The impact of anti-inflammatory cytokines on the pancreatic β-cell

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Keywords: Type 1 diabetes, interleukin-4, interleukin-10, interleukin-13, STAT3, STAT6.

Abstract

Considerable efforts have been invested to understand the mechanisms by which pro-inflammatory cytokines mediate the demise of beta-cells in type 1 diabetes but much less attention has been paid to the role of anti-inflammatory cytokines as potential cytoprotective agents in these cells. Despite this, there is increasing evidence that anti-inflammatory molecules such as interleukin (IL)-4, IL-10 and IL-13 can exert a direct influence of beta-cell function and viability and that the circulating levels of these cytokines may be reduced in type 1 diabetes. Thus, it seems possible that targeting of anti-inflammatory pathways might offer therapeutic potential in this disease. In the present review, we consider the evidence implicating IL-4, IL-10 and IL-13 as cytoprotective agents in the beta-cell and discuss the receptor components and downstream signalling pathways that mediate these effects.

Introduction

Human type 1 diabetes (T1D) is characterized by islet inflammation (“insulitis”) and the subsequent selective destruction of pancreatic β-cells. The triggering factors have yet to be elucidated in full but it is well-established that genetic predisposition plays an important role. However, the rate at which T1D is increasing among young people in many Western countries implies that genetic factors cannot be solely responsible and that an environmental trigger also exists. A number of candidates have been suggested (e.g. cow’s milk, gluten), however the strongest link implies the involvement of one or more viruses; most notably enteroviruses[1-4]. In this model, it is hypothesised that an initial (acute) viral infection of the β-cells may lead, secondarily, to the establishment of a more sustained infection in which the β-cells survive but have altered properties such that they display islet antigens inappropriately. This then promotes autoimmunity and initiates the insulitic attack[5].

Few studies have characterised the insulitic lesions fully in human pancreas but it is accepted that CD8+ cytotoxic T-cells, CD4+ T-helper (Th) cells, B-cells and macrophages are all present[6-8]. These secrete an array of pro-inflammatory cytokines and *in vitro* studies have shown that such molecules can induce apoptosis in rodent and human β-cells[9-11], thereby providing one mechanism by which β-cell

death might ensue. However, an increased generation of pro-inflammatory cytokines may not be sole factor that drives beta-cell demise since a concomitant loss of anti-inflammatory cytokine signalling could also contribute.

Anti-inflammatory cytokines are broadly antagonistic to their pro-inflammatory counterparts and are able to diminish inflammatory responses and to protect cells from otherwise cytotoxic insults. The importance of anti-inflammatory cytokines in protecting beta-cells is still open to debate although there is evidence that the production of these molecules may be reduced in type 1 diabetes[12-15]. If this is also the case within the islet milieu, then this would tend to exacerbate any detrimental effects of pro-inflammatory cytokines. In the present review, we assess the current understanding of the effects of anti-inflammatory cytokines on the pancreatic β-cell, specifically focussing on three key molecules (IL-4, IL-13 and IL-10) which have been implicated in the control of beta-cell viability. We note that other immune factors with anti-inflammatory properties may also be important in control of beta-cell function (e.g. TGF-β, IL-1ra, IL-11 and IL-35) and that the actions of some of these molecules have been reviewed elsewhere [16, 17].

Anti-inflammatory cytokines and type 1 diabetes

Anti-inflammatory cytokines are secreted by a number of immune cell subtypes including CD4+ Th2 cells, regulatory T cells, M2 macrophages, mast cells and regulatory B-cells. Many of these have been implicated as mediators of beneficial responses in the context of type 1 diabetes although most emphasis has been placed on the influence of T-helper and T-regulatory cells. For example, it is suggested that during the pathogenesis of human type 1 diabetes, a polarisation of CD4+ T-helper cells occurs, leading to a predominance of the Th1 phenotype with a concomitant down-regulation of the Th2 response[18, 19]. Under such conditions, PBMCs isolated from the blood of T1DM patients (or their first degree relatives) exhibit a reduction in anti-inflammatory cytokine secretion when compared to healthy controls[12-15]. The significance of this switch has been emphasised by the demonstration that administration of a cocktail of cytokines secreted from Th2 cells (including IL-4, IL-10 and IL-13) was protective against diabetes progression in rodents. Hence, numerous studies have revealed that treatment of NOD mice (a rodent model of type 1 diabetes) with IL-4, IL-13 or IL-10 delays the onset of

