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# Limited GADD45α expression and function in IL-1β toxicity towards insulin-producing cells

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**Growth arrest and DNA damage-inducible (GADD) 45 proteins are regulators of cell death and survival. The proinflammatory cytokine IL-1β strongly increases the level of the transcript encoding GADD45α in rat insulin-producing INS-1E cells. The activation of Gadd45α gene is clearly dependent on JNK and NF-κB activation and the synthesis of the secondary mediator nitric oxide (NO). Interestingly, the observed twelve-fold increase in the GADD45α-coding transcript level is not followed by increased expression of GADD45α at the protein level. An analysis of IL-1β toxicity in INS-1E cells overexpressing GADD45α revealed no correlation between the GADD45α protein level and the sensitivity to IL-1β toxicity. These findings suggest that the potential engagement of GADD45α in IL-1β toxicity towards beta cells is limited to the effects induced by the basal expression level of this protein.**

**Keywords**: GADD45; IL-1β; insulin-producing cells; type 1 diabetes

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## INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease with pancreatic beta cell death caused by proinflammatory cytokines (Cnop *et al*., 2005; Lenzen, 2008). The cytokine IL-1β originating from activated immune cells in the infiltrated pancreatic islets is a crucial mediator of autoimmune-mediated beta cell destruction in type 1 diabetes (Mandrup-Poulsen *et al*., 1993).

Exposure of beta cells to IL-1β leads to a rapid phosphorylation of mitogen-activated protein kinases (MAPKs): JNK (C-Jun N-terminal kinase), p38 and ERK1/2 (extracellular signal-regulated protein kinases) (Cnop *et al*., 2005; Saldeen *et al*., 2001) and the activation of the transcription factor NF-κB (Cnop *et al*., 2005). The activation of p38 or JNK cascades is deleterious for beta cells (Abdelli *et al*., 2007; Eckhoff *et al*., 2003; Saldeen *et al*., 2001), while the role of ERK1/2 activation is controversial (Pavlovic *et al*., 2000; Saldeen *et al*., 2001). NF-κB activation is widely known to promote cell survival (Gilmore *et al*., 2004; Papa *et al*., 2009; Zheng *et al*., 2003) as well as cancer cell growth (Fan *et al*., 2008; Orlowski & Baldwin, 2002). Interestingly, a proapoptotic role of NF-κB activation in beta cells has been reported (Ortis *et al*., 2008). Inhibition of cytokine-induced NF-κB activation counteracts the IL-1β mediated toxicity, mainly by down-regulation of the expression of genes engaged in apoptosis (Eldor *et al*., 2006; Heimberg *et al*., 2001).

GADD45α belongs to the family of growth arrest and DNA damage-inducible (GADD) proteins, consisting of three highly conserved isoforms: GADD45α, GADD45β and GADD45γ. GADD45 proteins play important roles in the regulation of cell cycle arrest, DNA repair, survival and apoptosis (reviewed in (Liebermann & Hoffman, 2008) and (Yang *et al*., 2009)). This important regulatory function is the result of complex interactions with multiple partners, as well as formation of homo- and hetero-complexes within the GADD45 family (Kearsey *et al*., 1995; Kovalsky *et al*., 2001; Tornatore *et al*., 2008; Vairapandi *et al*., 1996; Vairapandi *et al*., 2002). Growing evidence shows that all GADD45 isoforms are modulators of JNK and p38 MAPK activity (Papa *et al*., 2007; Takekawa & Saito, 1998; Zhu *et al*., 2009). Conversely, it is known that MAP kinases regulate the expression of GADD45 family members (Satomi & Nishino, 2009). GADD45 proteins have also been shown to be regulated by NF-κB transcriptionally, post-transcriptionally and post-translationally (Song *et al*., 2006; Zerbini *et al*., 2004; Zheng *et al*., 2005).

Up-to-date reports describe GADD45 proteins as important elements controlling the crosstalk between MAP kinases and NF-κB (Yang *et al*., 2009; Zerbini and Libermann, 2005), thereby regulating cell survival and apoptosis. All GADD45 isoforms were initially categorized as proapoptotic proteins (Takekawa & Saito, 1998). Later, however, it has been shown that GADD45β has an antiapoptotic effect on insulin-producing cells (Larsen *et al*., 2006). Similarly, it has been proposed that IL-1β-induced expression of GADD45α mediates the repair of nitric oxide-induced beta cell damage (Hughes *et al*., 2009). In the present study we show that the potential pro-survival effect of GADD45α protein on insulin-producing cells is limited to the effect of the basal protein expression, since IL-1β did not increase the level of GADD45α protein. Moreover, overexpression of GADD45α protein in the rat pancreatic beta cell line INS-1E results in increased caspase 3 and 7 activity, but has no statistically significant effect on cell fate following IL-1β treatment. This suggests that the protective effect of GADD45α against NO-induced DNA damage described before is counterbalanced by proapoptotic properties of this pro-

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**Abbreviations**: ERK1/2, extracellular signal-regulated protein kinases; GADD, growth arrest and DNA damage-inducible proteins; JNK, C-Jun N-terminal kinase; MAPKs, mitogen-activated protein kinases; T1DM, type 1 diabetes mellitus.

tein, which limits its potential as a prospective target for diabetes therapy.

