Influence of cytokines on Dmt1 iron transporter and ferritin expression in insulin-secreting cells

S Lortz, S Schröter[†], V Stückemann[‡], I Mehmeti and S Lenzen

Hannover Medical School, Institute of Clinical Biochemistry, 30623 Hannover, Germany

([†]S Schröter is now at Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany)

([‡]V Stückemann is now at Twincore Centre for Experimental and Clinical Infection Research, 30625 Hannover, Germany and Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, 30623 Hannover, Germany)

Correspondence
should be addressed
to S Lortz

Email

Lortz.Stephan
@mh-hannover.de

Abstract

Free intracellular ferrous iron (Fe^{2+}) is essential for the generation of the extremely toxic hydroxyl radicals, which contribute to β -cell destruction by cytokines. Therefore the expression of the different divalent metal transporter 1 (Dmt1) isoforms and ferritin (Ft) subunits, responsible for iron import and chelation, was analyzed under pro-inflammatory conditions (IL1 β alone or together with TNF α +IFN γ). The Dmt1 isoforms (1A/1B and +IRE/–IRE) and the total *Dmt1* expression in insulin-producing cells (RINm5F and INS-1E), in primary rat islets and, for comparison, in the neuroendocrine PC12 cell line were quantified by qRT-PCR. In addition, the expression of the light (L-Ft) and heavy Ft (H-Ft) subunits and the mitochondrial Ft isoform (*Mtft*) in insulin-producing cells under control conditions and after cytokine treatment was estimated. The 1B isoform was the predominant *Dmt1* mRNA in all insulin-producing cells, accounting for almost 100% of the 1A/1B isoform expression. For the IRE variants, +IRE expression was higher than –IRE expression. Pro-inflammatory cytokines accelerated the expression of *Dmt1* isoforms significantly with an overall 2.5- to 3-fold increase in the total *Dmt1* expression. In contrast, the expression of the iron-buffering ferritin subunits L- and H-Ft was unaffected by IL1 β and only slightly induced by the cytokine mixture. *Mtft* expression was also not increased. *Dmt1* expression was significantly elevated through pro-inflammatory cytokines, whereas Ft expression was marginally increased. This imbalance between the increased iron transport capacity and the almost unaffected iron storage capacity can foster cytokine-mediated formation of hydroxyl radicals and thus pro-inflammatory cytokine toxicity through elevated free iron concentrations.

Key Words

- ▶ insulin-secreting cells
- ▶ iron transport and storage
- ▶ Dmt1
- ▶ ferritin
- ▶ reactive oxygen species
- ▶ pro-inflammatory cytokines
- ▶ Haber–Weiss reaction

*Journal of Molecular
Endocrinology*
(2014) 52, 301–310

Introduction

The destruction of insulin-secreting β -cells by infiltrating immune cells is mediated via soluble pro-inflammatory cytokines, in particular interleukin 1 β (IL1 β), tumor necrosis factor- α (TNF α), and interferon- γ (IFN γ)

(Jörns *et al.* 2005, Thomas *et al.* 2009). Activation of the respective receptors initiates the intracellular signaling cascades leading ultimately to β -cell dysfunction and apoptosis (Cnop *et al.* 2005, Novotny *et al.* 2012).

One major component of this process is the oxidative destruction of cellular structures by an increased generation of reactive oxygen species (ROS), especially of highly toxic hydroxyl radicals (Gurgul-Convey *et al.* 2011, Mehmeti *et al.* 2011a). Hydroxyl radicals are formed in the Haber–Weiss reaction in the presence of catalytically active free ferrous iron (Fe^{2+}) from intracellular hydrogen peroxide (Halliwell & Gutteridge 2007).

Circulating iron is sequestered by transferrin within the bloodstream. Through interaction with the transferrin receptors this transferrin-bound iron can enter the cell by endocytosis. The subsequent transport step from the endosome to the cytosol is mediated by the divalent metal transporter 1 (Dmt1), which is expressed in four known isoforms, resulting from two different 5'-variants, called 1A and 1B, and two 3'-variants, which are different in their 3'-translated and UTR, containing or not an iron-responsive element (+IRE/–IRE; Gunshin *et al.* 1997, Hubert & Hentze 2002, MacKenzie *et al.* 2008). These elements are important for the regulation of the Dmt1 expression by influencing mRNA stability (Galy *et al.* 2008) and each of these 3'-variants can occur with both 5'-types of RNA. Due to the central role of iron for the synthesis of heme prosthetic groups and iron-sulfur clusters, a high portion is further transferred into mitochondria, although the exact transport mechanisms are only poorly characterized (Horowitz & Greenamyre 2010).

