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Novel Experimental Strategies to Prevent the Development of Type 1 Diabetes Mellitus

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ABSTRACT

Type 1 diabetes is an autoimmune disease leading to extensive destruction of the pancreatic β-cells. Our research focusses on the role of β-cells during the course of the disease, aiming at finding novel strategies to enhance β-cell resistance against the cytotoxic damage inflicted by the immune system. Special attention has been paid to the possibility that cytokines released by the immune cells infiltrating the pancreatic islets can directly suppress and kill β-cells. Certain cytokines (interleukin-1β, tumor necrosis factor-α and interferon-γ) either alone or in combination, are able to activate signal transduction pathways in β-cells leading to transcription factor activation and de novo gene expression. In this context, it has been found that induction of inducible nitric oxide synthase mediates an elevated production of nitric oxide, which impairs mitochondrial function and causes DNA damage eventually leading to apoptosis and necrosis. However, other induced proteins SUCH AS heat shock protein 70 and superoxide dismutase may reflect a defense reaction elicited in the β-cells by the cytokines. Our strategy is to further seek for proteins involved in both destruction and protection of β-cells. Based on this knowledge, we plan to apply gene therapeutic approaches to increase expression of protective genes in β-cells. If this is feasible we will then evaluate the function and survival of such modified β-cells in animal models of type 1 diabetes such as the NOD mouse. The long-term goal for this research line is to find novel approaches to influence β-cell resistance in humans at risk of developing type 1 diabetes.

Abbreviations: ASL; Argininosuccinate lyase, **ASS**; Argininosuccinate synthetase, **COX**; cyclooxygenase, **FACS;** fluorescence activated cell sorter, **GLUT 2**; Glucose transporter 2, **hsp70**; heat shock protein 70, **IFN-γ**; Interferon-γ; **IL-1β**; Interleukin-1β, **IL-1 ra**; Interleukin-1 receptor antagonist, **NF-κB**; Nuclear factor kappa B; **NO**; Nitric oxide, **eNOS/NOS 3**; endothelial nitric oxide synthase, **iNOS/NOS 2**; inducible nitric oxide synthase, **NOD mouse;** nonobese diabetic mouse, **nNOS/NOS 1**; neuronal nitric oxide synthase; **TGF-β**;

Transforming growth factor-β, **TNF-α;** Tumor necrosis factor-α, **PC2**; Proinsulin converting enzyme 2, **PDX-1**; Pancreatic and duodenal homeobox gene-1, **SOD**; Superoxide dismutase, **y^+CAT**; Cationic amino acid transporter

BACKGROUND

Despite extensive worldwide research efforts the pathogenesis of human type 1 diabetes is not fully understood. At present the prevailing view is that an autoimmune reaction causes destruction of the insulin-producing β-cells in the pancreas. Over the last three decades it has been proposed that viruses, specific toxins, autoreactive antibodies, macrophages, T-cells, cytokines, nitric oxide and free oxygen radicals mediate the destruction of β-cells. Based on a number of reports from Europe and NorthAmerica, it can be estimated that if a sibling has developed type 1 diabetes, the risk to get the disease for another sibling is 25–35% for a monozygotic twin, ≈10–15% for a dizygotic twin and ≈5% for a non-HLA-identical sibling (5,58, 68,71,84). In the whole Swedish population the risk is ≈0.3%. Thus there is a clear genetic risk with a major influence by genes within the HLA, and a less marked influence by several genes outside the HLA region. Another conclusion is that one or several environmental factors contribute to the susceptibility for the disease. In this context it is of great interest that the highest incidence of type 1 diabetes is found in Scandinavia. In the age group 0–14 years a recent report postulated for the year 2010 an increased incidence of type 1 diabetes to 50.2 and 32.2 new cases/ 100 000/year in Finland and Sweden, respectively (66). The corresponding figures for Japan and Peru, low incidence countries, are 4.1 and 1.3.

