Cytokine signalling in the β-cell: a dual role for IFNγ

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Abstract

IFNγ (interferon γ), a cytokine typically secreted by infiltrating immune cells in insulin in Type 1 diabetes, is by itself not detrimental to β-cells, but, together with other cytokines, such as IL-1β (interleukin 1β) and TNFα (tumour necrosis factor α), or dsRNA (double-stranded RNA), it induces β-cell apoptosis. The complex gene and protein networks that are altered by the combination of cytokines clearly point towards synergisms between these agents. IFNγ acts mostly via JAK (Janus kinase) activation, with the transcription factors STAT-1 (signal transducer and activator of transcription-1) and IRF-1 (IFNγ regulatory factor-1) playing a central role in the downstream pathway. The study of mice with a disruption of these transcription factors has revealed a possible dual role for IFNγ in β-cell destruction by cytokines or dsRNA. We demonstrated that the absence of STAT-1 from β-cells completely protects against IFNγ + IL-1β- and IFNγ + dsRNA-mediated β-cell death in vitro, whereas absence of IRF-1 does not prevent cytokine-induced β-cell apoptosis. In vivo, a lack of the IRF-1 gene in pancreatic islets even promotes low-dose streptozotocin-induced diabetes, whereas lack of STAT-1 confers resistance against β-cell death following low-dose streptozotocin-induced diabetes. Additionally, IRF-1+/− islets are more sensitive to PNF (primary islet non-function) after transplantation in spontaneously diabetic NOD (non-obese diabetic) mice, whereas STAT-1−/− islets are fully protected. Moreover, proteomic analysis of β-cells exposed to IFNγ or IFNγ + IL-1β confirms that very different pathways are activated by IFNγ alone compared with the combination. We conclude that IFNγ may play a dual role in immune-induced β-cell destruction. Transcription factors drive this dual role, with STAT-1 driving β-cell destruction and IRF-1 possibly playing a role in up-regulation of protective pathways induced by IFNγ.

Introduction

Type 1 diabetes results from an autoimmune destruction of the insulin-producing β-cells of the islets of Langerhans in the pancreas. β-Cells under attack die mainly through apoptosis. One of the actual effectors in the process of β-cell death is the CTL (cytotoxic T-lymphocyte), which is capable of killing antigen-expressing islet cells through secretion of perforin and granzyme-containing granules [1]. Interestingly, in the absence of perforin, the Fas/FasL pathway takes over and leads to β-cell death via up-regulation of islet cell Fas expression by the inflammatory cytokines IFNγ (interferon γ) and TNFα (tumour necrosis factor α) [2,3]. These cytokines are released by CTLs or nearby macrophages and bind to their corresponding receptors on the islet cells. The direct action of inflammatory cytokines is another or extra factor that triggers β-cell death. Then again, IL-1β (interleukin 1β), TNFα, IFNγ or dsRNA (double-stranded RNA) in the form of poly(I:C) alone fail to induce lysis of islet cells, but their combinations result in dysfunction and death of β-cells [4–7]. Although well studied in vitro, the precise in vivo contribution of these mechanisms is still ill defined, since redundancy of these pathways has been observed in many animal models of diabetes. On the basis of data from microarray and proteomic studies conducted in insulinoma INS-1E cells and FACS-purified rat β-cells exposed to IFNγ, in synergy with IL-1β, TNFα or poly(I:C), it has also become clear that β-cells that are under assault are not passive bystanders, but actively participate in their own destruction process [8–11]. β-Cells exposed to damaging agents trigger up- and down-regulation of a complex pattern of genes/proteins. This altered gene/protein expression profile is probably responsible for activation of apoptotic pathways, e.g. induction of Fas expression, up-regulation of MHC class I expression and secretion of chemokines and NO (nitric oxide), which eventually elicit a specific destruction of islet β-cells [9–11]. Moreover, IFNγ + IL-1β inhibits the expression of the SERCA (sarcoplasmic/endoplasmic reticulum Ca2+-ATPase) 2 pump and deplete ER (endoplasmic reticulum) Ca2+ stores in β-cells, leading to ER stress and ultimately β-cell death [12]. Taken together, these data suggest that β-cells under attack present changes in their gene/protein...
Cytokines, such as IL-1β, TNFα and IFNγ, and viral components, such as poly(I:C), are key contributors to β-cell death by apoptosis through activation of diverse networks of transcription factors, such as NF-κB, STAT-1 and IRF-3. The duration of STAT-1 function is tightly controlled by several families of negative regulators, including transacting proteins such as PTPs, SOCS, SLIM-1 and PIASs. Disruption of ER homeostasis, as induced by changes in ER Ca^{2+} concentrations, triggers accumulation of unfolded proteins and activation of a specific ER stress response. Cytokines are known to induce ER stress in β-cells via activation of IRE1α and PERK (PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase) and increased expression of CHOP (CCAAT/enhancer-binding protein)-homologous protein, as well as activation of the stress-activated protein JNK (c-jun N-terminal kinase) and caspase 12. Cytokines also modify the expression of other genes involved in the ER stress response, including up-regulation of TRAF2 (TNF-receptor-associated factor 2). On the other hand, poly(I:C) stimulates TLR3 expression and signalling in pancreatic β-cells. TLR3-generated signals activate NF-κB and co-operate with IFNγ, which is produced during the response to infection and participates in enhancing immune responses, to activate STAT-1. ATF, activating transcription factor; BiP, immunoglobulin heavy-chain-binding protein; eIF2α, eukaryotic initiation factor 2α; GAS, IFNγ-activated sequence; λBα, inhibitor of NF-κB α; IKK, λB kinase; NEMO, NF-κB essential modulator; NIK, NF-κB-inducing kinase; TANK (TRAF-associated NF-κB activator)-binding kinase 1.