In order to avoid an excess of free iron in the cell, in the form of the so-called labile iron pool (LIP), cellular iron homeostasis is precisely balanced by regulating iron import, its intracellular handling, and chelation (Pantopoulos *et al.* 2012). Intracellular iron storage and chelation are mediated by ferritin (Ft), which prevents in this way an excess of free intracellular iron and its damaging effects (Hentze *et al.* 2004). Ft in its cytosolic form consists of two different subunits, called the H (heavy) and L (light) subunits. Twenty-four of these subunits form, in a variable ratio of H:L subunits, the apoferritin molecule (Harrison & Arosio 1996). At variance from the cytosolic Ft molecule, the mitochondrial Ft (Mtft) molecule has a homopolymeric structure with a high homology to the Ft-H molecule (Levi *et al.* 2001).

Pro-inflammatory cytokines, however, favor pro-oxidative conditions and the generation of hydroxyl radicals in insulin-secreting cells (Lortz *et al.* 2000). The extremely low expression of the hydrogen peroxide-inactivating enzymes catalase and glutathione peroxidase, together with a relatively high superoxide dismutase expression (Lenzen *et al.* 1996, Tiedge *et al.* 1997, Lenzen 2008), leads to an accumulation of hydrogen peroxide under the

influence of pro-inflammatory cytokines (Gurgul-Convey *et al.* 2011). Although catalase expression in human islets (Welsh *et al.* 1995) is somewhat higher than that in rodent islets (Lenzen *et al.* 1996, Tiedge *et al.* 1997, Lenzen 2008), the catalase activity of human β -cells is, when compared with other tissues, very low (for discussion see Lenzen (2008)) and therefore similar damaging effects of ROS could be observed in human islets (Benhamou *et al.* 1998).

Together with an induction of iron import via Dmt1 through pro-inflammatory cytokines (Hansen *et al.* 2012), this would support hydroxyl radical formation, promoting serious damage to many cellular structures (Mehmeti *et al.* 2011b, Hansen *et al.* 2014). We therefore characterized the influence of pro-inflammatory cytokines on the expression of the different Dmt1 isoforms and whether enhanced Dmt1 expression could be compensated, probably by increased expression of the iron-chelating protein Ft.

Materials and methods

Tissue culture of insulin-producing cells

Insulin-producing RINm5F tissue culture cells were cultured in RPMI-1640 medium supplemented with 10 mM glucose, 10% (v/v) FCS, penicillin, and streptomycin as described previously (Lenzen *et al.* 1996, Tiedge *et al.* 1997). Insulin-secreting INS-1E cells (kindly provided by Prof. C Wollheim) were cultured as previously described (Asfari *et al.* 1992). The neuroendocrine PC12 cells were cultured in RPMI-1640 medium supplemented with 10 mM glucose, 5% (v/v) FCS, 10% (v/v) horse serum, penicillin, and streptomycin (Greene & Tischler 1976). All cell lines were cultured at 37 °C in a humidified atmosphere of 5% CO_2 .

Rat islet isolation

Pancreatic islets were isolated from 250 to 300 g adult male Lewis rats by collagenase digestion, separated by Ficoll gradient, and handpicked under a stereo microscope (Tiedge *et al.* 1997). Isolated islets were cultured overnight in RPMI-1640 medium supplemented with 5 mM glucose, 10% FCS, penicillin, and streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 .

Exposure to pro-inflammatory cytokines

The cells were seeded in 6 cm tissue culture plates at a density of 1×10^6 cells and allowed to attach for a period of 24 h. Thereafter the cells were exposed to

600 U/ml human IL1 β or a combination of cytokines (cytokine mixture) consisting of 60 U/ml IL1 β , 185 U/ml human TNF α , and 14 U/ml IFN γ (PromoCell, Heidelberg, Germany) for the indicated incubation times.