OBJECTIVE AND SIGNIFICANCE

The objective of the present research project is to investigate cellular and molecular mechanisms involved in pancreatic β-cell damage and repair in type 1 diabetes. We anticipate that a deeper knowledge of these issues will lead to new strategies for intervention in the autoimmune β-cell destructive process in type 1 diabetes, as well as methods to enhance β-cell resistance against cytotoxic damage. From a number of experiments performed on β-cells exposed to cytotoxic agents and cytokines in vitro, and β-cells retrieved from prediabetic NOD mice, together with clinical observations on variations in islet cell autoantibody titers and β-cell function in humans, we have postulated that after certain types of damage, β-cell function can be restored (25,26,28,86,87,94). Moreover, we believe that the β-cell is not a passive victim during a situation of potentially harmful exposure, but depending on gene expression and functional activity of the β-cell, the outcome can be influenced (Fig. 1).

We hope to be able to differentiate between the pathophysiological role of β-cell necrosis and apoptosis in type 1 diabetes. By studying cytokine-induced cell signaling and the mechanisms leading to β-cell apoptosis/necrosis, we will elucidate which factors that are crucial for β-cell survival and possibly indentify candidate

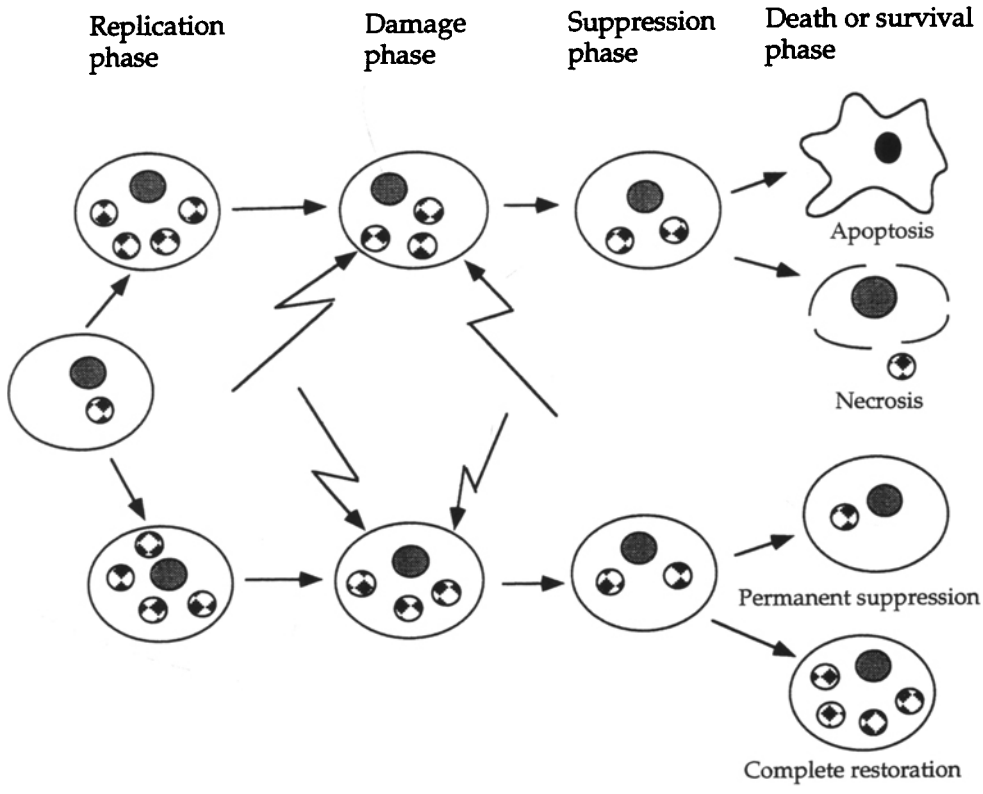

Fig. 1. Schematic view of the β-cell outcome following different immunologic or toxic assaults. In fetal and neonatal life, β-cell replication is increased, but later it becomes much restricted. Soon after birth β-cells acquire the full capacity to synthesise and release insulin (speckled symbols) upon appropriate stimuli. At one or several occasions in life, β-cells in some individuals are subject to damage (irregular arrows) which will lead to suppressed β-cell function and a reduction in insulin secretion. Depending on the genetic predisposition an autoimmune reaction will be launched which in certain individuals will cause extensive cell death leading to type 1 diabetes. In other individuals β-cells will survive, but their secretory function is impaired, which may have consequences for the glucose homeostasis. In some other individuals the β-cells may completely recover and the glucose tolerance will only be transiently disturbed. The latter outcome is most likely also dependent on genes regulating β-cell resistance to damage and β-cell repair.

genes conferring β-cell susceptibility or resistance to destruction in type 1 diabetes. Finally, by adapting newly developed gene transfer techniques for β-cells, in vivo gene therapy trials aiming at rescuing β-cells from destruction can be envisaged.

Early findings suggesting cytokine-induced inhibtion of pancreatic islet β-cells

In 1984 Schwizer et al. reported that coculture of macrophages and isolated mouse pancreatic islets caused inhibition of β-cell function and cell lysis (82). Soon there-