## MATERIALS AND METHODS

**Materials and reagents**. Human recombinant IL-1β was purchased from PromoKine (Heidelberg, Germany). Plastic materials were from BD Falcon (San Jose, CA, USA).

**Cell culture**. The permanent rat INS-1E cell line (a kind gift of Prof. C. Wollheim, University of Geneva, Geneva, Switzerland) from passages 80–95 was cultured at 37°C as described before (Ximenes *et al*., 2007) in fully supplemented RPMI 1640 medium with 10 mM glucose, 10% (v/v) fetal calf serum (FCS), penicillin, and streptomycin in a humidified atmosphere of 5% $CO_2$.

**Real-time PCR**. Total RNA was isolated using a modified Chomczynski-Sacchi method, reverse-transcribed and subjected to real-time PCR, as described before (Skalniak *et al*., 2009). For the normalization of each sample, the amount of cyclophilin A (NCBI mRNA sequence entry XM_345810) cDNA was measured (primers *cyclo*F and *cyclo*R). All samples were run in duplicates. Primers used in the real-time PCR are listed in Table 1. The specificity of primers targeting *Gadd45a* transcript was verified by RT-PCR and real-time PCR in order to check for cross-contamination by amplicons from GADD45β- or GADD45γ-coding transcripts (Supplementary Fig. 1 at www.actabp.pl).

**Cell stimulation**. Cells were seeded on 96-, 12- or 6-well plates or 6 cm² dishes. The experimental procedures were performed after two days without a serum-starvation step. For cytokine stimulation 60 U/μl of human recombinant IL-1β was used. For inhibition of signal transduction the following inhibitors and concentrations were used: JNK1/2/3 inhibitor SP600125 (Calbiochem): 5 μM and 20 μM, p38α/β/β2 inhibitor SB203580 (Calbiochem): 5 μM and 20 μM, IκBα inhibitor (NF-κB inhibitor) BAY 11-7082 (Calbiochem): 1.25 μM and 5 μM, nitric oxide synthesis inhibitor L-$N^G$-monomethylarginine (L-NMMA, Santa Cruz Biotechnology) and its inactive isoform D-$N^G$-monomethylarginine (D-NMMA, Santa Cruz Biotechnology): 1 mM. Inhibitors SP600125, SB203580 and BAY 11–7082 were administered 60 minutes before IL-1β stimulation, L-NMMA and D-NMMA were administered 24 hours before stimulation. BAY 11-7082 was removed from the medium at the time of stimulation with IL-1β.

**Western blotting**. Total cell lysates were prepared using RIPA buffer (25 mM Tris/HCl, pH 7.6, 150 mM NaCl, 1% Nonidet, 1% sodium deoxycholate, 0.1% SDS) with protease inhibitor cocktail (Roche) and separated on SDS-Page 12% polyacrylamide gel. Following the electrotransfer onto PVDF membrane (Millipore) and blocking in 2% BSA (BioShop) in Tris-buffered saline containing 0.1% Nonidet, membranes were incubated with primary antibody at 4°C overnight. After addition of secondary antibodies, the chemiluminescence detection was performed using Luminata Crescendo (Millipore). The following antibodies and dilutions were used: rabbit anti-GADD45α (1:1000, Cell Signaling), mouse anti-α-tubulin (1:2000, Calbiochem), peroxidase-conjugated anti-rabbit (1:3000; Cell Signaling) and peroxidase-conjugated anti-mouse (1:20 000, Sigma).

**Cytotoxicity assays**. IL-1β cytotoxicity towards INS-1E cells was measured by MTT test, intracellular ATP content and caspase 3 and 7 activity analysis. For MTT 40 000 cells were seeded in each well of a 96-well plate. Following a 24-hour stimulation with 60 U/ml IL-1β thiazolyl blue formazan (MTT, Sigma) was added for an additional 2 hours at a final concentration of 500 ng/ml. Plates were centrifuged at 300×*g* for 5 minutes at room temperature, medium was removed by suction and MTT crystals were dissolved in acidic (40 mM HCl) isopropanol. Absorbance was measured using a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 570 nm with the reference wavelength 650 nm.

For intracellular ATP content analysis cells were plated on 96-wells plates (40 000 per well). After 24-hour stimulation with IL-1β ATP content assay was carried out according to the manufacturer's instructions (ATPlite, Luminescence ATP Detection Assay System; Perkin Elmer). The luminescence was measured using Infinite M200 microplate reader (Tecan Group Ltd.).