Hypoxic incubation

For incubation under hypoxic conditions, the cells were cultured 24 h after seeding at 37 °C in a humidified atmosphere of 1% O₂ balanced with N₂ for hypoxia for the indicated incubation times (6 and 24 h hypoxia or 24 h hypoxia followed by 6 h normoxia). Hypoxia was generated in an oxygen-regulated incubator (CB210 incubator with O₂ control option, Binder, Tuttlingen, Germany). After incubation, total RNA was isolated immediately.

Real-time quantitative RT-PCR

Total RNA was isolated as previously described (Chomczynski & Sacchi 1987). For cDNA synthesis, random hexamers were used to prime the reaction of the RevertAid H- M-MuLV reverse transcriptase (Fermentas, St Leon-Rot, Germany). QuantiTect SYBR Green technology (Qiagen), which uses a fluorescent dye that only binds double-stranded DNA, was employed. The reactions were carried out using the Opticon Realtime-PCR-System (Bio-Rad). The samples were first denatured at 94 °C for 3 min, followed by 40 PCR cycles comprising a melting step at 94 °C for 30 s, an annealing step at 62 °C for 30 s, and an extension step at 72 °C for 30 s. Optimal parameters for the PCRs were empirically defined and the purity and specificity of the amplified PCR product for each experiment was verified by melting curve analysis. All transcripts showed C_t-values, which were at least ten C_t-values lower than the values for blanks. Each PCR amplification was carried out in triplicate. The primer sequences are listed in [Supplementary Table 1](#), see section on [supplementary data](#) given at the end of this article. Data are expressed as relative gene expression after normalization to the housekeeping gene β -actin using the Qgene96 (Institute of Biochemistry and Genetics, Research Group Cardiovascular Genetics, University of Basel, Switzerland) and LineRegPCR (Heart Failure Research Center, Amsterdam, the Netherlands) software.

Western blot analyses

After 48 h of incubation, whole-cell extracts were sonified in ice-cold PBS on ice for 15 s at 60 W with a Braun-Sonic 125 sonifier. Protein content was determined by the BCA assay (Pierce, Rockford, IL, USA). 20 μ g protein per lane was

fractionated by 12.5% SDS-PAGE and transferred to polyvinylidene fluoride membranes. Nonspecific binding sites of the membranes were blocked by 5% non-fat dry milk for 1 h at room temperature. Then the membranes were incubated overnight at 4 °C with the specific primary antibody (Dmt1, sc-30120, diluted 1:200; actin, sc-1615, 1:250, Santa Cruz Biotechnology). The excess of primary antibody was removed by three washes with wash buffer (PBS, 0.1% Tween 20, 0.1% BSA), subsequently the membrane was incubated with the peroxidase-labeled secondary antibodies at a dilution of 1:20 000 at room temperature for 1 h. The protein bands were visualized by chemiluminescence using the ECL detection system (GE Life Sciences, Freiburg, Germany). The intensity of the bands was quantified through densitometry with the Gel-Pro Analyzer 6.0 program (Media Cybernetics, Silver Spring, MD, USA).

Statistical analyses

Data are expressed as mean $\pm$ s.e.m. Statistical analyses were performed using ANOVA plus Bonferroni's test for multiple comparisons (Graphpad, San Diego, CA, USA).

Results

Expression of the different Dmt1 isoforms in insulin-producing cells and primary rat islets

For the quantification of the four characterized Dmt1 isoforms in different insulin-producing cell lines and primary rat islets, qRT-PCR analyses with isoform-specific primers were carried out. The 1B transporter isoform was the predominately expressed Dmt1 isoform in all insulin-producing cell lines and rat pancreatic islets, responsible for almost 100% of total Dmt1 expression (Fig. 1). In contrast to this, expression of the 1A isoform was extremely low in all investigated insulin-producing cells and accounted for <1% of the total Dmt1 expression. Regarding the +/− IRE splice variants, the expression level of +IRE variant was markedly higher than that of −IRE in both RINm5F and INS-1E cells (approximately 40–60% +IRE and 20% −IRE). However, in primary rat islets both variants were almost equally expressed (approximately 30% each, Fig. 1), which probably results from a different expression pattern of Dmt1 in non-beta islet cells.

