IFN- α induces a preferential long-lasting expression of MHC class I in human pancreatic beta cells

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Abstract

Aims/hypothesis IFN- α , a cytokine expressed in human islets from individuals affected by type 1 diabetes, plays a key role in the pathogenesis of diabetes by upregulating inflammation, endoplasmic reticulum (ER) stress and MHC class I overexpression, three hallmarks of islet histology in early type 1 diabetes. We tested whether expression of these mediators of beta cell loss is reversible upon IFN- α withdrawal or IFN- α pathway inhibition.

Methods IFN- α -induced MHC class I overexpression, ER stress and inflammation were evaluated by flow cytometry, immunofluorescence and real-time PCR in human EndoC- β H1 cells or human islets exposed to IFN- α with or without the presence of Janus kinase (JAK) inhibitors. Protein expression was evaluated by western blot.

Results IFN- α -induced expression of inflammatory and ER stress markers returned to baseline after 24–48 h following cytokine removal. In contrast, MHC class I overexpression at the cell surface persisted for at least 7 days. Treatment with JAK inhibitors, when added with IFN- α , prevented MHC class I overexpression, but when added 24 h after IFN- α exposure these inhibitors failed to accelerate MHC class I return to baseline.

Conclusions/interpretation IFN- α mediates a long-lasting and preferential MHC class I overexpression in human beta cells, which is not affected by the subsequent addition of JAK inhibitors. These observations suggest that IFN- α -stimulated long-lasting MHC class I expression may amplify beta cell antigen presentation during the early phase of type 1 diabetes and that IFN- α inhibitors might need to be used at very early stages of the disease to be effective.

Keywords IFN- α · JAK inhibitors · MHC class I · Pancreatic beta cells · Pancreatic islets · Type 1 diabetes

Abbreviations

BIP	Binding immunoglobulin protein	CHX	Cycloheximide
CHOP	CCAAT/enhancer-binding protein homologous protein	CXCL10	C-X-C motif chemokine ligand 10
		ER	Endoplasmic reticulum
		JAK	Janus kinase

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Research in context

What is already known about this subject?

- IFN- α , a cytokine expressed in human islets from individuals affected by type 1 diabetes, plays a key role in the pathogenesis of the disease
- IFN- α upregulates inflammation, endoplasmic reticulum (ER) stress and MHC class I overexpression, three hallmarks of islet histology in early type 1 diabetes
- IFN- α -induced MHC class I overexpression may contribute to presentation of beta cell autoantigens and amplification of the autoimmune assault

What is the key question?

- Is expression of ER stress and MHC class I overexpression reversible upon IFN- α withdrawal or IFN- α pathway inhibition?

What are the new findings?

- IFN- α -induced expression of inflammatory and ER stress markers returned to baseline after 24–48 h following cytokine removal
- In contrast, MHC class I overexpression at the cell surface persisted for at least 7 days
- Treatment with Janus kinase (JAK) inhibitors (key mediators of IFN- α signalling), when added with IFN- α , prevented MHC class I overexpression. However, when inhibitors were added 24 h after IFN- α exposure they failed to accelerate the return of MHC class I levels to baseline

How might this impact on clinical practice in the foreseeable future?

- JAK inhibitors, already used to treat other autoimmune diseases, may protect beta cells in type 1 diabetes. The present data suggest, however, that these agents must be introduced early—probably ahead of the clinical onset of disease

MX1	MX dynamin-like GTPase 1
STAT	Signal transducer and activator of transcription
TYK2	Tyrosine kinase 2

To evaluate the possibility of targeting the IFN- α pathway for the treatment of type 1 diabetes, we tested whether the effects of IFN- α are reversible upon cytokine withdrawal or IFN- α pathway inhibition by the JAK inhibitors ruxolitinib and cerdulatinib.

Introduction

The overexpression of MHC class I in pancreatic islets, probably secondary to local IFN- α production, is a key feature in the pathogenesis of type 1 diabetes [1, 2]. The primary function of MHC class I is to transport intracellular (endogenous or viral) peptides to the cell surface, where they are presented and recognised by cytotoxic CD8⁺ T cells, leading to killing of the antigen-expressing cells or, in some cases, to development of tolerance [3].