Activity of caspases 3 and 7 was measured using Caspase-Glo 3/7 Assay (Promega). Protein extracts from INS-1E cells stimulated with IL-1β for 24 hours were isolated with RIPA buffer (Sigma) and 2.5 μg of protein was mixed with 25 μl of Caspase-Glo 3/7 Reagent on a white 96-well plate. After 60 minutes of incubation luminescence was measured as above.

**Nitrite accumulation**. For NO concentration measurement the Griess method of nitrite detection was used (Guevara *et al*., 1998). Media from INS-1E cells stimulated for other experiments (as described in figure legends) were collected. On a microplate 100 μl of culture medium was mixed with 100 μl of Griess reagent (0.5% sulphanilic acid, 0.05% *N*-(1-naphthalenediamine) and 2.5% phosphoric acid). The absorbance at 545 nm was measured using a microplate reader.

**Stable GADD45α overexpression**. The coding sequence for rat GADD45α (NCBI mRNA sequence entry NM_024127) was amplified with the primers *R45aOEf1* and *R45aOEr1* (Table 1). The PCR product was cloned into the pcDNA3 vector (Invitrogen) with XhoI and HindIII restriction enzymes. The construct was verified by restriction analysis and sequencing. INS-1E cells were seeded on 10 cm² dishes and transfected with GADD45α-coding vector or empty pcDNA3 vector (mock control cells) using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. One day following transfection INS-1E growth medium was supplemented with 300 μg/ml G418 disulphate salt (Sigma). After antibiotic-resistance selection single clones were picked, trypsinized and cultured for further experiments in a constant presence of 300 μg/ml G418 disulphate salt. The overexpression of *Gadd45a* mRNA was quantified using real-time PCR and verified by western blot.

## RESULTS

### Activation of *Gadd45a* expression by IL-1β in INS-1E cells

To characterize the induction profile of *Gadd45a* INS-1E cells were exposed to IL-1β for different time periods. At each time point untreated control cells were collected as well. The *Gadd45a* transcript level was measured by real-time PCR. The level of the transcript encoding GADD45α markedly increased following IL-1β exposure in a time-dependent manner (Fig. 1A). This stimulation of *Gadd45a* transcription was significant already after 3 hours and increased constantly up to 30 hours. The final

transcript level after a 30-hour stimulation was 14 times higher than in untreated cells.

In contrast, no increase in GADD45α protein amount was observed following IL-1β stimulation (Fig. 1B, C). Thus, *Gadd45a* transcription is highly activated by IL-1β, but the resulting transcript level increase does not translate into a change in protein expression.

Similar results were observed when GADD45α expression was analysed in the mouse pancreatic beta cell line MIN6 (Supplementary Fig. 2 at www.actabp.pl). Stimulation with IL-1β resulted in a 2.5-fold increase in *Gadd45a* transcript level (Supplementary Fig. 2A at www.actabp.pl), while the expression GADD45α protein was not enhanced (Supplementary Fig. 2B and C at www.actabp.pl).

### Involvement of MAP kinases and NF-κB in the IL-1β-dependent elevation of *Gadd45a* expression

IL-1β modulates gene expression via several signaling pathways. To investigate the involvement of MAP kinas-