Investigation of the neuroendocrine PC12 cell line showed a completely different Dmt1 expression profile. Although the expression of the 1B variant was also predominant in PC12 cells, the expression of the 1A transcript represented up to 40% of the total Dmt1

Figure 1

Expression of the different *Dmt1* isoforms in insulin-producing cells, primary rat islets, and PC12 cells. Total RNA was isolated 24 h after culture under control conditions from RINm5F cells, INS-1 cells, primary rat islets, and PC12 cells. The gene expression of all investigated *Dmt1* isoforms was analyzed by qRT-PCR with *Dmt1* 1A, 1B (grey bars), +IRE and -IRE

(white bars)-specific primers and with a primer pair detecting a *Dmt1* consensus sequence. The expression levels were normalized to the housekeeping gene β -actin. The gene expression of total *Dmt1* in each cell type was set as 100%. Data are mean $\pm$ s.e.m. from 6 to 14 individual experiments.

expression, a multiple of that in insulin-producing cells. The proportion of the +IRE transcript of total DMT1 expression was nearly 80% and therefore twofold higher than in islets or INS-1E cells, whereas the -IRE proportion of total DMT1 expression was <20% (Fig. 1).

iron supply, and metabolism are activated. Therefore, the effects of temporary hypoxia on *Dmt1* isoform expression were analyzed.

Incubation of RINm5F cells for 6 h at 1% O₂ had no effect on the expression of all investigated *Dmt1* isoforms or on total *Dmt1* expression (Fig. 2). Although an extension of the incubation time to 24 h resulted in a threefold induction of *Dmt1* 1A, while the expression of 1B, of +/–IRE, and of the total *Dmt1* remained unchanged. A transient hypoxic period of 24 h followed by 6 h under normoxic conditions increased *Dmt1* 1A

Effects of hypoxia on *Dmt1* gene expression in insulin-producing RINm5F cells

Under oxygen deprivation, a number of signaling pathways involved in systemic iron transport, cellular

Figure 2

Effects of hypoxia on *Dmt1* gene expression in insulin-producing RINm5F cells. RINm5F cells were incubated for up to 24 h with 1% O₂, for 24 h under hypoxic conditions followed by a 6 h normoxic incubation (24+6 h) or for 24 h under control conditions. After incubation, total RNA was isolated and the *Dmt1* isoform expression and total *Dmt1* expression was analyzed by

qRT-PCR with specific primers. The expression levels were normalized to the housekeeping gene β -actin and the expression level of each *Dmt1* isoform or the total *Dmt1* expression under control conditions was set as 100%. Data are mean $\pm$ s.e.m. from 4 to 14 individual experiments. * P <0.05, ** P <0.01 compared with the control incubation.

isoform expression approximately threefold, similar to the induction after 24 h hypoxia alone. The expression of all other investigated isoforms and the total *Dmt1* expression were ~30–50% higher after 24 h hypoxia followed by 6 h normoxic incubation, but not significantly different from the control values (Fig. 2).

Effects of pro-inflammatory cytokines on *Dmt1* gene expression in insulin-producing RINm5F cells

In earlier studies, we could show that the production of ROS, especially of hydroxyl radicals, is a central element in the cytokine-mediated destruction process of insulin-producing cells (Gurgul-Convey *et al.* 2011, Mehmeti *et al.* 2011a). Therefore, the effects of pro-inflammatory cytokines on the different *Dmt1* isoforms were investigated. Both, IL1 β and a mixture of pro-inflammatory cytokines, consisting of IL1 β , TNF α and IFN γ , caused a

fivefold induction of the 1A and a 1.5-fold induction of the 1B isoform expression after 6 h incubation. After 24 h, the IL1 β - and cytokine mixture-mediated increase in the expression of 1A isoform was already 20-fold and further increased to 40-fold after the 48 h treatment. An increase in the expression of 1B isoform was also observed, although the induction was lower than that of the 1A isoform (threefold after 24 h and fourfold after 48 h incubation; Fig. 3).