IFN- α induces MHC class I overexpression, inflammation and endoplasmic reticulum (ER) stress in human beta cells [4, 5]. These effects are mediated via activation of the Janus kinase (JAK)–tyrosine kinase 2 (TYK2)/signal transducer and activator of transcription (STAT) pathway [4], with a key role for the type 1 diabetes candidate gene *TYK2*, whose encoded protein phosphorylates and activates STATs [4, 5]. JAK/TYK2 inhibitors have been recently approved for the treatment of other autoimmune diseases, such as rheumatoid arthritis [6].

Methods

Culture of human EndoC- β H1 cells and human islets, and cell treatments The human beta cell line EndoC- β H1 (kindly provided by R. Scharfmann, University of Paris, France) was cultured as previously described [4]. These cells were free from mycoplasma infection, as evaluated by the MycoAlert Mycoplasma Detection kit (Lonza, Basel, Switzerland).

Isolation of human islets from three non-diabetic organ donors (see electronic supplementary material [ESM] Table 1) was performed in accordance with the local ethics committee in Pisa. After arrival in Brussels, islets were dispersed and cultured as previously described [4]. All experiments with EndoC- β H1 or human islet cells provide independent biological data (i.e. considering EndoC- β H1 cells from different passages or human islets from different donors as $n = 1$). Where indicated, cells were treated with human IFN- α (PeproTech, Rocky Hill, NJ, USA) 20 U/ml or 1000 U/ml [4] for 24 h and then either studied

immediately or after 1–7 days of culture following removal of the cytokine. Cells were also treated with the JAK/TYK2 inhibitors ruxolitinib (kindly provided by Calibr, La Jolla, CA, USA), cerdulatinib (Selleckchem, Munich, Germany) and Bayer-18 (Synkinase, San Diego, CA, USA), added before or after IFN- α , or cycloheximide (CHX; Sigma-Aldrich, Darmstadt, Germany) (see ESM Methods for details of study design).

Flow cytometry, immunofluorescence, immunoblot and ELISA EndoC- β H1 cells were plated in 24-well plates (150,000 cells/well). After treatment, cells were incubated with mouse anti-MHC class I antibody (W6/32) (1:1000) for 2 h and subsequently with a BV421-conjugated secondary antibody before analysis using flow cytometry (FACSCanto; BD Biosciences, San Jose, CA, USA) as previously described [4]. The cell populations were separated based on size and granularity and further analysed for BV421 fluorescence.

See ESM methods for details of western blot analysis and ELISA.

mRNA extraction and real-time PCR EndoC- β H1 cells were plated in 96-well plates (45,000 cells/well). After treatment, poly(A)⁺ mRNA was isolated using the Dynabeads mRNA DIRECT kit (Thermo Fisher, Waltham, MA, USA) and reverse transcribed as previously described [5]. Quantification by real-time PCR was carried out using SYBR Green. Gene expression values were corrected by the housekeeping gene β -actin, as its expression is not affected by the conditions used in this study [5]. The following primers were used: human β -actin, human *HLA-ABC*, human *CXCL10*, Human *MX1*, Human *CHOP* and Human *BIP*. See ESM Table 3 for details of primers.

Statistical analysis Data are expressed as means $\pm$ SEM. A significant difference between experimental conditions was

Fig. 1 IFN- α induces a specific and prolonged MHC class I overexpression in EndoC- β H1 cells. EndoC- β H1 cells were left untreated (NT) or treated with IFN- α (1000 U/ml) for 24 h. Then the culture medium was changed to remove IFN- α and the cells were cultured in the absence of IFN- α ('wash') for 24 h, 48 h, 72 h, 96 h or 7 days. MHC class I protein levels were measured by FACS. The percentage of positive cells (a) and the mean fluorescence intensity (MFI; expressed as fold-change relative to the untreated sample) (b) were quantified. Data are presented as means $\pm$ SEM of 4–18 independent measurements per condition ($n = 18$ for NT and IFN- α , and $n = 4$ –6 for the other conditions). mRNA expression of