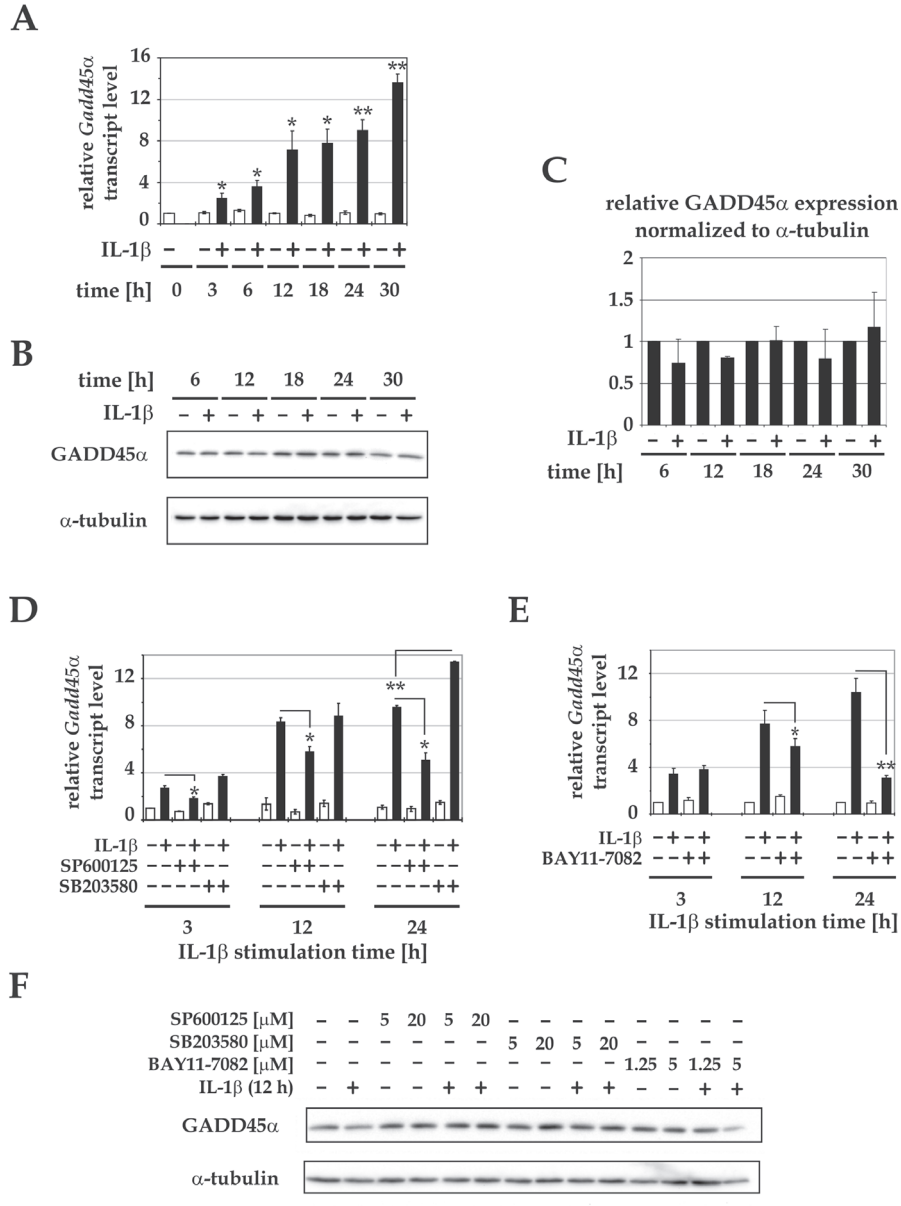


**Figure 1. Expression of *GADD45a* in INS-1E cells after IL-1β stimulation.**
(**A**) A real-time PCR analysis of the *Gadd45a* transcript level time course. Graphs represent the fold-change of the *Gadd45a* transcript level normalized to untreated cells at the time point "0". (**B**) Western blot analysis of the time course of the GADD45α protein level. A representative blot from three independent experiments is shown. (**C**) Densitometry analysis of western blot results from the time course of GADD45α protein, normalized to α-tubulin expression and untreated controls. Shown are means ±S.D. of three experiments. (**D**) Effect of JNK and p38 inhibition on *Gadd45a* transcript level after stimulation with IL-1β. The JNK inhibitor (SP600125) and p38 inhibitor (SB203580) were added to the culture medium 1 hour before stimulation and maintained in the medium for the stimulation period. (**E**) Effect of NF-κB inhibition on *Gadd45a* transcript level after stimulation with IL-1β. The NF-κB inhibitor (BAY11-7082) was added to the culture medium 1 hour before stimulation and removed prior to IL-1β addition. (**F**) Effect of JNK, p38 and NF-κB inhibition on GADD45α protein level. A representative blot from three independent experiments is shown. Data points in graphs A, D and E are presented as fold stimulation normalized to untreated controls (graph A) or untreated controls at the time point "3 h" (graphs C and D). The culture media from experiments presented in Fig. 1D and E were used for nitrite accumulation measurements (Fig. 2D and E, respectively). The results presented in graphs A, D and E are means ±S.D. of three experiments. Student's *t*-test was used for statistical analyses: **p*<0.5, ***p*<0.01.

es in the *Gadd45a* transcriptional regulation two inhibitors were used: SP600125 (an inhibitor of JNK MAPK) and SB203580 (an inhibitor of p38 MAPK). Real-time PCR analysis revealed that the inhibition of JNK phosphorylation diminished the IL-1β-induced increase of the *Gadd45a* mRNA level, suggesting an involvement of JNK in the stimulation of *Gadd45a* gene expression by IL-1β (Fig. 1D). The inhibition of p38 MAPK had no significant effect on the IL-1β-induced *Gadd45a* transcript level (Fig. 1D), except at 24 hours of stimulation when a significant increase in the transcript level was observed. Neither the inhibition of JNK nor of p38 affected the GADD45α protein level (Fig. 1F).

To investigate the role of NF-κB in the induction of the *Gadd45a* gene by IL-1β cells were incubated with BAY11-7082 (an inhibitor of cytokine-induced IκB-α phosphorylation) and stimulated with IL-1β. Except for the 3-hour time point, the inhibition of NF-κB reduced the IL-1β-stimulated *Gadd45a* transcription as compared to cells stimulated with IL-1β only. The strongest effect was observed after 24 hours (Fig. 1E).