Parallel to the changes in expression of the 1B isoform, the IRE-containing mRNA variant showed a 2.5- to 3-fold increase after 6 h incubation with IL1 β alone and the cytokine mixture. However, thereafter the gene expression increase was ten- and 14-fold, respectively, after 24 and 48 h incubation with pro-inflammatory cytokines. The expression of the –IRE isoform was enhanced threefold after 6 h, sixfold after 24 h, and eightfold after 48 h incubation with both IL1 β alone and the cytokine mixture.

Figure 3

Effects of pro-inflammatory cytokines on the *Dmt1* gene expression in insulin-producing RINm5F cells. RINm5F cells were incubated for 24 h with IL1 β (600 U/ml) alone (grey bars) or a cytokine mixture (IL1 β 60 U/ml, TNF α 185 U/ml, IFN γ 14 U/ml, black bars). After incubation, total RNA was isolated and the *Dmt1* isoform expression and total *Dmt1* expression was

analyzed by qRT-PCR with specific primers. The expression levels were normalized to the housekeeping gene β -actin and the expression level of each *Dmt1* isoform or the total *Dmt1* expression under control conditions was set as 100%. Data are mean $\pm$ S.E.M. from five to six individual experiments. * P < 0.05, ** P < 0.01, *** P < 0.005 compared with the control incubation.

These changes resulted also in a significantly increased total *Dmt1* expression, by 2.5-fold after 24 h and 3.3-fold after 48 h of cytokine treatment (Fig. 3).

Effects of different pro-inflammatory cytokines on *Dmt1* gene expression in insulin-producing INS-1E cells

To investigate the role of the different pro-inflammatory cytokines in the induction of the *Dmt1* expression, INS-1E cells were incubated with the different cytokines alone or in two different combinations, namely IFN γ together with TNF α and IL1 β together with TNF α and IFN γ . The expression pattern of all investigated *Dmt1* isoforms and the total *Dmt1* expression in response to the employed cytokines were similar, although the expression levels were different (Fig. 4).

IL1 β alone or the complete cytokine mixture, containing IL1 β , TNF α , and IFN γ , caused the highest induction up to 12.5-fold for the *Dmt1* 1A isoform and 5.5-fold

for the +IRE isoform. In contrast, exposure of INS-1E cells to TNF α or IFN γ alone did not change the expression of any *Dmt1* isoform or the total *Dmt1* expression significantly. The mixture of both cytokines (TNF α and IFN γ) also did not significantly increase the *Dmt1* expression. Thus, total *Dmt1* expression was only (approximately 2.5-fold) induced by IL1 β and the complete cytokine mixture ($P < 0.05$), whereas all other cytokines had no significant effects on the overall *Dmt1* expression (Fig. 4). This indicates that the IL1 β - and the cytokine mixture-induced increase in the *Dmt1* expression is most probably NF- κ B mediated.

Effects of pro-inflammatory cytokines on the *Dmt1* protein expression in insulin-producing INS-1E cells

To show that the cytokine-induced increase in *Dmt1* gene expression results also in detectable changes in the *Dmt1* protein level, the *Dmt1* protein expression was analyzed by western blot analyses.

Figure 4

Effects of different pro-inflammatory cytokines on *Dmt1* gene expression in insulin-producing INS-1E cells. INS-1E cells were incubated for 24 h under control conditions (white bars), with IL1 β (600 U/ml), IFN γ (140 U/ml), or TNF α (1850 U/ml) alone (light grey bars), with IFN γ (14 U/ml) together with TNF α (185 U/ml, dark grey bars) or with the cytokine mixture (IL1 β 60 U/ml, TNF α 185 U/ml, IFN γ 14 U/ml, black bars). After incubation total RNA was

isolated and the *Dmt1* isoform expression and total *Dmt1* expression were analyzed by qRT-PCR with specific primers. The expression levels were normalized to the housekeeping gene β -actin and the expression level of each *Dmt1* isoform or the total *Dmt1* expression under control conditions was set as 100%. Data are mean $\pm$ s.e.m. from three individual experiments. * $P < 0.05$, ** $P < 0.01$ compared with the control incubation.