spontaneous diabetes and also reduces its incidence[20-24]. Furthermore, T-cells isolated from the blood of such mice exhibit a more Th2-like phenotype, releasing higher levels of anti-inflammatory cytokines than those of control NOD mice[22, 25]. The incidence of diabetes can also be delayed in the NOD mouse by generating animals that specifically express IL-4 in β-cells[26] or by the injection of dendritic cells which constitutively express this cytokine[27]. However, other studies have found that overexpression of IL-10 in islet endocrine cells can have little effect on diabetes progression[28], and in some cases it can accelerate the disease process[29]. This paradoxical effect may be explained by differences in factors such as the local concentration and localisation of IL-10 between the study designs[30], and highlights the complexity in the function of these cytokines in diabetes. Given the body of evidence above, it may be significant, for disease pathogenesis that *in situ* RT-PCR analysis has revealed that anti-inflammatory cytokines are expressed at only low levels in the immune cell infiltrates of four rodent models (NOD mouse, BB rat, Komeda rat, LEW.1AR1-iddm rat) and in human patients with type 1 diabetes[31].

Whilst many of their beneficial effects undoubtedly stem from the anti-inflammatory impact of the molecules on various immune cells, it is also evident that such cytokines can also exert a positive impact on the islet cells directly. Importantly, islets are a heterogeneous group of cells which contain resident immune cells, endocrine cells and possibly other cell types. Thus careful interpretation of results is required when examining data from isolated islets. That said, treatment of human islets or clonal β-cell lines with IL-4, IL-13 or IL-10 protects against a variety of cytotoxic insults[9, 32-35]. Furthermore, additional data have revealed that these cytokines can also reverse the detrimental effects of some pro-inflammatory mediators on glucose-stimulated insulin secretion (GSIS) in both clonal β-cells and primary islets[9, 36, 37]. Evidence also points towards a potential pro-proliferative role for certain anti-inflammatory cytokines on islet cells. In these experiments, adoptive transfer of various T-cell populations led to an increase in β-cell proliferation through the secretion of soluble factors including IL-10[38].Finally, anti-inflammatory molecules are reported to inhibit changes in the microvasculature associated with diabetes progression in the NOD mouse[24]. Taken together, it can be concluded from such evidence that, whilst pro-inflammatory factors are likely to be of primary importance in diabetes pathogenesis, any simultaneous reduction in the level of anti-

inflammatory cytokines may serve to exacerbate their negative effects on islet cell viability and function.

IL-4, IL-13 and IL-10

IL-4 and IL-13 are distantly related multifunctional cytokines which are encoded by genes located in a cytokine gene cluster on human chromosome 5 at region 5q31. In humans these two molecules share approximately 30% protein sequence homology and they also display clear similarities in secondary structure. IL-4 (~17 kDa) and IL-13 (~16 kDa) share a cognate cell surface receptor and they induce similar (but not identical) downstream signalling cascades. By contrast, the gene encoding IL-10 is structurally distinct and is located on human chromosome 1 at 1q31-32. It is comprised of 5 exons, and generates a protein of approximately 18 kDa. IL-10 signals via a unique set of receptor components which are not shared with IL-4 or IL-13.

In a wider functional context, IL-4, IL-10 and IL-13 are known to promote humoral immunity and to exert profound and diverse anti-inflammatory effects including inhibition of pro-inflammatory cytokine production, promotion of the differentiation of naïve T-cells towards a Th2 phenotype and increased expression of various anti-inflammatory and anti-apoptotic genes (e.g. heme oxygenase-1, Bcl-xL, Mcl-1 and survivin) in target cells. Despite these positive effects, anti-inflammatory cytokines, particularly IL-13, are also implicated in some pathological conditions. As an example, they are important mediators of allergic airway responses where they induce the production of allergy associated chemokines, mucus hypersecretion, immunoglobulin class switching to IgG and IgE, airway hyper responsiveness and fibrosis[39].

