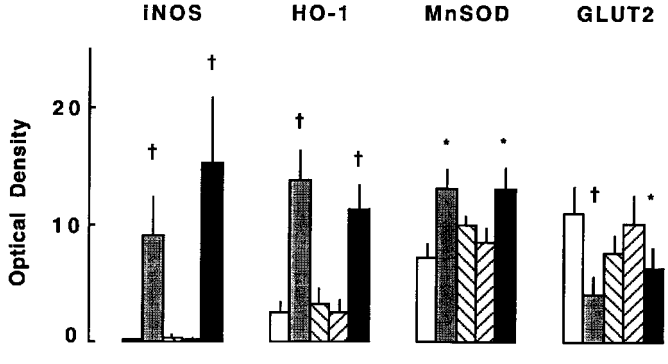


FIG. 2. Protein expression in β-cell aggregates cultured for 24 h with or without cytokines: □, control; ■, IL-1β (30 U/ml); ▨, TNF-α (100 U/ml); ▨, IFN-γ (100 U/ml); and ■, the combination of these three cytokines. Data are mean OD ± SE from four or five independent experiments. *$P$ < 0.05 between test and control conditions; †$P$ < 0.01 between test and control conditions.

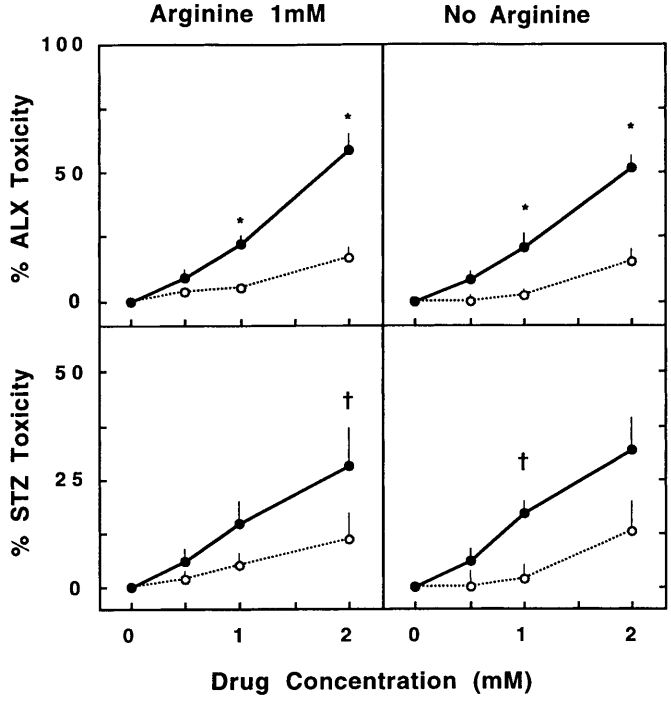


FIG. 3. Effect of IL-1β pretreatment on ALX- or STZ-induced toxicity in β-cells. Single β-cells were precultured for 24 h with (○) or without (●) IL-1β (30 U/ml) in a medium supplemented with or without 1 mmol/l ARG. Cells were then exposed for 30 min to 0–2 mmol/l ALX or STZ and were subsequently cultured for 4 days before determining the percentage of dead cells. Data are means ± SE from four or five independent experiments. *$P$ < 0.05 between IL-1β–pretreated and control cells; †$P$ < 0.01 between IL-1β–pretreated and control cells.