HLA-ABC (c), *CXCL10* (d), *MX1* (f), *CHOP* (g) and *BIP* (h) were analysed by real-time PCR, normalised by β -actin and then by the highest value of each experiment considered as 1. Data are presented as means $\pm$ SEM of 3–9 independent experiments (i.e. using cells from different passages) per condition ($n = 9$ for NT and IFN- α , and $n = 3$ –5 for the other conditions). CXCL10 protein secretion in the supernatant fraction was determined by ELISA (e). Data are presented as means $\pm$ SEM of six independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs NT; † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ vs IFN- α (one-way ANOVA)

assessed by one- or two-way ANOVA followed by paired or unpaired *t* test with Bonferroni correction using GraphPad Prism 6.0 (www.graphpad.com). A $p < 0.05$ was considered to be statistically significant.

Results

IFN- α , tested at two concentrations (1000 U/ml and 20 U/ml), strongly induced the production of MHC class I (Fig. 1a–c and ESM Fig. 1a–c), the inflammatory markers chemokine C-X-C motif chemokine ligand 10 (CXCL10) and the antiviral MX dynamin-like GTPase 1 (MX1) (Fig. 1d, f and ESM Fig. 1d,e), and the ER stress markers CCAAT/enhancer-binding protein homologous protein (CHOP, also known as *DDIT3*) and the binding immunoglobulin protein (BIP, also known as *HSPA5*) (Fig. 1g, h and ESM Fig. 1f,g) in EndoC- β H1 cells. When IFN- α was removed from the medium ('wash') and the cells cultured in its absence for 24 h–7 days, MHC class I protein and, to a less extent, mRNA remained overexpressed for at least 7 days (Fig. 1a–c and ESM Fig. 1a–c), while *CXCL10* and *MX1* (Fig. 1d, f and ESM Fig. 1d,e) and *CHOP* and *BIP* (Fig. 1g, h and ESM Fig. 1f,g) expression had already started to decrease by 24–48 h. CXCL10 secretion to the medium, (ELISA) also decreased by 24 h, returning to near basal (control) levels by 72 h (Fig. 1e). IFN- α -mediated MHC class I overexpression also persisted for at least 7 days in dispersed human islets (Fig. 2).

Pretreatment with the JAK inhibitors ruxolitinib and cerdulatinib prevented IFN- α -induced *HLA-ABC*, *CXCL10*, *MX1* and *CHOP* expression in a dose-dependent manner (ESM Fig. 2a–h) and also prevented IFN- α -induced CXCL10 secretion (ESM Fig. 2m). In contrast, the TYK2 inhibitor Bayer-18 showed no effect on IFN- α -induced gene expression (ESM Fig. 2i–l) and therefore was not used further. Bayer-18 also failed to inhibit IFN- α -induced MHC class I expression in two other cell lines (HeLa and PANC-1; data not shown). This unexpected observation emphasises the need to validate the different JAK/TYK2 inhibitors in human beta cells ahead of future clinical trials. Despite their ability to prevent IFN- α signalling, ruxolitinib and cerdulatinib did not accelerate MHC class I return to baseline when added after IFN- α stimulation and its subsequent removal (ESM Fig. 3), suggesting that continuous IFN- α signalling is not necessary for the long-lasting MHC class I overexpression observed in human beta cells.

The protein synthesis inhibitor CHX significantly reduced MHC class I basal expression, while it did not affect IFN- α -induced MHC class I expression over 16 h (ESM Fig. 4a,b). After 48 h in the continuous presence of CHX, IFN- α -induced MHC class I overexpression remained unchanged and similar to non-CHX-treated cells (data not shown). These results suggest that IFN- α both induces a marked MHC class I

Fig. 2 IFN- α induces a prolonged MHC class I overexpression in dispersed human islets. Dispersed human islets were left untreated or treated with IFN- α (1000 U/ml) for 24 h. Then the culture medium was changed to remove IFN- α and the cells were cultured in the absence of IFN- α (wash) for 48 h or 7 days. Immunocytochemistry analyses of MHC class I (red), insulin (green) and Hoechst 33342 (HO; blue) were performed to establish the MHC class I content in three independent human islet preparations (magnification $\times 40$)

overexpression and stabilises the protein at the cell surface. CHX decreased β -catenin, β -actin and BIP levels over time, confirming its efficacy (ESM Fig. 4c,d).