Although, as stated previously, cytokines originate most commonly from cells with an immune origin, islet endocrine cells may also be a potential source. Thus, various cytokines are synthesised and released from islet cells, including IL-1β, IL-6, IFNγ and IL-12[40-42]. The capacity of islet cells to generate anti-inflammatory cytokines has been less well-studied but there is evidence that they may be elaborated from islet cells under some circumstances. Thus, with the recent interest in the possibility of a viral trigger for type 1 diabetes, several studies have set out to identify genes which show altered expression in response to a viral challenge. Intriguingly, two studies

have reported an elevation of IL-10 mRNA and protein production from isolated human islets following infection with an enterovirus[2, 43] although, in a third study, no increase was detected[44]. Immunological evidence deposited in the human protein atlas (www.proteinatlas.org; last accessed 11/04/14) also suggests that certain islet cells stain very intensely for IL-10, implying that it may be produced within these cells. Additionally, it was found that IL-13 can also be generated in human islets and that its production is down-regulated during viral infection[43]. By contrast, human islets may not be a source of IL-4 since levels of this cytokine were below the limits of detection in two studies[2, 44]. Despite this evidence, it is not yet clear which specific cell types within the islets are responsible for cytokine release. In addition, the underlying mechanisms mediating the synthesis and release of cytokines from islet cells also require examination.Overall, these data show that, under appropriate conditions, islet cells have the capacity to synthesize, release and respond to various anti-inflammatory cytokines and this concept then opens up the possibility that under relevant conditions, anti-inflammatory cytokine signalling in islets may be regulated in an immune cell independent autocrine or paracrine manner. However, it cannot be excluded that resident immune cells within the islet might also contribute to the release of these factors. In either case, it is possible that anti-inflammatory cytokines produced from the islet may offer a level of protection in the face of local stressors under a variety of (patho)physiological conditions.

Anti-inflammatory cytokine receptor expression in islet cells

Classically, cytokines induce their biological effects by binding to cognate cell surface receptors which then initiate relevant intracellular signal transduction cascades. The receptor subunits responsible for eliciting the effects of IL-4, IL-13 and IL-10 are depicted in Fig 1. IL-4 and IL-13 are known to be capable of binding to at least one common component, although other receptor subunits have been identified which bind these cytokines individually[45, 46]. IL-4 binds to the IL-4Rα subunit, and upon cytokine binding, this molecule then recruits either the IL-13Rα1 subunit or the common γ-chain (which is also capable of forming complexes with a host of other cytokine receptors including IL-2Rα, IL-7Rα or IL-21R) to form a functional receptor. IL-13 can bind to the IL-13Rα1 subunit (which then dimerizes with IL-4Rα) or to an IL-13Rα2 monomer which has been described as a decoy receptor because of its short cytoplasmic tail (although a potential signalling role for

this receptor has recently been described[47]). IL-10, on the other hand, interacts with a completely different set of receptor components, consisting of the IL-10Rα and IL-10Rβ subunits[48]. Two molecules of each subunit are recruited to form a heterotetrameric functional receptor. Although IL-10Rα plays a unique role in IL-10 signalling, IL-10Rβ may also interact with additional receptor subunits to facilitate signal transduction induced by molecules such as IL-22, IL-26, IL-28 and IL-29[49]. Thus, there is considerable promiscuity among cytokine receptor components and the precise combinations that are assembled under any given circumstance may dictate which responses ensue.

Few studies have examined the expression of the cognate receptors for IL-13, IL-4 and IL-10 in primary islet cells and much of the current information comes from rodent islet cell lines (Table 1). Messenger RNA encoding IL-4Rα, IL-13Rα1 and the common γ-chain were detected in BRIN-BD11 cells, and expression of these receptor subunits was also reported at the protein level in these cells by ICC[33]. In a separate study, IL-4Rα and IL-13Rα1 mRNA expression were found in INS-1E cells[32] and, importantly, in the same study, both IL-4Rα and IL-13Rα1 mRNA were also detected in isolated human islets. Furthermore, IHC analysis of normal adult pancreas revealed the presence of IL-4Rα throughout the islet[34]. These data suggest that IL-4Rα is expressed in β-cells (and possibly also in other islet endocrine cells) although this still requires more complete confirmation.

IL-13Rα2 is known to be expressed in the pancreatic islets of rodents, and high fat diet treatment of these animals induced an elevation in the mRNA encoding this subunit[50]. In other systems, elevated IL-13Rα2 expression derives from stimulation with various cytokines, including TNFα[51], suggesting that it may be subject to acute regulation according to the cellular milieu. IL-13Rα2 has a higher affinity for IL-13 than does IL-13Rα1 and, as a consequence, increased expression of IL-13Rα2 might lead to reduced signalling through the IL-13Rα1/IL-4Rα receptor complex[52], and thereby dampen any anti-inflammatory effects mediated through IL-13Rα1. It might be speculated, therefore, that if an elevation of IL-13Rα2 occurred in the islet beta-cells in diabetes, this could increase their vulnerability to apoptosis; a possibility that warrants further investigation.