after Mandrup-Poulsen and colleagues demonstrated that addition of supernatants from human monocyte cultures to rat islet cultures induced both functional and structural damage (62). In a subsequent study they suggested that the inhibitory factor in the supernatants was mainly interleukin-1β (IL-1β) (7). In a following series of investigations it was found that the inhibitory action of IL-1β was related to a mitochondrial perturbation and inhibition of the oxidative metabolism (80,81). Moreover, other macrophage-derived cytokines were found to potentiate the actions of IL-1 (22,61,72). Finally, it was found that the inhibitory action of IL-1β could be reversed if the cytokine exposure was discontinued and the β-cells allowed to recover (15,28). The findings referred to above have attracted much interest, since they suggest that cytokines released by immune cells in the vicinity of pancreatic islets might cause β-cell inhibition and cell death, a phenomenon which could be of relevance in the pathogenesis of type 1 diabetes.

Nitric oxide producing enzymes

Nitric oxide (NO) is a small gaseous radical produced by nitric oxide synthases (NOSs). The radical has widespread actions, ranging from the control of blood flow and memory formation in the brain, to being an important factor in the hosts defense against microbial infections (100). There are three mammalian isoforms of NOSs (37,38,89). Two isoforms are constitutively expressed and synthesize small amounts of NO. The nNOS/NOS1 isoform is preferentially expressed in neurons, whereas eNOS/NOS3 isoform is mainly found in endothelial cells. The third isoform is not expressed by cells during normal conditions, but will become so in a number of different cell types in response to pro-inflammatory cytokines and bacterial lipopolysaccaride in vitro. This isoform has also been found to be expressed during inflammatory reactions and microbial infections, and it will produce large amounts of NO. Due to its lack of tissue specific expression and its inducibility this isoform has been denoted inducible NOS, or iNOS/NOS2.

NOSs utilize the amino acid L-arginine and several cofactors to yield NO and citrulline (37,38,89). The generated NO is highly reactive, and will react preferentially with molecules containing an un-paired electron such as iron, oxygen, nitrogen and sulphur. Indeed, it is through this mechanism the radical exerts its bioregulatory and cytotoxic actions.

The production of NO by iNOS depends both on the level of expression of the iNOS gene and on the stability of the iNOS mRNA, which will determine how much iNOS protein is being translated (37,38,65). The human, rat and mouse promoters have all been cloned and sequenced. In all species, the 5' untranslated region contains a multitude of consensus sequences for the potential binding of different transcription factors. The cellular access to the substrate L-arginine and the necessary cofactors will also determine the amount of NO produced.