Expression, rendering them more susceptible to the deadly effects of cytokines and poly(I:C).

IL-1β and TNFα exert their effects in β-cells mainly through the NF-κB (nuclear factor κB) pathway, whereas IFNγ acts mostly via the JAK (Janus kinase)/STAT (signal transducer and activator of transcription)-1 pathway [5,10,13,14]. On the other hand, poly(I:C) stimulates TLR3 (Toll-like receptor) 3 expression and signalling in pancreatic β-cells. TLR3-generated signals activate NF-κB and co-operate with IFNγ, which is produced during the response to infection and participates in enhancing immune responses, to activate STAT-1 (Figure 1) [15].

The diverse biological effects of IFNγ rely on its actions in regulating gene transcription. The transcription factor STAT-1 is a key mediator of biological responses to IFNγ. Upon triggering of the IFNγ receptor, JAKs are activated and phosphorylate STAT-1 at Tyr701. Thereafter, STAT-1 is released from the receptor, forms a homodimer and moves into the nucleus, where it binds to GASs (IFNγ-activated sequences) within the promoters of target genes [16]. As a...
result, STAT-1 modulates the expression of primary response genes, among them other transcription factors, such as IRF-1 (IFNγ regulatory factor-1). IRF-1 is strongly inducible after IFNγ stimulation and participates in the transcription of many IFNγ-regulated secondary genes whose promoters contain ISREs (IFNγ-stimulated regulatory elements) [17]. Regulation of STAT-1 activation also involves phosphorylation of Ser277 [18] and, as described more recently, SUMOylation at Lys56 [19]. It is clear that STAT-1 is needed for IFNγ signalling [20], but a role for other STAT proteins, including STAT-3 [21], is emerging as well.

The STAT activation process is transient and its duration is tightly controlled by several families of negative regulators, including transacting proteins such as PTPs (protein tyrosine phosphatases), SOCS (suppressor of cytokine signalling) and SLIM-1 (STAT-interacting LIM protein 1), as well as by phosphatases), SOCS (suppressor of cytokine signalling) and apoptosis remains established. Overall, it is clear that many of the cytokine- and virus-induced effects involved in inhibition of β-cell function and survival are regulated at the transcriptional and post-transcriptional/translational level. Novel insights into the role of IFNγ in β-cell destruction came from studies either intensifying or inhibiting IFNγ action on the β-cells. In this review, we compare the transcriptional effects of a global disruption of IFNγ signalling to those of a selective disruption of the JAK/STAT-1 or the JAK-1 pathway, on β-cell sensitivity to immune destruction in vitro and in vivo.