IL-10Rβ expression has been studied recently in human islets in the context of IFNλ (IL-28) production by the endocrine cells in response to Coxsackievirus infection[53] since this component can also form part of the IL-28 receptor. Healthy human pancreatic islets were shown to express IL-10Rβ at the mRNA level and further investigation by IHC revealed that this receptor subunit is present in both α- and β-cells, but absent from δ-cells[38]. We have studied the expression of IL-10 receptor subunits in INS-1E cells and in isolated human islets by RT-PCR (Fig 2) and have confirmed that IL-10Rα and -β are each expressed in human islets, although we were unable to detect IL-10Rα in INS-1E cells. This might imply that IL-10Rα is not found in beta-cells or, more cautiously, it could simply mean that INS-1E cells are not an ideal model in which to study IL-10 signalling. Further experiments are required to firmly establish the pattern of IL-10Rα expression in rodent and human islet cells.

A number of naturally occurring polymorphisms have been identified within the genes encoding IL-4, IL-13 and IL-10, their respective receptor subunits as well as in components of their downstream signalling cascades. Many of these have been associated with allergic or inflammatory disorders[54], although certain studies have also linked SNPs in some of the genes (or specific inherited haplotypes) to diabetes risk. Indeed, a number of relatively small-sized studies have reported an association between SNPs within IL-4R, IL-4 and IL-13 and type 1 diabetes risk[55-57]. However, others have not confirmed this association[58], and one larger study revealed an association between IL-4R SNPs and T1D in only a single cohort among the type 1 diabetes genetics consortium collection[59]. In this case, the authors mooted the idea that their own association data and those obtained elsewhere might be false positives. Nevertheless, they did not go as far as to draw the firm conclusion that SNPs within the IL-4R are not associated with T1D susceptibility. Genes encoding the IL-10 receptor components have not been linked to diabetes risk, however recent data has linked a SNP in the promoter of the IL-10 gene itself to risk of type 2 diabetes or to gestational diabetes[60].

Signal transduction pathways activated by anti-inflammatory cytokines in islet cells

The canonical intracellular signalling pathways deriving from treatment of cells with IL-13, IL-4 or IL-10 have been reviewed elsewhere[45, 46, 48, 61], and will not be described in detail here. Briefly, however, it is thought that binding of IL-13, IL-4 or

IL-10 to their cognate receptors leads to the formation of functional protein complexes in the plasma membrane. Members of the Janus kinase (Jak) family of proteins are associated with each receptor constitutively and these become trans-phosphorylated (and activated) in response to formation of the active complex. Active Jaks then phosphorylate key tyrosine residues on the cytoplasmic tail of the cytokine receptor with which they are associated. Signal transducer and activator of transcription (STAT) family monomers are recruited to the phosphorylated residues by virtue of an interaction with their C-terminal SH2 domains. Classically, IL-4 or IL-13 are considered to recruit STAT6, whereas IL-10 usually signals via STAT3.

Once bound to the receptor, the STAT protein is, itself, phosphorylated on target tyrosine residues by activated Jak, thereby facilitating the disengagement of STATs from the receptor. Upon release, STAT molecules dimerise (and/or possibly form larger oligomeric complexes) with other phosphorylated STATs and translocate to the nucleus where they bind to consensus sequences present within defined target genes to promote transcription.

In addition to activation of the Jak/STATs, other pathways have also been implicated in cytokine receptor signalling, with the best-studied of these being the PI-3K/Akt pathway. In this case, IRS1 or IRS2 is recruited to the tyrosine phosphorylated cytoplasmic tail of the cytokine receptor where it becomes phosphorylated by Jak. The phosphorylated IRS protein then binds to the regulatory p85 subunit of PI-3K which liberates the catalytic p110 subunit to catalyse the conversion of phosphatidylinositol (4,5) bisphosphate (PIP_2) to phosphatidylinositol (3,4,5) trisphosphate (PIP_3). Serine/threonine kinases, such as PDK and Akt, are then recruited to the newly formed PIP_3 molecules via their PH domains and this association leads to an initial threonine phosphorylation of Akt by PDK, which precedes full Akt activation and the initiation of subsequent downstream signalling events[62]. Additional pathways activated in response to IL-4 or IL-13 include MEK/ERK1/2, Akt and the induction of TGF-β via the IL-13Ra2-dependent stimulation of AP-1; however these have not yet been examined in β-cells[45, 47].