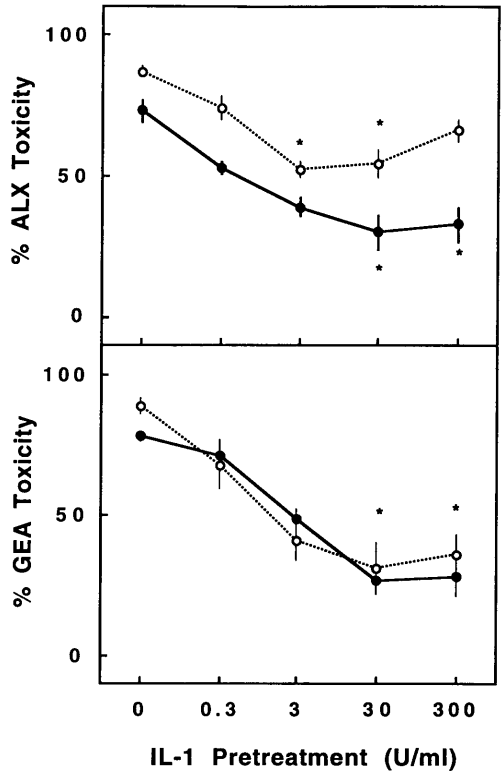


FIG. 4. Dose and time dependency of IL-1β pretreatment on ALX- or GEA-induced toxicity in β-cells. Single β-cells were precultured for 24 h (●) or 4 days (○) with or without different concentrations of IL-1β. Cells were then exposed to ALX (2 mmol/l for 30 min) or GEA (50 µmol/l for 60 min) before culture for 4 days. At the end of culture, the percentage of dead cells was calculated. Data are means ± SE from four or five independent experiments. *$P$ < 0.05 between IL-1β–pretreated and control cells.

caused a dose-dependent increase in the percentage of dead cells. The agent was less toxic after IL-1β pretreatment, either in the presence or absence of ARG (Fig. 2). The cytotoxicity of GEA was also markedly reduced in IL-1β–pretreated cells (3–300 U/ml for 24 h) (Fig. 4), but in contrast to the ALX and STZ toxicities, only when ARG was present during the pretreatment (Table 2). Addition of the ARG analog NMA during IL-1β exposure (30 U/ml for 24 h) also prevented the protective effect of the IL-1β pretreatment: toxicity of GEA (50 µmol/l for 60 min) was 65 ± 8 and 73 ± 8% in the NMA-control with and without IL-1β, respectively ($P$ > 0.05; $n$ = 5). A similar protection against GEA was measured when IL-1β pretreatment was prolonged to 4 days (Fig. 4). By using a fluorescence toxicity assay, cell death by ALX, STZ, or GEA was identified to occur through necrosis.

Sensitivity of GEA-pretreated β-cells to the toxic effects of ALX and high concentrations of GEA. We examined whether NO could reduce the β-cell sensitivity to toxic agents. To test this possibility, β-cells were pretreated with a nontoxic concentration of the NO donor GEA (i.e., 1–10 µmol/l for 24 h). In this concentration range, the 24-h culture medium contained 0.5–2.5 µmol/l nitrite, which is comparable to the nitrite concentrations during IL-1β exposure (30 U/ml for 24 h) (1.6 ± 0.3 µmol/l). After a subsequent 4-day culture period, β-cell survival (75 ± 2%) was comparable to that in control preparations (76 ± 2%; $P$ > 0.05). Higher GEA concentrations (50 µmol/l for 60 min) were cytotoxic (Figs. 4 and 5; Table 3). This GEA toxicity was reduced when the β-cells were pretreated with the nontoxic GEA concentrations (Fig. 5). The ALX toxicity was also reduced by this pretreatment (Fig. 5). After 10 µmol/l pretreatment, the toxicity of 2 mmol/l ALX and of 50 µmol/l GEA was only minor (<15%), whereas it was >60% in control preparations (Fig. 5).

## DISCUSSION

This study confirms that IL-1β alone is not necessarily cytotoxic for rat β-cells (6). In such in vitro conditions, the cytokine was found to alter the cellular phenotype, as indi-

TABLE 2
Effects of IL-1β pretreatment on β-cell sensitivity to the cytotoxic action of ALX and GEA

| Pretreatment | Cytotoxicity | |
|---|---|---|
| | ALX | GEA |
| ARG$^+$ control | 55 ± 7 | 57 ± 10 |
| ARG$^+$ IL-1β (30 U/ml) | 17 ± 6* | 27 ± 7* |
| ARG$^-$ control | 51 ± 3 | 61 ± 9 |
| ARG$^-$ IL-1β (30 U/ml) | 13 ± 6† | 56 ± 11 |

Data are means ± SE of five independent experiments and express toxicity caused by exposure to 2 mmol/l ALX for 30 min or 50 µmol/l GEA for 60 min. *$P$ < 0.05 between IL-1β and corresponding control; †$P$ < 0.01 between IL-1β and corresponding control.