Inhibiting IFNγ action on the β-cell

Transgenic (Tg) mice overexpressing IFNγ have a more active inflammatory destruction of islet β-cells [24], whereas inactivation of IFNγ by specific antibodies prevents induction of autoimmune diabetes by cyclophosphamide as well as adoptive transfer of diabetes by splenocytes from spontaneously diabetic NOD (non-obese diabetic) mouse donors [25,26]. Also in the diabetes model based on LCMV (lymphocytic choriomeningitis virus) infection, IFNγ action on β-cells appears to be essential [27]. Here, the IFNγ-knockout mutation blocked insulinitis and diabetes without affecting the generation of autoreactive CTLs. Surprisingly, a targeted mutation of the IFNγ gene in NOD mice appeared to have little effect on insulitis and spontaneous diabetes [28], but IFNγ-deficient NOD mice were resistant to diabetes after transfer of spleen cells from diabetic NOD mice.

In vitro, full IFNγ receptor-knockout islets were resistant to cytokine-induced damage, as suggested by a near complete reduction in iNOS (inducible NO synthase) and MHC class I expression following 24 h of exposure to IL-1β + IFNγ compared with wild-type islets (D. Pavlovic, M. C. Chen, C. A. Gysemans, C. Mathieu and D. L. Eizirik, unpublished work), but in vivo, IFNγ receptor-mutated islets were destroyed as rapidly as wild-type islets [29]. Moreover, diabetes still appears in NOD mice genetically deficient in the β-chain of the IFNγ receptor [30], as well as in NOD mice with a dominant-negative mutant IFNγ receptor α-chain present selectively on β-cells [31]. In contrast, NOD mice with a global deficiency of the IFNγ receptor α-chain had a marked inhibition of insulitis and decreased incidence of disease [32]. In this study, defects in antigen-presenting cells or in islet β-cell targets were put forward to explain the reduced final frequency of Type 1 diabetes in NOD mice [32]. Later, it was demonstrated that autoimmune diabetes was not inhibited in NOD mice by lack of the α-chain of the IFNγ receptor, but rather by a closely linked Idd resistance allele from the 129 congenic donor strain [33].

Taken together, these observations suggest that, although IFNγ is necessary for β-cell-specific up-regulation of MHC class I [31] and iNOS [34] proteins, among others, the IFNγ response seems not to be essential for insulinis and diabetes development in NOD mice. It is conceivable that, in the absence of IFNγ signalling, the immune system utilizes other mechanisms to damage β-cells. Nevertheless, a contributing role of IFNγ in immune-attack-induced β-cell dysfunction and apoptosis remains established.

Selective inhibition of the JAK/STAT-1 signalling pathway on the β-cell

Interfering with STAT-1

We and others have suggested a key role for STAT-1 in pancreatic β-cell death induced by cytokines and viral triggers on the basis of the following observations. IFNγ treatment together with IL-1β, TNFα or poly(I:C) synergistically induced both expression and phosphorylation of STAT-1 in pancreatic islet cells; STAT-1 was also expressed in pancreatic islets from diabetic NOD mice and co-localized with apoptotic β-cells. Moreover, transfection with phosphorylation-defective STAT-1 inhibited β-cell death by IFNγ + TNFα [5,9,10]. These data, however, do not fit with the observed resistance of STAT-1-overexpressing selected INS-1 cells against cytokine-induced β-cell death [35]. Nevertheless, most findings support the idea that inhibition of the JAK/STAT-1 pathway protects β-cells against cytokine-induced cell death. Substantial insights into the role of STAT-1 in governing the biological responses of IFNγ came following the development of STAT-1−/− (full knockout) mice, harbouring a null allele for the STAT-1 gene [20]. The absence of STAT-1 from β-cells prevents IFNγ + IL-1β-induced β-cell death in vitro [36]. In addition, STAT-1−/− islets, exposed to IFNγ plus poly(I:C), a model for virus-induced cell death, are protected against β-cell death [15]. In vivo, STAT-1−/− mice are partially resistant to development of diabetes after MLDS (multiple low-dose streptozotocin) treatment [36]. Moreover, NOD mice with full STAT-1 deficiency, generated despite a critique on the purity of the knockout [37], were free from insulitis and diabetes [38].