In pancreatic β-cells the downstream signalling events that follow from anti-inflammatory cytokine receptor activation have received only limited investigation and IL-10 signalling is still largely unexplored. In BRIN-BD11 cells, IL-4 treatment

increased the phosphorylation of STAT6 and Akt[34]. Similarly, IL-13 was shown to increase the levels of pJak2, pSTAT3 and pSTAT6[32]. Importantly, in the same report, elevations in pSTAT3 and pSTAT6 were also confirmed in IL-13 treated human islets. Deployment of a pan-Jak inhibitor (pyridine 6) dose-dependently reduced the IL-13 mediated phosphorylation of STAT6 and STAT3 in rodent beta-cells, confirming that these effects are Jak dependent[32]. Surprisingly, however, the complement of Jaks expressed in β-cells has not been established, and it is not yet clear which Jak isoform(s) is responsible for mediating these effects, although Jak2 may play a role[32]. Taken together, these data obtained in islet cells are consistent with the canonical model of IL-4/13 signalling seen in other cell types[46]. Fig 3 illustrates the current understanding of IL-4/13 signalling in pancreatic β-cells based on the knowledge gained from such studies.

One intriguing observation was the (very unexpected) detection of three separate protein bands on Western blots when INS-1E cell lysates were probed with antisera directed against pSTAT6[32] following exposure to anti-inflammatory cytokines. One of these migrated at the expected molecular weight of native pSTAT6 (105kDa) whereas the two additional bands ran more slowly. None was present in lysates recovered from unstimulated cells but all three were induced in response to either IL-4 or IL-13. A similar pattern was observed in BRIN-BD11 cells and in the human beta-cell line, 1.1B4 upon exposure to IL-13, whereas only a single band was found in extracts of human islets exposed to IL-13. The precise identity of the slowly-migrating (higher molecular weight) bands seen in the cell lines has not been established but they do not appear to represent any of the previously described STAT6 isoforms since these are of lower molecular weight than the native protein[63]. It seems probable that the novel bands must all contain a residue equivalent to the Tyr at position 641 in native STAT6 since this forms the target site for Jak-mediated phosphorylation and the phospho-specific antiserum employed in the studies detects an epitope which encompasses phospho-Tyr641. Conceivably, the variants may represent forms of STAT6 that are post-translationally modified, leading to their altered migration characteristics on SDS gels. In support of this, STAT6 is known to be undergo a range of post-translational modifications, including phosphorylation (serine and tyrosine), methylation, acetylation, sumoylation and *O*-linked *N*-acetylglucosaminylation[63]. Many of these modifications have significant effects on

STAT6 function[63] and they may contribute to differences in the overall profile of biological effects elicited but it remains unclear why and how these unusual forms of STAT6 are generated in cytokine-treated beta-cells.

Cytokine signalling can be negatively regulated by a number of molecules including members of the suppressor of cytokine signalling (SOCS) family and various tyrosine phosphatases (PTPs). A number of PTPs, including PTP-BL and PTPN2 (TCPTP), are reportedly expressed in rodent β-cells[64, 65] and STAT6 has been identified among their substrates[66, 67]. In INS-1E cells, over-expression of PTP-BL abrogated the phosphorylation of STAT6 after IL-4 treatment[34]. SNPs associated with the gene encoding PTPN2 have been identified as pre-disposing to Type 1 diabetes[68], and knockdown of PTPN2 in INS-1 cells enhances the toxicity of pro-inflammatory cytokines[69]. These data highlight the probable importance of the Jak/STAT pathway in the pathogenesis of diabetes and emphasise the potential involvement of anti-inflammatory cytokines.