Given an adequate supply of cofactors, arginine availability will be rate limiting for NO production in iNOS expressing cells (65,101). Mammalian cells use a number of different transporters for uptake of L-arginine from the extracellular space, with the members of the cationic amino acid transporter system y^+CAT's being the

most important (60,101). In some cell types L-arginine can also be intracellularly recycled from citrulline, one of the end products in the NOS catalyzed reaction, through an intracellular biosynthetic pathway denoted the citrulline-NO cycle. In this cycle two enzymes, argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL), contribute to the conversion of citrulline into L-arginine which then can be utilized as substrate for NO generation by the NOSs (101).

Generation of NO from iNOS in islet cells

Ten years ago, NO was proposed to be a mediator of cytokine-induced β-cell destruction. Southern et al. reported that NO was responsible for the inhibition of cellular functions in rat β-cells exposed to the cytokines interleukin-1 and tumor necrosis factor-α (83). The studies have been confirmed and extended by our group and others (17,96) and it is now generally acknowledged that NO, produced endogenously by a cytokine-exposed β-cell or given exogenously through NO-donors, is detrimental to rodent pancreatic β-cells and can cause cell death (23). Large amounts of NO can also be deleterious to human islets and β-cells, although these cells appear to be less susceptible to the radical than rodent islets and β-cells. Moreover, non-NO dependent mechanisms account for the majority of cytokine-induced damage in human β-cell (21,27,75).

Human and rodent pancreatic β-cells express iNOS and produce NO in response to cytokines in vitro. In addition, iNOS has been found to be expressed by β-cells and activated macrophages, localized in the pancreatic islets during the pre-diabetic insulitis in animals developing the disease (23,73,76). We and others have made the interesting finding that whereas rodent β-cells will express iNOS mRNA upon exposure to IL-1 as a single stimuli, human β-cells must be exposed to combinations of cytokines, such as IL-1 + IFN-γ, to express the iNOS gene (18,27). It is unclear why there is a difference between rodent and human cells, but the requirement for transcription factors activating the iNOS promoter in pancreatic β-cells may differ between humans and rodents. We have observed that the activation of the transcription factor NF-κB is an early and necessary event in the cascade of signals finally resulting in the transcription of iNOS mRNA (6,36,78). This holds true for both human and rodent islet cells and transcription factor binding elements are present in both the human and the rodent iNOS promoters (23).

Early studies by other groups pointed out another transcription factor, IRF-1, as being indispensable for the expression of iNOS in murine macrophages (48). We and others have found that IRF-1 mRNA expression precedes iNOS mRNA expression in human and rodent β-cells exposed to IL-1β and IFN-γ (1,32). Later studies using an IRF-1-/- mouse showed, however, that this transcription factor is not required for the expression of iNOS mRNA in purified pancreatic β-cells. Nevertheless, IRF-1 appeared to contribute to the overall iNOS mRNA expression in whole islet preparations (69). The latter observation indicates a role for IRF-1 in iNOS mRNA expression by non-endocrine cells present in the islet preparations.

Substrate regulation of iNOS mediated NO synthesis from islet cells

In examining how substrate availability can regulate NO formation in cytokine-stimulated β-cells we found primary rat β-cells to express the two y^+CAT transporters CAT-2A and CAT-2B (33). In cell types other than β-cells, the expression levels of some of the y^+CAT transporters will increase in response to stimuli concomitantly inducing the expression of the iNOS gene (60,101). We did not find such an increase in y^+CAT transporters in rat β-cells exposed to IL-1β (33). Nevertheless, we observed an increase in arginine accumulation in IL-1β-stimulated cells, suggesting that cytokine-exposed β-cells will adapt to an increased need for arginine by other mechanisms than increasing the numbers of y^+CAT transporters.