TABLE 3
Effects of cytokine pretreatment on β-cell sensitivity to the cytotoxic action of ALX and GEA

| Pretreatment | Cytotoxicity | |
|---|---|---|
| | ALX | GEA |
| Control | 73 ± 2 | 74 ± 4 |
| IL-1β (30 U/ml) | 32 ± 4* | 25 ± 6* |
| TNF (100 U/ml) | 65 ± 4 | 67 ± 5 |
| IFN (100 U/ml) | 72 ± 3 | 66 ± 6 |
| IL-1β + TNF + IFN | 36 ± 3* | 63 ± 5 |

Data are means ± SE of five to seven independent experiments and express toxicity caused by exposure to 2 mmol/l ALX for 30 min or 50 µmol/l GEA for 60 min. *$P$ < 0.01 vs. control pretreatment.

cated by changes in cell shape (6) and in cellular proteins (7). A 24-h exposure reduced the expression of β-cell–specific proteins, such as PDX-1, GLUT2, proinsulin convertase PC2, and insulin and increased that of other proteins such as HO-1, HSP70, MnSOD, and iNOS. Induction of these nonspecific proteins has been previously described (7,12,14,15); it is now shown that they occur independently of cytotoxic effects. Suppression of transcription factor PDX-1 is expected to result in lower expression of β-cell–specific genes, such as insulin, IAPP, GLUT2, and glucokinase (31–34). These data are consistent with the concept that IL-1β can shift the functional state of β-cells to that of a glucose-unresponsive population, which explains the loss of their characteristic property, namely the production of insulin in response to glucose (6). This IL-1β–induced alteration in β-cell phenotype appears in part to be NO-dependent; it cannot be completely reproduced by an exogenous NO donor.

The observation that IL-1β shifts the rat β-cell toward a phenotype with reduced expression of its specific traits (6,7, and present data) led us to test whether this change reduces its sensitivity for ALX and STZ, two agents with β-cell–selective toxicity. We found that IL-1β–pretreated β-cells were less sensitive to both toxins. In view of the profound alteration in protein synthesis and gene expression, several nonmutually exclusive processes might account for this lower sensitivity. The decreased GLUT2 expression is expected to lower the cellular uptake of ALX and STZ and, consequently, their cytotoxicity (35,36). The altered functional state may reduce depletion in cellular $NAD^+$, as after heat shock (37). The increase in MnSOD could provide a higher capacity for scavenging oxygen radicals (38). On the other hand, the lower sensitivity to the two toxic agents appeared to be independent of an increased expression of HO-1 or HSP70, since it was also observed in the ARG-free condition in which these proteins were not elevated.

The IL-1β–pretreated β-cells were also more resistant to cytotoxic concentrations of the NO donor GEA. Induction of this resistance appeared to be NO dependent, because it did not develop in an ARG-free medium or in the presence of the iNOS inhibitor NMA; it could be mimicked by an NO donor used at nontoxic concentrations. Low levels of NO have been previously shown to confer cytoprotection in other tissues (16,17,39). In β-cells and these other cell types, this protective effect is associated with an increased expression of HO-1 (16). It is not yet unequivocally established that this enzyme provides an increased cellular resistance to NO damage. Previous studies have suggested that bilirubin, which is produced by HO-1,