The culmination of downstream signalling events in cells exposed to IL-4, IL-13 and IL-10 is a change in target gene expression. Whilst this change often results in an increased expression of the target gene, STAT-dependent negative regulation of some genes has also been described[70], Examples of reportedly STAT6 responsive genes include those encoding proteins involved in regulation of cell viability (such as Bcl-xL or sIL-1ra) as well as receptor components driving cytokine responses, such as IL-4Rα and IL-13Rα2[70-72]. By contrast, STAT3-induced genes include c-Myc, c-Fos and SOCS3[73, 74]. The expression of STAT3- and STAT6-responsive genes in β-cells following anti-inflammatory cytokine treatment remains almost completely unexplored, and the understanding of which genes (and their products) are altered in response to these molecules will provide important mechanistic insights into their functions in the pancreatic β-cell.

Impact of anti-inflammatory cytokines on islet cell viability and function

As outlined above, exposure of cells to anti-inflammatory cytokines can elevate the expression of anti-apoptotic genes, and this response might contribute to cytoprotection. In this context, several studies have revealed that human islets and rodent clonal β-cells are less vulnerable to a range of cytotoxic stimuli following exposure to IL-13, IL-4 and IL-10 either alone or in combination[9, 32, 33, 35]. The data

confirm that the protective response is dose-dependent[32, 33] and often, but not always, a cumulative effect is observed when more than a single cytokine is administered[35]. Mechanistically, studies from our laboratory have indicated that the Jak/STAT6 pathway may be an important mediator of the cytoprotective response since pharmacological inhibition of Jak2, Jak3 or all Jak family members, diminishes the protective effect of IL4 or IL-13[32, 34]. Additional evidence derives from studies with an INS-1E cell line conditionally expressing PTP-BL, a phosphatase which antagonises STAT6 phosphorylation[66]. Over-expression of PTP-BL not only reduced pSTAT6 levels in cells exposed to IL-4, but it also attenuated the improvement in mitochondrial metabolism (as measured by MTS assay) associated with cytokine exposure[34]. In addition, induction of PTP-BL antagonised the improved viability of serum starved cells caused by IL-13 (serum deprived cells: 37.1±1.1% cell death, IL-13 (20ng/ml): 27.2±0.5%, IL-13 + PTP-BL: 33.1±1.7%; $p<0.01$ (calculated by Student's *t*-test) vs IL-13 treatment alone n=5). These data are consistent with the involvement of STAT6 in mediating the responses to IL-4 and IL-13 although it should also be noted that other STAT isoforms are dephosphorylated by PTP-BL[66]. This may be important since, for example, STAT3 also becomes phosphorylated in response to IL-13 in beta-cells[32]. However, we consider it unlikely that STAT3 contributes directly to the cytoprotective actions of IL-13 for two principal reasons. Firstly, both IL-13 and a different cytokine, IL-6, cause a similar transient elevation in pSTAT3 levels in beta-cells, but they exert entirely opposite effects on cell viability. Thus while IL-13 is cytoprotective, IL-6 enhances toxicity. Furthermore, using a STAT3 specific reporter, it was shown that IL-13 fails to promote STAT3 mediated transcription in INS-1E cells whereas this was strongly induced by IL-6. Thus, it seems likely that cytokine-induced STAT6 activation is responsible for mediating cytoprotection in beta-cells.

Whilst, as has already been intimated, the gene targets of STAT6 remain unknown in the β-cell, various groups have independently verified that sIL-1ra is regulated in a STAT6 dependent manner [72, 75, 76]. sIL-1ra, rather than inducing downstream signalling cascades, elicits its anti-inflammatory effects by interacting directly with the interleukin-1 receptor thereby blocking IL-1β binding. Considering the recent finding that sIL-1ra can be released directly from islet cells[77], it is an intriguing possibility that

STAT6 may regulate the secretion of IL-1ra from the islet which could contribute to the cytoprotection offered by IL-4 and IL-13 in the presence of IL-1β.

It was noted above that the PI-3K/Akt pathway is activated in response to IL-13 and IL-4 in islet cells and, intriguingly, inhibition of this pathway with wortmannin exerted a differential effect on the protection offered by IL-4[34] and IL-13[32]. IL-4 induced responses were sensitive to wortmannin whereas those mediated by IL-13 were not. These results imply that there may be important differences in the signalling pathways induced by these cytokines. This could reflect the fact that, in the receptor complex, IL-4Rα can interact with either IL-13Rα or the common γ-chain, thereby leaving open the possibility that differential signalling might ensue when IL-4 or IL-13 is present. This differential effect of the two cytokines has also been described elsewhere, particularly in the context of asthma[78].