A 48-h incubation with 600 U/ml IL1 β induced Dmt1 protein expression only slightly by 15%. However, INS-1E cells exposed to the cytokine mixture showed a 33% higher Dmt1 protein expression than untreated cells ($P < 0.05$, Fig. 5). After 24 h of incubation with cytokines, quantification of Dmt1 protein expression revealed no significant changes (data not shown).

Effects of pro-inflammatory cytokines on *Ft* gene expression in insulin-producing RINm5F cells

In addition to the iron import through Dmt1, iron chelation by Ft is another important element of intracellular iron homeostasis and the prevention of iron-catalyzed production of hydroxyl radicals. Thus, the effects of pro-inflammatory cytokines on the *Ft* gene

expression were quantified by qRT-PCR after 8 and 24 h incubation.

Incubation of RINm5F cells with 600 U/ml IL1 β had no influence on the expression of *Ft-H*, *Ft-L*, and *Mtft* after 8 or 24 h incubation. But the cytokine mixture caused a slight significant increase (30%) in the expression of *Ft-H* after 8 h and a twofold increase after 24 h (Fig. 6A). For the *Ft-L* subunit after 24 h incubation only, a slight significant induction (25%) was observed with the cytokine mixture (Fig. 6B), whereas for the expression of *Mtft* no changes after incubation with IL1 β alone or the cytokine mixture were seen (Fig. 6C).

Discussion

Development of type 1 diabetes mellitus is associated with a progressive cellular dysfunction and damage, leading ultimately to β -cell death (Eizirik & Mandrup-Poulsen 2001, Novotny *et al.* 2012). As shown in previous studies, many steps in this process are mediated by ROS including also interactions with nitric oxide (Gurgul-Convey *et al.* 2011). Through its extremely low half-life and its high reactivity, the hydroxyl radical is a key element in the β -cell destruction process. A high concentration of intracellular free iron behaves as a catalyst in the Haber-Weiss reaction during the generation of hydroxyl radicals and can therefore contribute to cellular damage (Kehrer 2000, Halliwell & Gutteridge 2007). The influence of β -cell toxic cytokines on the expression balance of iron-importing and intracellular chelating proteins was therefore in the focus of the present study.

Insulin-producing cell lines and primary rat islets exhibited a highly selective expression of *Dmt1* 1B mRNA and a slight preference for the +IRE variant. After transient hypoxia, only the weakly expressed 1A isoform was induced, whereas the 1B isoform was not and the expression of both 3'-variants, +IRE and -IRE respectively, was only slightly increased. These findings are in agreement with those reported for PC12 cells (Lis *et al.* 2005) and highlight the role of regulatory sequences in the 5' regulatory region of the *Dmt1* gene as binding sites for HIF-1 α (Lee *et al.* 1998). However, due to the near absence of the expression of the 1A isoform, no significant changes in total *Dmt1* expression were detected, showing the limited adaption possibilities of insulin-producing cells to hypoxic conditions in contrast to other cell types (Carlsson & Palm 2002).

Pro-inflammatory cytokines, however, were able to induce overall *Dmt1* expression, as well as the expression of every single isoform significantly. Furthermore, after

Figure 5

Effects of pro-inflammatory cytokines on Dmt1 protein expression in insulin-producing INS-1E cells. INS-1E cells were incubated for 48 h with IL1 β (600 U/ml) alone (grey bars) or a cytokine mixture (IL1 β 60 U/ml, TNF α 185 U/ml, IFN γ 14 U/ml, black bars). After incubation protein expression of Dmt1 was quantified by western blot analysis and normalized to the housekeeping protein β -actin. Protein expression of untreated INS-1E cells was set as 100%. Data are mean $\pm$ s.e.m. from eight to ten individual experiments. * $P < 0.05$ compared with the control incubation.

Figure 6

Effects of pro-inflammatory cytokines on *Ft* gene expression in insulin-producing RINm5F cells. RINm5F cells were incubated for 24 h under control conditions (white bars) or for 8 and 24 h with IL1 β (600 U/ml) alone (grey bars) and with the cytokine mixture (IL1 β 60 U/ml, TNF α 185 U/ml, IFN γ 14 U/ml, black bars). After incubation total RNA was isolated and *Ft*-H (A), *Ft*-L (B), and *Mftt* expression (C) was analyzed by qRT-PCR with specific primers. The expression levels were normalized to the housekeeping gene β -actin and the expression level of each *Ft* subunit under control conditions was set as 100%. Data are mean $\pm$ s.e.m. from six individual experiments.