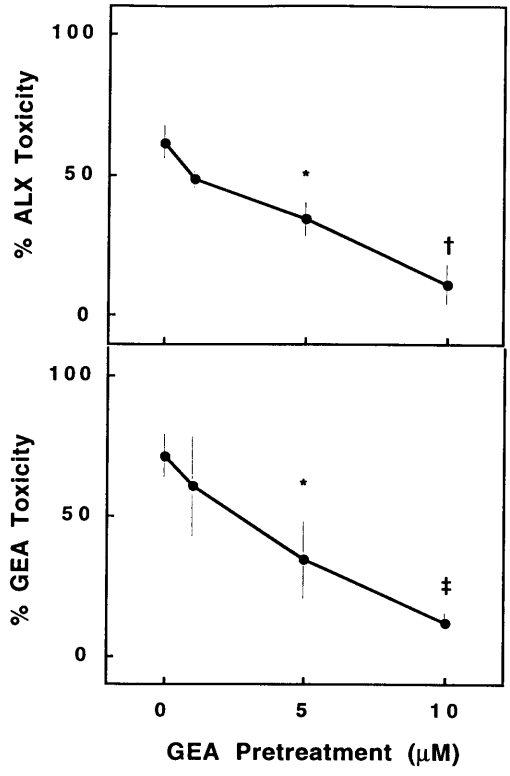


FIG. 5. Dose dependency of GEA pretreatment on cytotoxicity of ALX-induced toxicity or a high concentration of GEA-induced toxicity in β-cells. Single β-cells were exposed to ALX (2 mmol/l for 30 min) or GEA (50 µmol/l for 60 min) after a 24-h preculture with or without different concentrations of GEA and were subsequently cultured for 4 days before determining the percentage of dead cells. Data are means ± SE from four independent experiments. *$P$ < 0.05 between GEA-pretreated and control cells; †$P$ < 0.01 between GEA-pretreated and control cells; ‡$P$ < 0.001 between GEA-pretreated and control cells.

can scavenge reactive oxygen species (18,19). This mechanism can explain why HO-1 induction by GEA also protects the cells against ALX toxicity. The increased HO-1 expression can be considered a marker for the cellular response to NO.

The IL-1β–induced protection against ALX and NO toxicity was also observed after a longer exposure period (4 days) to the cytokine. The effect against ALX, however, lost significance at the highest IL-1β concentration (300 U/ml); it is unknown whether this failure results from a downregulation of the IL-1 receptors. This IL-1β–induced protection against cytotoxic agents appears to be specific for this cytokine, at least when compared with the effects of TNF-α or IFN-γ. Neither of these cytokines induced a lower cellular sensitivity to ALX, nor did they cause an alteration in protein expression as seen with IL-1β. Their addition to IL-1β did not counteract the protective action of IL-1β, at least during a 1-day pretreatment protocol. As shown previously (25), longer pretreatment periods (4 days) with the cytokine combination resulted in death of isolated rat β-cells, which indicates that exposure of rat β-cells to IL-1β does not protect them against the cytotoxic effect of a cytokine combination. Under this experimental condition, the combination of IL-1β with TNF-γ and IFN-γ leads to apoptosis (25). We can thus conclude that IL-1β can protect rat β-cells against agents that cause necrosis but not against cytokine-induced apoptosis. IL-1β alters the characteristic β-cell phenotype so that the cells become less

sensitive to the β-cell–specific toxins ALX and STZ. It also induces expression of NO synthase, which mediates an NO-dependent protection against oxygen and NO radicals. This IL-1β protection was preserved in the presence of other proinflammatory cytokines, namely IFN-γ and TNF-α, at least during a 24-h treatment period. It was detected in purified single β-cell preparations in which IL-1β did not cause the cytotoxicity that has been described in isolated islet preparations (3–5). In isolated islets, local NO accumulation might rapidly reach cytotoxic concentrations that make β-cell necrosis the preponderant phenomenon. The present data are therefore not in contradiction with those previously described in isolated islets; they should not be taken as evidence against the possibility that IL-1β is cytotoxic in vivo. They do, however, indicate that this cytokine is not necessarily cytotoxic for β-cells and that it can even protect these cells against certain agents that cause β-cell necrosis. The consequence for such IL-1β–induced protection is, however, the loss of β-cell normal homeostatic function.

## ACKNOWLEDGMENTS

This work was supported by grants from the Belgian Fonds voor Wetenschappelijk Onderzoek (FWO 3.0057.94), the services of the Prime Minister (Interuniversity Attraction Pole P4/21), and the Juvenile Diabetes Foundation (JDF-DIRP 95–97). M.V.d.C. is a research fellow of the Belgian National Fund for Scientific Research.

We thank René De Proft, Johan Guns, and Alexandra Smets for technical assistance.