Extracellular arginine concentrations may be significantly reduced at inflammatory sites (2,14). This is mainly due to the release of the enzyme arginase by activated macrophages. Arginase competes with NOS for the substrate L-arginine, and at inflammatory sites the extracellular space can become depleted from arginine (2). Initial studies indicated that cytokine-exposed pancreatic islets were dependent on an exogenous source of arginine for NO to be synthesized (4,31,33,34). This was indeed an important finding considering that iNOS is expressed by islet cells during the pre-diabetic insulitis, an inflammatory condition in which activated macrophages are present. With this information in mind we questioned whether pancreatic islet cells would be able to synthesise NO when extracellular arginine is scarce. Interestingly, when adding L-citrulline to the L-arginine free media, the immunostimulated β-cells regained their ability to generate NO (31,33,34). This observation suggested that the β-cells have the capability to regenerate L-arginine from L-citrulline and use this as a substrate for NO synthesis. When examining the presence of the two enzymes ASS and ALS in β-cells, it was found that both enzymes were expressed in unstimulated cells, and that the expression of ASS was increased in parallel with the induction of iNOS expression. These findings together with the studies on arginine transport, indicate that the pancreatic β-cells make adaptive changes to guarantee an adequate arginine supply for NO production during conditions when iNOS is expressed (34).

Role of iNOS in β-cell destruction

Some of our efforts have also been to study the mechanisms by which NO, endogenously produced or provided from an exogenous source, will cause cellular dysfunction and death of β-cells. In rodent β-cells, a cytokine-induced, NO-dependent impairment of insulin release appears to be due to an inactivation of the Krebs cycle enzyme aconitase (96). NO will also cause DNA damage and cell death (20,21,30). In a recent study using iNOS -/- mice, we found cytokine-induced β-cell necrosis to be NO-dependent whereas apoptosis was mostly NO-independent (56). In human β-cells, the cytokine-induced cellular dysfunction and death are mainly NO-independent, whereas NO added from exogenous sources can cause mitochondrial damage and DNA strand breaks resulting in cell death (23). The species differences may be explained by the fact that human, rat and mouse cells are equipped with different

levels of defense and/or repair responses to cytotoxic stimuli (97). The dichotomy seen in human β-cells responding to NO, produced endogenously after exposure to cytokines, or supplied from an exogenous source, may be explained by the fact that cytokine-stimulated cells will, in parallel with expressing iNOS, up-regulate protective defense mechanisms. Most likely a direct stimulation with exogenous NO will not allow the time necessary for such responses to become activated.

Although NO is clearly damaging to pancreatic β-cells in vitro, its role in the pathogenesis of type 1 diabetes is still controversial. It has been reported that blocking of NOSs, with more or less specific inhibitors, will reduce or delay or fail to affect the incidence of type 1 diabetes in rodent models of the disease (10,18,42,59,88). We have also shown that a mouse lacking iNOS (i.e. an iNOS knock-out mouse) is resistant to the induction of diabetes by multiple low doses of streptozotocin (35). In a study by Takamura et al iNOS was ectopically expressed by β-cells (90). These mice developed a rapid and early diabetes. Some recent studies have proposed that the NO produced during the early stages of insulitis may have immunoregulatory actions and a recent study suggests that small amounts of NO can activate some level of defense, protecting β-cells to a later exposure to noxious agents, including NO (55).

Altogether these findings suggest that the early description of NO as being a double-edged sword, having both important bioregulatory actions and cytotoxic properties (39), can be used when its role in the pathogenesis of type 1 diabetes is described. Future studies, aiming to discriminatie between the "good" and the "bad" actions of the radical, may give direction as to how a successful preventive therapy for type I diabetes can be developed.