Previously, it has been demonstrated that NF-κB may influence the expression of GADD45α protein at post-transcriptional stages (Song *et al*., 2006; Zerbini *et al*., 2004; Zheng *et al*., 2005). To investigate whether activation of NF-κB may be involved in the inhibition of GADD45α synthesis observed in cells stimulated with IL-1β, in which the significant increase of *Gadd45a* transcript level was observed, we performed western blot analyses with extracts from cells treated with the NF-κB inhibitor and stimulated with IL-1β. Again, no effect on GADD45α protein amount was observed (Fig. 1F).

### Changes in *Gadd45a* expression after IL-1β treatment are dependent on nitric oxide production

IL-1β induces the production of nitric oxide (NO) by INS-1E cells (Fig. 2A). To investigate the importance of NO in the IL-1β-stimulated increase of the *Gadd45a* transcript level, an inhibitor of nitric oxide synthase (NOS) was used. Inhibition of nitrite production by the iNOS inhibitor, L-NMMA significantly reduced the expression of *Gadd45a* gene in IL-1β-treated cells in comparison to cells incubated with an inactive isoform D-NMMA or cells only stimulated with IL-1β (Fig. 2B). The inhibition of *Gadd45a* gene expression paralleled decreased nitrite formation (Fig. 2C).

The inhibition of p38 or JNK had no effect on nitrite production by INS-1E cells (Fig. 2D), while the inhibition of NF-κB significantly reduced the nitrite concentration in the culture medium both after 12 and 24 hours of stimulation (Fig. 2E).