## REFERENCES

1. Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14:619–633, 1965
2. Pipeleers D, Van De Winkel M: Pancreatic β-cells possess defense mechanisms against cell-specific toxicity. *Proc Natl Acad Sci U S A* 83:5267–5271, 1986
3. Rabinovitch A: An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. *Diabetes Metab Rev* 14:129–151, 1998
4. Rabinovitch A: Roles of cytokines in IDDM pathogenesis and islet β-cell destruction. *Diabetes Rev* 1:215–240, 1993
5. Mandrup-Poulsen T: The role of interleukin-1 in the pathogenesis of IDDM. *Diabetologia* 39:1005–1029, 1996
6. Ling Z, In't Veld PA, Pipeleers DG: Interaction of interleukin-1 with islet β-cells: distinction between indirect, specific cytotoxicity and direct, specific functional suppression. *Diabetes* 42:56–65, 1993
7. Ling Z, Chen MC, Smismans A, Pavlovic D, Schuit F, Eizirik DL, Pipeleers DG: Intercellular differences in interleukin 1-induced suppression of insulin synthesis and stimulation of non-insulin protein synthesis by rat pancreatic β-cells. *Endocrinology* 139:1540–1545, 1998
8. Southern C, Schulster D, Green IC: Inhibition of insulin secretion by interleukin-1 beta and tumour necrosis factor-alpha via an L-arginine-dependent nitric oxide generating mechanism. *FEBS Lett* 276:42–44, 1990
9. Corbett JA, McDaniel ML: Does nitric oxide mediate autoimmune destruction of β-cells? Possible therapeutic interventions in IDDM. *Diabetes* 4:897–903, 1992
10. Corbett JA, McDaniel ML: Intraislet release of interleukin 1 inhibits beta cell function by inducing beta cell expression of inducible nitric oxide synthase. *J Exp Med* 181:559–568, 1995
11. Eizirik DL, Welsh M, Strandell E, Welsh N, Sandler S: Interleukin-1 beta depletes insulin messenger ribonucleic acid and increases the heat shock protein hsp70 in mouse pancreatic islets without impairing the glucose metabolism. *Endocrinology* 127:2290–2297, 1990
12. Helqvist S, Polla BS, Johannesen J, Nerup J: Heat shock protein induction in rat pancreatic islets by recombinant human interleukin 1 beta. *Diabetologia* 34:150–156, 1991
13. Borg LA, Cagliero E, Sandler S, Welsh N, Eizirik DL: Interleukin-1 beta increases the activity of superoxide dismutase in rat pancreatic islets. *Endocrinology* 130:2851–2857, 1992
14. Andersen HU, Larsen PM, Fey SJ, Karlsen AE, Mandrup-Poulsen T, Nerup J: Two-dimensional gel electrophoresis of rat islet proteins: interleukin 1β–induced changes in protein expression are reduced by L-arginine depletion and nicotinamide. *Diabetes* 44:400–407, 1995
15. Strandell E, Buschard K, Saldeen J, Welsh N: Interleukin-1β induces the expression of hsp70, heme oxygenase and Mn-SOD in FACS-purified rat islet β-cells, but not in α-cells. *Immunol Lett* 48:145–148, 1995
16. Motterlini R, Foresti R, Intaglietta M, Winslow RM: NO-mediated activation of heme oxygenase: endogenous cytoprotection against oxidative stress to endothelium. *Am J Physiol* 270:H107–H114, 1996
17. Kim YM, de Vera ME, Watkins SC, Billiar TR: Nitric oxide protects cultured rat hepatocytes from tumor necrosis factor-γ-induced apoptosis by inducing heat shock protein 70 expression. *J Biol Chem* 272:1402–1411, 1997
18. Llesuy SF, Tomaro ML: Heme oxygenase and oxidative stress: evidence of involvement of bilirubin as physiological protector against oxidative damage. *Biochim Biophys Acta* 1223:9–14 , 1994
19. Otterbein LE, Kolls JK, Mantell LL, Cook JL, Alam J, Choi AM: Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. *J Clin Invest* 103:1047–1054, 1999
20. Bellmann K, Jaattela M, Wissing D, Burkart V, Kolb H: Heat shock protein hsp70 overexpression confers resistance against nitric oxide. *FEBS Lett* 391:185–188, 1996