* $P < 0.05$, ** $P < 0.01$ compared with the control incubation; # $P < 0.05$, ## $P < 0.01$ compared with IL1 β incubation.

48 h also an increase in protein expression was obvious, whereas after 24 h a significant increase had not yet been observed. Although the delay in protein translation was unusually long, these findings are consistent with the observed kinetics of cytokine-induced cell toxicity in earlier experiments (Gurgul-Convey *et al.* 2011) and indicate that changes in *Dmt1* mRNA expression are followed by changes in protein expression. Though the absolute gene expression level of the 1A isoform was very low under control conditions, the massive induction after 24 and 48 h cytokine treatment could contribute to the changes in overall *Dmt1* gene and protein expression and might serve also as an additional marker for cytokine-mediated changes in gene expression in insulin-secreting cells.

Further incubations with single cytokines or with selective combinations revealed the importance of IL-1 β for the increase in the *Dmt1* expression, mediated most probably by the NF- κ B signaling pathway. With these results, we could confirm the data of Hansen *et al.* (2012), reporting that an enhanced NF- κ B transcriptional activity through increased Pdx1 activity potentiated cytokine-induced toxicity through upregulation of *Dmt1* expression. Also in other inflammatory diseases, such as rheumatoid arthritis, an accelerated cellular iron uptake has been observed (Telfer & Brock 2004).

A high iron transport capacity through an increased *Dmt1* expression does not necessarily result in enhanced oxidative stress, because an effective chelation by Ft should maintain a balanced iron homeostasis and inhibit an increase of the LIP (Harrison & Arosio 1996). However, the expression of the cytosolic *Ft* H- and L-subunits was only moderately induced after a 24 h incubation with the cytokine mixture, whereas the expression of the mitochondrially located *Ft* remained unchanged after incubation with cytokines. For the macrophage-derived U937 cell line it has been reported that TNF α and IFN γ induced the *Ft* H mRNA, whereas IL1 β showed no effect (Fahmy & Young 1993). In addition to TNF α interleukin 1 α induced also the expression of *Ft*, which is in mesenchymal cells also selective for the H-isoform (Torti *et al.* 1988). These reports indicate that the regulation of the *Ft* expression by pro-inflammatory cytokines is not unique to insulin-secreting cells, but common to many different cell types. However, in β -cells increased *Dmt1* expression was associated with a higher cell death rate under pro-inflammatory conditions and suppression of *Dmt1* expression by siRNA or chelation of iron under these conditions resulted in significant protection against β -cell toxic cytokines (Hansen *et al.* 2012), showing the

relevance of augmented iron import and the increasing Fe^{2+} -driven radical formation for the pathogenesis of type 1 diabetes mellitus.

The extremely low H_2O_2 inactivation capacity of insulin-secreting cells (for review, see Lenzen (2008)) goes along with a pronounced increase in *Dmt1* expression and even though to a somewhat lesser extent also of iron chelation by Ft in the cytosol, which may, to a certain extent, at least neutralize increased cytokine-mediated iron uptake capacity. However, such a compensation dampening ROS toxicity through increased iron binding is not possible in the mitochondria apparently, since *Mtft* expression did not increase under the influence of cytokines. This observation may help to explain why pro-inflammatory cytokine toxicity to the mitochondria in insulin-secreting cells has been found to be particularly prominent (Gurgul *et al.* 2004, Mehmeti *et al.* 2011a). Future studies will have to show whether iron uptake and chelation may qualify as protective targets for pharmacotherapy in the prevention of β -cell destruction in type 1 diabetes mellitus.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JME-13-0261>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the European Union (Collaborative Project Natural immunomodulators as novel immunotherapies for type 1 diabetes (NAIMIT) in the 7th Framework Programme, Contract No. 241447).

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Received in final form 5 March 2014

Accepted 10 March 2014