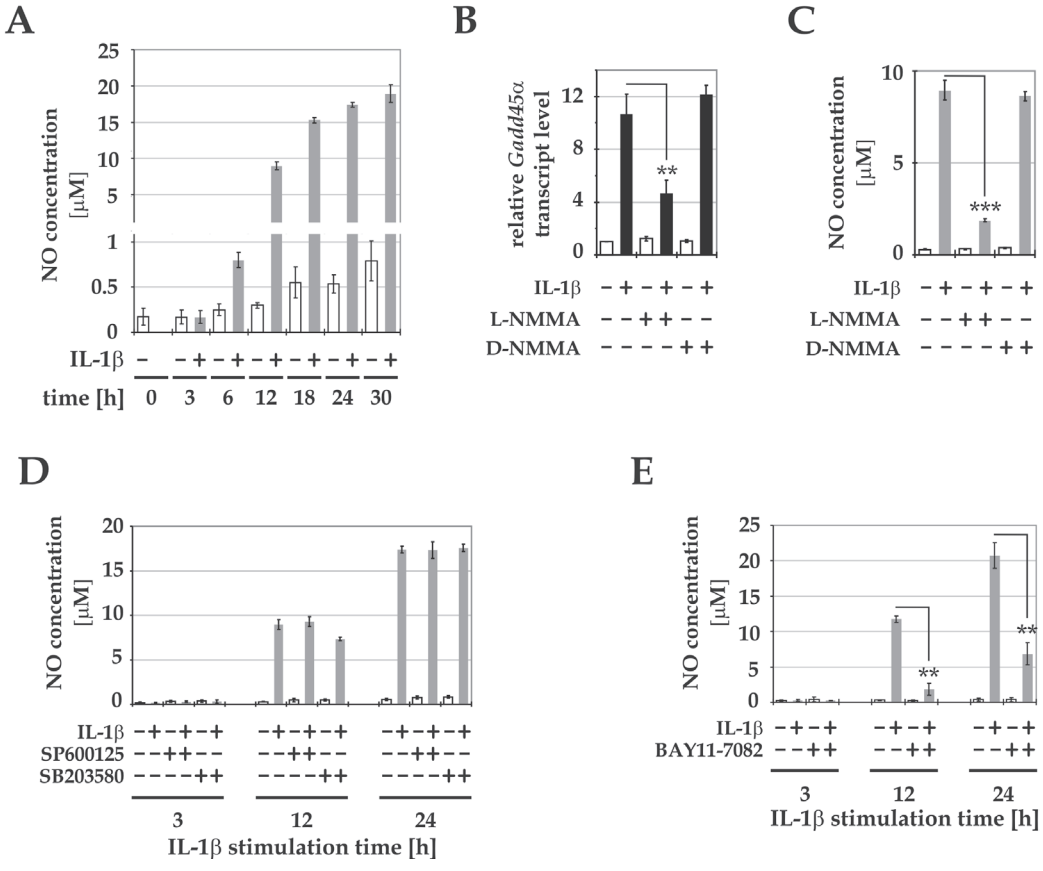


**Figure 2. The engagement of nitric oxide in the regulation of *Gadd45a* expression in INS-1E cells following IL-1β stimulation.**
(**A**) Time course of nitrite accumulation by INS-1E cells following IL-1β stimulation. Cells were seeded on 6-well plates and stimulated for the indicated time periods with 60 U/ml of IL-1β. Medium was used for nitrite determination and cells for a real-time PCR analysis (Fig. 1A). (**B**) Effect of inhibition of nitrite accumulation on the *Gadd45a* transcript level after stimulation with IL-1β. Cells were stimulated with 60 U/ml of IL-1β for 12 h. The NO synthesis inhibitor (L-NMMA) and its inactive isoform (D-NMMA) were added to the culture medium 24 hours before stimulation and retained in the medium for the stimulation period. Data points in graphs are presented as fold-stimulation normalized to untreated control. The culture medium from these cells was used for nitrite determination (Fig. 2C). (**C**) Influence of NO synthesis inhibition on nitrite accumulation. (**D**) Effect of JNK and p38 inhibition on nitrite accumulation. (**E**) Effect of NF-κB inhibition on nitrite accumulation. Data in graphs C, D and E are means ± SD of three independent experiments. Student's *t*-test was used for statistical analysis: ***p*<0.01, ****p*<0.001.

### Effect of *Gadd45a* overexpression on INS-1E cell metabolism and viability

To investigate the effect of GADD45α on the viability of IL-1β-treated INS-1E cells, GADD45α protein was overexpressed. For this, seventeen INS-1E transfected cell clones characterized by different levels of stable *Gadd45a* expression (Fig. 3A) and in two control clones (mock-transfected) were generated. The clones were divided into four groups (designated "C" for control clones and "I", "II" and "III" for GADD45α-overexpressing clones), depending on the level of overexpression of the *Gadd45a* transcript (Fig. 3A). Additionally, to check for a general tendency, calculations were performed for all 17 clones together (Fig. 3, designated "All clones") and compared with control. IL-1β toxicity was analysed by MTT assay (Fig. 3D), ATP content measurement (Fig. 3E) and activity of caspases 3 and 7 (Fig. 3F). The increased expression level of GADD45α protein in generated clones was verified by western blot-