The hypothesis that STAT-1 is a crucial transcription factor in the cytokine- and virus-mediated aspects of β-cell
destruction in vivo was strengthened by our recent observations that STAT-1−/− mice were unaffected by MLDS injections after syngeneic islet transplantation, reflecting resistance of the STAT-1−/− islets to MLDS-induced immune attack in a fully competent immune system [39]. Moreover, we demonstrated that STAT-1−/− islets were fully protected against PNF (primary islet non-function) after transplantation in spontaneously diabetic NOD mice. Despite this protection against immediate islet graft dysfunction, there was no protective effect of STAT-1 inactivation against long-term allograft destruction following transplantation into animal models of diabetes. This suggests that the early beneficial effects of STAT-1 deletion were not sufficient to prevent the late and more complex phenomena involved in islet graft destruction.

Interfering with SOCS proteins

The SOCS family of proteins has the ability to negatively regulate growth factor and cytokine signalling. SOCS-1/3 act through inhibition of the JAK/STAT-1 pathway, repressing IFN signalling, among others. Under basal conditions, endogenous SOCS expression in pancreatic islet cells is almost undetectable. In vitro, overexpression of SOCS-1 in insulin-producing cells prevents β-cell death after combined exposure to IFNγ + IL-1β [40], whereas SOCS-1−/− islets were more vulnerable to IFNγ + TNFα-induced β-cell death than wild-type islets [41]. In vivo, NOD–RIP (rat insulin promoter)–SOCS-1-Tg mice had reduced diabetes frequency, correlating with decreased IFNγ-induced STAT-1 activation in β-cells [42]. Moreover, T-cell receptor-Tg NOD8.3 mice with SOCS-1 overexpression in β-cells did not progress to diabetes [43]. It was proposed that SOCS-1 protects against diabetes by inhibiting the effector pathways used by the NOD8.3 T-cells to kill β-cells. In addition, SOCS-1−/−Tg islets had delayed islet allograft rejection, but were not protected from β-cell destruction in spontaneously diabetic NOD mice [44].

Overexpression of SOCS-3 suppressed both IFNγ and IL-1β signal pathways in β-cells [45]. On the basis of microarray analysis, it became clear that SOCS-3 prevented β-cell death upon cytokine exposure probably due to reduced NF-κB activation [46]. Multiple IL-1β-induced NF-κB-dependent apoptotic genes were found to be inhibited by SOCS-3 expression. Surprisingly, SOCS-3−/− (female) mice had a reduced β-cell volume, possibly related to decreased β-cell proliferation through inhibition of the growth hormone/prolactin signalling pathway. In spite of the reduced β-cell mass, the Tg females had enhanced glucose clearance, indicating that SOCS-3 overexpression in β-cells did not interfere with normal function of these cells [47].

