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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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.
Supplementary Figure 1. Specificity of primers used for the detection of Gadd45α transcript.

(A) Alignment of partial rat nucleotide sequences of transcripts encoding three isoforms of GADD45 proteins, α, β and γ. For each sequence a pair of primers was chosen for RT-PCR and real-time PCR studies. (B) DNA gel electrophoresis of RT-PCR products for all three pairs of designed primers. (C-E) Real-time PCR analysis of the time courses of transcripts encoding GADD45 proteins. INS-1E cells were cultured in basal glucose concentration (11 mM) or elevated glucose (30 mM) and stimulated with IL-1β for indicated time periods (n=3). The differences in mRNA profiles indicate no cross-priming of Gadd45 transcripts by the selected primer pairs.
Supplementary Figure 2. The expression of GADD45α in MIN6 cell line after IL-1β treatment. MIN6 cells were cultured at 37°C in DMEM medium supplemented with 25 mM glucose, 10% (v/v) fetal calf serum (FCS), penicillin, and streptomycin in a humidified atmosphere of 5% CO2. (A) Time course of the expression level of Gadd45α mRNA following IL-1β treatment, analyzed by real-time PCR. Graphs represent the fold-change of the Gadd45α transcript level normalized to untreated cells. Mouse Gadd45α-specific primers used were: forward: CAGAGCAGAAGACCGAAAGGATGG, reverse: CGTTATCGGGGTCTACGTTGAGC. (B) Western blot analysis of the GADD45α protein level following IL-1β treatment. A representative blot from three experiments is shown. (C) GADD45α expression in IL-1β-treated MIN6 cells, estimated by densitometry analysis of western blot results shown in (B) normalised to α-tubulin expression and untreated controls. Shown are means ± S.D. of three experiments.