Discussion

MHC class I overexpression is induced by cytokines such as IFN- α [2] and IFN- γ [1] in human islets from type 1 diabetes patients. IFN- α also induces human beta cell ER stress and production of chemokines [4], suggesting that this cytokine is a key player in the early stages of type 1 diabetes and in the transition between innate and adaptive immune responses. We have shown that MHC class I remains overexpressed at the cell surface of beta cells even 7 days following IFN- α withdrawal, while markers of inflammation and ER stress rapidly return to baseline. When added alongside IFN- α , JAK inhibitors prevent these effects of the cytokine. However, they fail to accelerate the return to baseline if added 24 h after IFN- α stimulation, suggesting that continuous IFN- α stimulation is not required for persistent MHC class I overexpression at the cell surface. These findings raise the possibility that IFN- α -stimulated MHC class I expression, together with other inflammatory mediators, amplifies beta cell antigen presentation in early type 1 diabetes and suggest that IFN- α inhibitors might need to be used very early in the disease to be effective. The window of opportunity for an eventual use of JAK inhibitors as therapeutic agents in type 1 diabetes will need to be

investigated further. Expression of a type I IFN response signature in circulating cells actually precedes the detection of autoantibodies in children at risk for type 1 diabetes [7, 8]. If detection of this signature becomes part of the routine follow up for at-risk children, its presence could indicate an ideal moment for intervention. Alternatively, normoglycaemic children positive for two or more autoantibodies could be considered, but it remains to be seen whether this will revert putative local manifestations of activated innate/adaptive immunity.

It is noteworthy that IFN- α not only induces a massive overexpression of MHC class I but also seems to stabilise the protein at the cell surface of human beta cells (present data). In line with this, IFN- γ induces both MHC class I expression and stability at the cell surface in murine myeloblast cells [9]. Ligand-free MHC molecules that reach the cell surface are rapidly degraded [10]. Thus, the prolonged maintenance of MHC class I expression at the beta cell surface may be due to the parallel abundance of antigenic peptides generated in response to IFN- α [11].

What is the biological significance of this prolonged IFN- α -induced expression of MHC class I mRNA and protein in human beta cells? Continuous expression of MHC class I complexes in the absence of co-stimulation might induce immune tolerance rather than immunity [12]. It is thus conceivable that, following a short local innate immune response (e.g. following brief danger signals provided by a limited viral infection or exposure to nuclear debris from dying cells), transitory IFN- α stimulation leads to prolonged MHC class I expression in the absence of parallel chemokine production or expression of costimulatory molecules, favouring the switch from immunity to immune tolerance. Another possibility is that the prolonged expression of MHC class I on the target cells enables the immune system to efficiently detect and delete beta cells expressing foreign peptides, for example, removing cells with a low level or early viral infection, thus preventing a second wave of infection. The role for the observed prolonged MHC class I expression in beta cells might be context dependent and additional studies are required to provide clarity on this issue.

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Data availability All data related to the manuscript are included in the text or in the supplementary material.

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Contribution statement ACdB and RSS contributed to the original idea and the design of the experiments, researched data, contributed to discussion, and wrote, revised, and edited the manuscript. LM, MLC, LoM and PM researched data and revised and edited the manuscript. RGM contributed to the original idea, experimental design and interpretation of the experiments, and revised and edited the manuscript. DLE contributed to the original idea and the design and interpretation of the experiments, contributed to discussion, and wrote, revised and edited the manuscript. All authors have read and approved the final version of the manuscript, and gave informed consent. ACdB and DLE are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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