21. Hohmeier HE, Thigpen A, Tran VV, Davis R, Newgard CB: Stable expression of manganese superoxide dismutase (MnSOD) in insulinoma cells prevents IL-1beta-induced cytotoxicity and reduces nitric oxide production. *J Clin Invest* 101:1811–1820, 1998
22. Pipeleers DG, In't Veld PA, Van De Winkel M, Maes E, Schuit FC, Gepts W: A new in vitro model for the study of pancreatic α and β cells. *Endocrinology* 117:806–816, 1985
23. Ling Z, Hannaert JC, Pipeleers D: Effect of nutrients, hormones and serum on survival of rat islet beta cells in culture. *Diabetologia* 37:15–21, 1994
24. Schuit FC, Pipeleers DG: Regulation of adenosine 3′,5′-monophosphate levels in the pancreatic B cell. *Endocrinology* 117:834–840, 1985
25. Hoorens A, Pipeleers D: Nicotinamide protects human beta cells against chemically-induced necrosis, but not against cytokine-induced apoptosis. *Diabetologia* 42:55–59, 1999
26. Pipeleers DG, Schuit FC, In't Veld PA, Maes E, Hooghe-Peters E, Van De Winkel M, Gepts W: Interplay of nutrients and hormones in the regulation of insulin release. *Endocrinology* 117:824–833, 1985
27. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: Analysis of nitrate, nitrite, and [$^{15}$H]nitrate in biological fluids. *Anal Biochem* 126:131–138, 1982
28. Munim A, Asayama K, Dobashi K, Suzuki K, Kawaoi A, Kato K: Immunohistochemical localization of superoxide dismutases in fetal and neonatal rat tissues. *J Histochem Cytochem* 40:1705–1713, 1992
29. Alarcòn C, Lincoln B, Rhodes CJ: The biosynthesis of the subtilisin-related proprotein convertase PC3, but not that of the PC2 convertase, is regulated by glucose in parallel to proinsulin biosynthesis in rat pancreatic islets. *J Biol Chem* 268:4276–4280, 1993
30. Serup P, Jensen J, Andersen FG, Jorgensen MC, Blume N, Holst JJ, Madsen OD: Induction of insulin and islet amyloid polypeptide production in pancreatic islet glucagonoma cells by insulin promoter factor 1. *Proc Natl Acad Sci U S A* 93:9015–9020, 1996
31. Waeber G, Thompson N, Nicod P, Bonny C: Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor. *Mol Endocrinol* 10:1327–1334, 1996
32. Watada H, Kajimoto Y, Miyagawa J, Hanafusa T, Hamaguchi K, Matsuoka T, Yamamoto K, Matsuzawa Y, Kawamori R, Yamasaki Y: PDX-1 induces insulin and glucokinase gene expressions in αTC1 clone 6 cells in the presence of betacellulin. *Diabetes* 45:1826–1831, 1996
33. Sander M, German MS: The beta cell transcription factors and development of the pancreas. *J Mol Med* 75:327–340, 1997
34. Madsen OD, Jensen J, Petersen HV, Pedersen EE, Oster A, Andersen FG, Jorgensen MC, Jensen PB, Larsson LI, Serup P: Transcription factors contributing to the pancreatic beta-cell phenotype. *Horm Metab Res* 29:265–270, 1997
35. De Vos A, Heimberg H, Quartier E, Huypens P, Bouwens L, Pipeleers D, Schuit F: Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. *J Clin Invest* 96:2489–2495, 1995
36. Schnedl WJ, Ferber S, Johnson JH, Newgard CB: STZ transport and cytotoxicity: specific enhancement in GLUT2-expressing cells. *Diabetes* 43:1326–1333, 1994
37. Bellmann K, Wenz A, Radons J, Burkart V, Kleemann R, Kolb H: Heat shock induces resistance in rat pancreatic islet cells against nitric oxide, oxygen radicals and streptozotocin toxicity in vitro. *J Clin Invest* 95:2840–2845, 1995
38. Fridovich I: Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 64:97–112, 1995
39. Wink DA, Hanbauer I, Krishna MC, De Graff W, Gamson J, Mitchell JB: Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proc Natl Acad Sci U S A* 90:9813–9817, 1993