One mechanism by which anti-inflammatory cytokines might offer cytoprotection to pancreatic β-cells is by reducing the level of oxidative and/or nitrosative stress. It is known that human islet cells are susceptible to oxidative stress and that, in particular, β-cells express many of the key anti-oxidant enzymes (e.g. glutathione peroxidase and catalase) at only very low levels[79, 80]. This is significant because pro-inflammatory cytokines, especially IL-1β, promote the expression of iNOS and thereby increase the local production of nitric oxide (NO). Importantly, treatment of clonal β-cells with IL-13, IL-4 or IL-10 reduced nitrite accumulation (an index of NO production) during exposure to IL-1β[32]. However, while this is suggestive of a possible mechanism of cytoprotection, examination of the wider literature yields a more equivocal picture. Hence, while one study reported that IL-4 inhibited IL-1β induced NO production in rat islets[81], this was not seen in separate work employing IL-10[36] despite the fact that IL-10 reduced basal NO accumulation[36]. Importantly, all three anti-inflammatory cytokines reduced iNOS expression in RINm5F cells[35]. By contrast, IL-10 or IL-4 treatment had no effect on the mRNA expression of iNOS or other antioxidant genes in a further report[24] although, somewhat surprisingly, eNOS levels were enhanced by IL-10 treatment[24]. Studies in other systems have shown that anti-inflammatory cytokines such as IL-4 or IL-10 can impede NFκB activation under certain circumstances, possibly through IκB stabilisation[82-84] and, if this happens in beta-cells, then this might account for some of the responses observed.

Finally, one recent study has revealed that pro-inflammatory cytokines reduced the expression of peroxiredoxin 6 in clonal β-cells, thereby rendering these cells more vulnerable to oxidative stress[85]. IL-4 reversed this effect.

Taken together (and allowing for the fact that not all data are fully concordant) it can be argued that anti-inflammatory cytokines are likely to inhibit IL-1β induced NO production upstream of iNOS expression in β-cells; conceivably by modulation of NFκB activation. The resultant dampening of nitrosative (and possibly oxidative) stress is then beneficial. However, this mechanism is unlikely to explain completely the effects of anti-inflammatory cytokines because these molecules are protective against a range of cytotoxic insults (e.g. serum deprivation or palmitate treatment), not all of which are highly dependent on the generation of oxidative or nitrosative stress for their deleterious effects. Rather, we speculate that IL-4, IL-10 and IL-13 may induce a more general 'cytoprotective phenotype' by influencing a distal step in the control of viability.

Effects of anti-inflammatory cytokines on beta-cell function

While it is well established that pro-inflammatory cytokines, particularly IL-1β, can inhibit glucose stimulated insulin secretion (GSIS), the impact of anti-inflammatory cytokines on β-cell function has received much less attention. Thus, there are few data addressing the direct impact of anti-inflammatory cytokines on GSIS, although a number of studies have revealed that these molecules reverse the inhibitory effects of pro-inflammatory factors on insulin secretion from clonal β-cell[37], rat islets[36] and human islets[9]. Furthermore, one study which correlated β-cell function with the levels of cytokines released from isolated human PBMCs, found that IL-10, IL-13 and IL-4 were all positively correlated with glucagon-stimulated C-peptide levels, used as a proxy of β-cell function[86]. The mechanism that underlies this effect has not been addressed, although it may reflect the modulation of IL-1β signalling as previously described in the context of NO generation. The effect of anti-inflammatory cytokines on the secretion of hormones from other endocrine islet cells has not been examined. However, it is worth highlighting that IL-6 enhances both insulin and glucagon secretion[87, 88] and that IL-6 can signal via STAT3. Conceivably, therefore, other cytokines which promote STAT3 phosphorylation (such as IL-10) might also influence hormone secretion in similar ways.