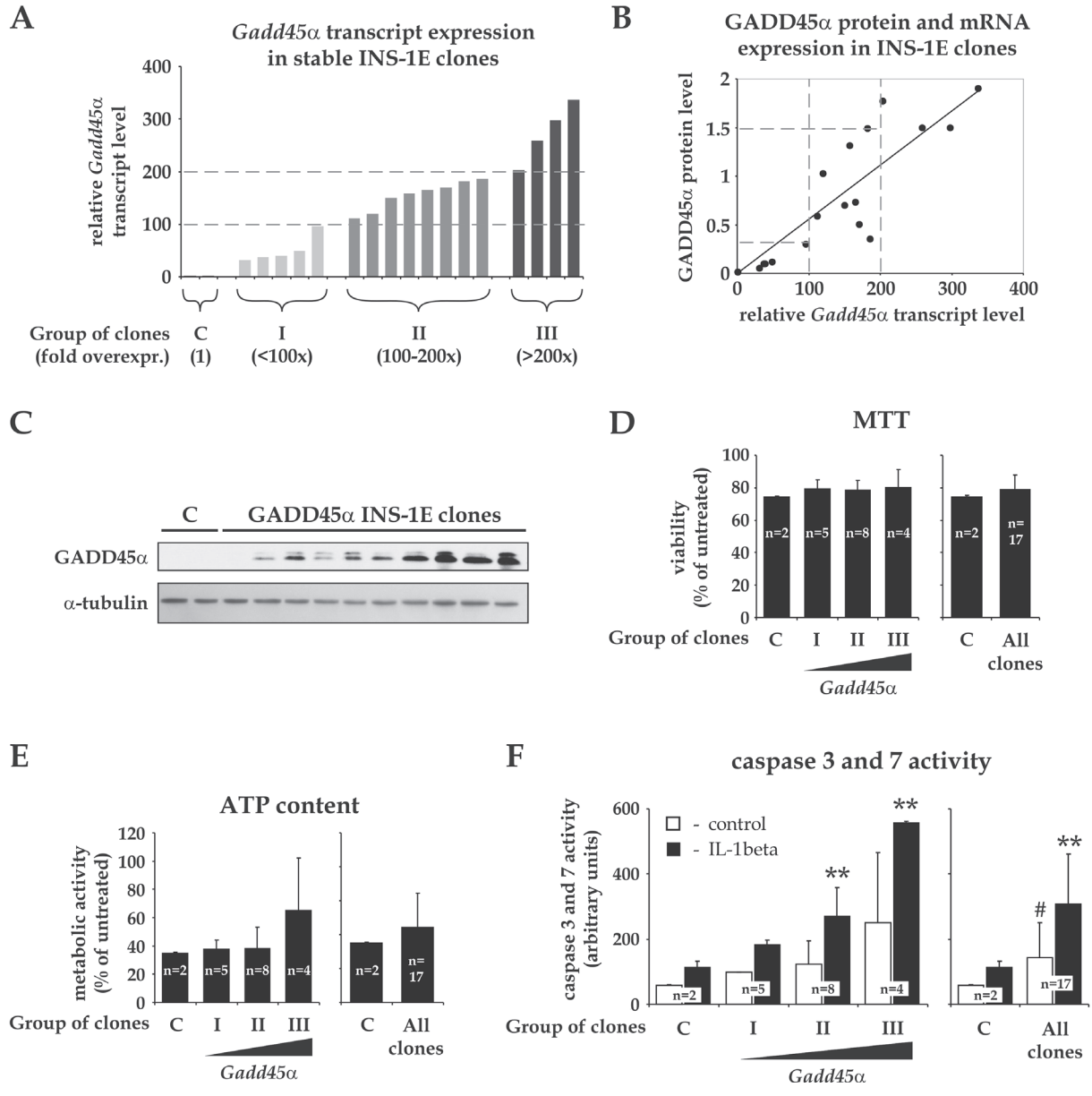


**Figure 3. The influence of stable overexpression of GADD45α on the IL-1β-induced INS-1E cell death.**
(**A**) Real-time PCR analysis of the expression of *Gadd45a* transcript in generated INS-1E clones. Clones were grouped depending on their *Gadd45a* expression level (group I — the lowest expression, group III — the highest expression of *Gadd45*a, group C — control clones). Data were normalized to mean expression in control clones. (**B**) Correlation between transcript and protein level in GADD45α-overexperssing INS-1E clones. *Gadd45a* mRNA expression was quantified by real-time PCR and GADD45α protein amount was estimated by densitometry of western blot results, normalized to α-tubulin content. Each black dot corresponds to a single INS-1E clone. (**C**) An example of western blot result presenting overexpression of GADD45α protein in selected INS-1E clones. Densitometry analysis was performed on this and other blots in order to semi-quantitatively evaluate the level of GADD45α expression. (**D**) Cell viability of GADD45α-overexpressing clones measured by MTT assay. Cells were stimulated for 24 hours with 60 U/ml of IL-1β. Results were normalized to untreated controls (% of untreated) and a mean value was calculated for each clone from four experiments. Presented data are means ± S.D. from results for each clone in a group (2–17 clones in a group, as indicated). (**E**) ATP content analysis for each group of GADD45α-overexpressing clones. Cells were stimulated for 24 hours with 60 U/ml of IL-1β. The data were analysed and presented identically as for MTT experiment (Fig. 3D). (**F**) Caspase 3/7 activity analysis for each group of GADD45α-overexpressing clones. The graphs represent means ± S.D. from three experiments. Student's *t*-test was used for statistical analysis: **p*<0.05, ***p*<0.01. (D–F) Number on each bar (n=...) represents the number of clones in respective group.

**Table 1. Sequences of primers used in experimental approaches.**
All sequences are given in the 5'→3' direction.

| | |
|---|---|
| Real-time PCR primers | |
| R45aF1 | CAGAGCAGAAGATCGAAAGGATGG |
| R45aR1 | CGTTGTCCGGGTCTACGTTGAGC |
| cycloF | TATCTGCACTGCCAAGACTGAG |
| cycloR | CACAATGCTCATGCCTTCTTTCA |
| Primers used for construct preparation | |
| R45aOEf1 | CCC**AAGCTT**AACATGACTTTGGAGGAATTCTCG |
| R45aOEr1 | CCG**CTCGAG**ATCACCGTTCGGGGAATCACCG |

ting (Fig. 3C). The level of GADD45α protein expression correlated well with the expression level of *Gadd45a* mRNA (Fig. 3B).

Incubation with 60 U/ml IL-1β led to a 25% loss of cell viability for control group ("C") after 24 hours of treatment (Fig. 3D), as measured by MTT assay. Measurements of cell viability for the group of all clones treated with IL-1β revealed similar toxicity ($p = 0.266$). No statistically significant effect of the magnitude of *Gadd45a* expression on IL-1β-mediated toxicity was observed (Fig. 3D, groups I, II and III *versus* C, $p = 0.429$, 0.549 and 0.649, respectively). Consistently, no significant correlation between *Gadd45a* overexpression magnitude and cell metabolism (measured by ATP content) was observed (Fig. 3E, $p = 0.535$, 0.660 and 0.450 for groups I, II and III *versus* C) Similarly, for the group of all 17 clones versus control group no significant difference in ATP content was observed following IL-1β treatment (Fig. 3E, $p = 0.205$). However, cells overexpressing *Gadd45a* presented significantly elevated caspase 3 and 7 activity, both at the basal state and following stimulation with IL-1β (Fig. 3F, $p = 0.031$ and 0.003 for "All clones" group versus control cells with and without IL-1β, respectively), suggesting an increased activation of apoptosis-related processes. The activation of caspases 3 and 7 was dose-dependent and statistically significant for groups II and III following IL-1β treatment (Fig. 3F, $p = 0.007$ and $<0.001$, respectively).