Interfering with the SUMO (small ubiquitin-related modifier) family of proteins

SUMO proteins are involved in the post-translational modification of cellular proteins and regulate various cellular processes such as nuclear transport, stress responses, inflammation and the response to viruses. In 2004 Guo et al. [48] identified a fourth SUMO protein, SUMO-4, of which a single nucleotide polymorphism, SUMO-4*M55V, was strongly associated with Type 1 diabetes susceptibility. Early PCR data suggested that expression of SUMO-4 was restricted to immune tissues and kidney [48,49], but expression was also found in pancreatic islet cells [50]. Expression of the conserved SUMO-4*M55 form was found to be associated with higher stress-inducible HSF (heat-shock factor) activity in contrast to the M55V substitution of SUMO-4 [49]. Interestingly, higher HSF activity often correlates with increased anti-apoptotic heat-shock protein levels. Furthermore, Gou et al. [48] showed that SUMO-4*M55 conjugated to IκBα (inhibitor of NF-κB) negatively regulated NF-κB signalling in contrast to the M55V variant which resulted in higher NF-κB transcriptional activity [48]. Wang and She [50] reported that SUMO-4 inhibited STAT-1 DNA-binding activity by direct SUMOylation. The authors postulated that the SUMO-4*M55 substitution could lead to stronger STAT-1 activity contributing to diabetes pathogenesis [50]. To date, no data exist on the biological effects of SUMO-4 overexpression or inhibition in insulin-producing islet cells. Nevertheless, the existing findings suggest that SUMO proteins, especially SUMO-4, may be implicated in β-cell destruction in Type 1 diabetes [51].

Selective inhibition of the IRF-1 secondary signalling pathway on β-cell

In vitro data reveal that IFNγ alone or in combination with IL-1β induced an early increase in IRF-1 expression, preceding iNOS and ICE (interleukin-converting enzyme) (or caspase 1) expression, in human and rat islets and in the rat insulinoma cell line RINm5F [52,53]. Later, these observations were confirmed by microarray analysis of INS-1E cells and primary rat β-cells exposed to either IFNγ + IL-1β or IFNγ + poly(IC) [8–10]. Inhibition of IRF-1 blocked IFNγ + TNFα-induced cell death in vivo [5] and islets isolated from IRF-1−/− mice were more resistant to IFNγ + IL-1β-induced cell death than wild-type islets [54]. However, FACS-purified β-cells from IRF-1−/− mice were as sensitive as β-cells from wild-type mice to cytokine-induced cell death [54]. We postulated that other islet cell types may contribute to cytokine-induced β-cell death in whole islets. In this respect, IRF-1 is probably more important for cytokine signalling in the immune system than in pancreatic β-cells. If this is true, the observation that NOD mice deficient in IRF-1 did not develop insulinis and diabetes [55] can probably be explained by blocking immune effector cells, rather than by inhibiting IFNγ effects at the β-cell level. Surprisingly, we observed that lack of the IRF-1 gene in pancreatic islets in vivo promoted MLDS-induced diabetes and accelerated early as well as late β-cell destruction after allogeneic islet transplantation ([29] and H. Callewaert, C. A. Gysemans, M. Nelson-Holte, L. Overbergh, D. L. Eizerik and C. Mathieu, unpublished work). These data suggest that disruption of the IRF-1 gene in pancreatic islets increases susceptibility to in vivo immune β-cell destruction.
Conclusions
A dual role for IFNγ signalling in β-cell destruction is suggested, with one hand, disruption of the STAT-1-mediated signalling cascade in β-cells completely preventing cytokine- and virus-induced β-cell death in vitro [15,36] and protecting β-cells against early β-cell destruction in vivo [36,39]. On the other hand, disruption of the transcription factor IRF-1 failed to prevent cytokine-induced β-cell death in vitro [54] and even aggravated susceptibility of the islets to MLDS-induced diabetes and accelerated islet allograft destruction in vivo [29]. Although STAT-1-regulated genes have mainly a pro-apoptotic role in β-cells, genes regulated by the downstream transcription factor IRF-1 seem to have a protective role against β-cell destruction.

In addition we want to point to the very diverse mechanisms which contribute to β-cell destruction in vivo. Whereas direct β-cell killing by CTLs is probably the way in which the final blow is delivered to the β-cell, cytokine-mediated mechanisms clearly contribute to β-cell dysfunction and death. Interfering with cytokine signalling cascades may help to make less susceptible β-cells. Our data suggest, however, that, in order to protect β-cells, long-term integrated approaches targeting different pathways involved in β-cell death will be needed.

C.M. is the recipient of an FWO (Research Foundation – Flanders) clinical fellowship. H.C. is the recipient of a FWO aspirant doctoral fellowship. C.G. is the recipient of an FWO postdoctoral fellowship.

References