Therapeutic potential of targeting anti-inflammatory cytokine signalling pathways in type 1 diabetes

Despite the evidence outlined in this review, the potential of anti-inflammatory cytokines and their signalling mechanisms as therapeutic targets in type 1 diabetes has received surprisingly little attention. This may be because the most strenuous efforts are being invested to understand the role played by pro-inflammatory mediators. Nevertheless, data from the NOD mouse have shown that the adoptive transfer of immunomodulatory cells preferentially secreting anti-inflammatory cytokines (e.g. M2 macrophages, regulatory T-cells), leads to a reduction in diabetes incidence[89, 90]. In addition, there are also data which imply that manipulation of anti-inflammatory cytokine signalling could be therapeutically effective. Among these are results revealing that helminth infections dramatically attenuate the progression of type 1 diabetes (at least in NOD mice)[91, 92]. Superficially, this seems an idiosyncratic observation but the importance may lie in the fact that such infections cause fundamental changes in the secretion of anti-inflammatory cytokines from circulating immune cells which may then impact on disease progression at the islet level. Thus, by study of such phenomena it might be possible to develop still more targeted approaches in which the actions of anti-inflammatory cytokines could be harnessed to combat the progression of disease.

Acknowledgements

Work in the authors' laboratory is funded by Diabetes UK (12/0004505), the European Union's Seventh Framework Programme PEVNET (FP7/2007-2013) under grant agreement number 261441 and with the support of the Network for Pancreatic Organ Donors with Diabetes (nPOD), a collaborative type 1 diabetes research project sponsored by the JDRF, and with a JDRF research grant awarded to the nPOD-V Consortium. Organ procurement organisations partnering with nPOD to provide research resources are listed at www.jdrfnpod.org/our-partners.php.

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Figure Legends

Fig 1. Canonical signal transduction via IL-4, IL-13 and IL-10 receptors. IL-4 interacts with either the IL-4Rα/common γ-chain or the IL-4Rα/IL-13α1 receptor complexes, whereas IL-13 binds to an IL-4Rα/IL-13α1 dimer or to an IL-13α2 monomer. IL-10 signals via IL-10Rα/IL-10Rβ. IL-4 and IL-13 are known to preferentially induce STAT6 signalling while IL-10 responses are usually mediated via STAT3.

Fig 2. IL-10 receptor components are expressed in pancreatic endocrine cells. The expression of IL-10Rα and IL-10Rβ was examined by RT-PCR in cDNA generated from human islets and INS-1E cells. Amplified products were separated on agarose gels and examined under UV illumination after staining with Gel Red. Arrows indicate the position of the 300bp marker. To confirm their identity, bands were extracted and sequenced. Primer pairs used for PCR analysis were: IL-10Rα (human) Fd: ATGACCTTACCGCAGTGACC Rv: TCCAGAGGTTAGGAGGCTGA, IL-10Rβ (human) Fd: CTCGGCTGCTTCGCCTTGCT Rv: CTAGCTTTGGGGCCCCTGCC, IL-10Rα (rat) Fd: CCTGCATGGCAGCACCGACA Rv: ACAACCATGGCCCAAGGCGG, IL-10Rβ (rat) Fd: CCCTCCCTGGATCGTGGCCA Rv: AGCTCCTGAGGCCCTGCCTC.

Fig 3. Possible pathways of IL-4 and IL-13 signalling in pancreatic β-cells. Following interaction of IL-4 with its cognate receptors, Jak kinases are phosphorylated. This leads to the recruitment and activation of STAT6 and to activation of the PI-3K/Akt pathway. IL-13 also activates both the STAT6 and PI-3K/Akt pathways but there is evidence that STAT3 may also become phosphorylated. IL-4 and IL-13 induced cytoprotection is probably mediated via STAT6 and the extent of phosphorylation of this molecule is also regulated by PTP-BL. The functional consequences of IL-13-induced STAT3 phosphorylation are unclear. In addition, the increase in Akt phosphorylation mediated by IL-13 is not obligatory for cytoprotection.

Tables:

Receptor Subunit	Cell/Tissue	Techniques
IL-4Rα	Human islets	RT-PCR, IHC (26, 27)
	INS-1E cells	RT-PCR (26)
	BRIN-BD11 cells	RT-PCR, ICC (27)
IL-13Rα1	Human islets	RT-PCR (26)
	INS-1E cells	RT-PCR (26)
	BRIN-BD11 cells	RT-PCR, ICC (27)
IL-13Rα2	Rodent islets	qPCR (43)
IL-10Rα	Human Islets	RT-PCR (Fig 2)
IL-10Rβ	Human α and β cells	IHC (46)
	Human islets	RT-PCR (Fig 2, 46)
	INS-1E cells	RT-PCR (Fig 2)

Table 1: Anti-inflammatory receptor component expression in primary islet cells and islet cell lines.