## DISSCUSSION

GADD45 proteins are important regulators of cell death and survival (Liebermann & Hoffman, 2008; Yang *et al*., 2009; Zerbini & Libermann, 2005). The proapoptotic function of GADD45α has been reported previously in numerous cell types (Al-Romaih *et al*., 2008; Hildesheim *et al*., 2002; Li *et al*., 2009; Okura *et al*., 2000; Zerbini *et al*., 2004). Recently, a novel mechanism that mediates pro-survival functions of GADD45α in hematopoietic cells has been proposed (Hoffman and Liebermann, 2009; Liebermann & Hoffman, 2007). The protection of these cells against genotoxic stress has been linked to the activation of the GADD45α-p38-NF-κB survival pathway. Therefore, the effect of GADD45α protein on the cell fate seems to be ambiguous.

The transcription of *Gadd45a* is increased by JNK kinase in several cell types (Hughes *et al*., 2009; Satomi & Nishino, 2009). In the present study this phenomenon was observed also in INS-1E cells. In these cells JNK exerts a constant positive effect on *Gadd45a* transcription, as inhibition of this kinase cascade resulted in a decrease of the GADD45α-coding transcript in comparison to control cells. This effect was independent of the synthesis of nitric oxide.

Previously, it has been shown that IL-1β induces an increase in the *Gadd45a* transcript level in insulin-producing INS 832/13 cells and that this increase is dependent on NO synthesis (Hughes *et al*., 2009). The authors suggested that the induction of GADD45α synthesis is crucial for the repair of NO-induced DNA damage, and thus protects against IL-1β-induced beta cell death (Hughes *et al*., 2009). Our results are in agreement with the finding that the *Gadd45a* transcript level is increased in insulin-producing cells in a NO-dependent manner. Inhibition of NO synthesis markedly decreased the amount of *Gadd45a* transcript after 12 and 24 hours of stimulation with IL-1β. Similarly, inhibition of NF-κB resulted in impairment of *Gadd45a* transcript induction after 12 and 24 hours of stimulation. This effect was accompanied by the inhibition of NO synthesis, presumably due to the inhibition of NF-κB-induced iNOS expression (Cardozo *et al*., 2001; Darville & Eizirik, 1998; Kleinert *et al*., 2003; Souza *et al*., 2008).

Although the activation of the transcription of *Gadd45a* by IL-1β was prominent in INS-1E cells (and consistent with the previous report on INS 832/13 cells (Hughes *et al*., 2009)), it was not accompanied by increased synthesis of the protein. Similarly, no GADD45α protein increase was observed in MIN6 cells after IL-1β treatment. This observation is in agreement with a previous study (Larsen *et al*., 2006), where no increase of GADD45α protein was observed in INS-1E cells or isolated rat islets. Thus, the GADD45α-mediated DNA repair is likely to be limited to the effect provided by the basal expression of this protein.

It has been reported that the GADD45α protein transcript half-life and translation are regulated by several mRNA-binding proteins, including nucleolin (Zhang *et al*., 2006; Zheng *et al*., 2005), Hur (Zhang *et al*., 2006), AUF1 (A+U-Rich Element Binding Factor 1) and TIAR (TIA-1-related protein) (Lal *et al*., 2006), which are targets of NF-κB activity. However, the inhibition of NF-κB did not result in a release of the synthesis of GADD45α. Similarly, inhibition of JNK or p38 did not increase GADD45α protein level.

In the present study the selection of GADD45α-overexpressing INS-1E clones failed to provide significant protection against IL-1β toxicity. On the other hand, overexpression of GADD45α did not result in increased susceptibility of INS-1E cells to IL-1β, although the activity of caspases 3 and 7 was significantly increased. This suggests that either GADD45α has no influence on insulin-producing cells death in response to IL-1β, or that the protective effects are counterbalanced by deleterious properties of GADD45α protein. Since silencing of GADD45α expression was shown to inhibit DNA repair in insulin-producing cells (Hughes *et al*., 2009), and overexpression of this protein activates effector caspases 3 and 7, it seems that optimal expression of this bimodal regulator may be crucial for the cell fate. This may explain the complex nature of the regulation of expression of GADD45α following IL-1β stimulation, where the increase in *Gadd45a* transcript is apparently not sufficient to stimulate the synthesis of the protein.

## CONCLUSIONS

IL-1β increases the amount of *Gadd45a* transcript in a JNK, NF-κB and NO-dependent manner. Interestingly, the GADD45α protein level remains constant after stim-

ulation with IL-1β, suggesting a tight translational regulation of GADD45α protein synthesis. Overexpression of GADD45α does not protect INS-1E cells against toxic effects of IL-1β.


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