Cytokine-inducible genes in pancreatic islets

In whole pancreatic islets and in insulin-producing cell lines cytokines induce several genes and proteins, including iNOS (23), ASS (34), ICE/caspase 1 (44,49); cyclooxygenase (COX) (16), manganese SOD (MnSOD) (9), heme oxygenase (40) and heat-shock protein 70 (hsp70) (29,54,99). IL-1β also induces expression of iNOS, MnSOD, hsp70 and heme oxygenase in FACS-purified adult rat β-cells (54,85), indicating that primary β-cells are a main cellular target for these previously described cytokine-induced effects on whole islets and tumoral insulin-producing cells. Although proteins such as caspase 1, iNOS, ASS and COX may exacerbate the inflammatory reaction and β-cell destruction, MnSOD, hsp70 and heme oxygenase are probably involved in β-cell defence and/or repair (23,26,63). Indeed, both hsp70 (46) and SOD (68) protect different cell types against apoptosis. Interestingly, IL-1β decreases the expression of proteins involved in the process of glucose recognition and insulin synthesis and release, such as GLUT 2, PDX-1 and the proinsulin converting enzyme PC2 (55).

Identification of new β-cell pro- and anti-apoptotic genes, and understanding of their regulation, may be instrumental in developing new approaches to preserve expression of cytokine-induced defence, repair and anti-apoptotic genes, while preventing the concomitant expression of genes and proteins contributing to β-cell

death. Up to now, the identification of cytokine-induced modifications in β-cell gene expression has been attempted by the gene-by-gene "candidate gene" approach (23). Since the process of cytokine-induced β-cell apoptosis is probably complex, involving up and down regulation of diverse groups of genes, the candidate gene approach will only provide a limited understanding of this phenomenon. To address this issue, differential display by reverse transcribed PCR has been utilized on FACS-purified β-cells exposed to control condition (no cytokines added) or IL-1β for 6 or 24 h (11,12). The following genes were found to be up-regulated by the cytokine: adenine nucleotide translocator-1, phospholipase D-1, neutrophil chemoattractant-1 and 3, monocyte chemotactic protein-1 and serine protease inhibitor-1, while expression of the β-cell autoantigen protein tyrosine phosphatase-like protein IA-2 was inhibited by IL-1β. In this context it is of interest that the adenine nucleotide translocator-1 has also been found to be up-regulated after exposure to the β-cell toxin strepotozotocin (98).

We believe that further characteriziation of the function of the genes referred to above in β-cells may be relevant for elucidation of mechanisms for β-cell susceptibiltity or protection against cell damage. Furthermore, by utilizing cDNA microarray analysis it is most likely that additional genes that are up- or down-regulated by cytokines in β-cells will be identified.

Genetic manipulation of β-cells to prevent their destruction

If the natural course of type 1 diabetes is interrupted by intervention with gene therapy, it could be anticipated that the β-cell mass is gradually restored to normal levels by β-cell repair and growth. Assuming that it is possible to target a viral vector to the β-cell *in vivo*, the remaining β-cell mass could be made to produce a local β-cell survival factor. This would not only save the β-cells, but also leave the immune system unaffected, as the transgene production is localized to the islets. In light of recent studies on the mechanisms involved in β-cell destruction, mainly in animal models, several promising candidates have emerged which may rescue β-cells. It could be macrophage mediated release of NO and cytokines that promote β-cell death, or it might be the cytotoxic T-cell that kills the β-cell by releasing the apoptotic signals perforin and Fas ligand (Fig. 2).

In both the cases of macrophage release of NO and cytotoxic T-cell killing, β-cell survival factors can be envisaged. The β-cell survival factors could be divided into at least five different categories: (i) cytokine antagonists; (ii) modulators of cytokine signaling; (iii) inhibitors of Fas ligand and perforin; (iv) anti-apoptotic factors; and (v) anti-stress factors (Fig. 3).

In the first category, transgene directed production of cytokine antagonists in β-cells could antagonize macrophage induced β-cell damage. An example of such an antagonist is the IL-1 receptor antagonist (IL-1ra). These factors would not only prevent cytokine-induced upregulation of the Fas receptor, which renders the β-cell sensitive to T-cell mediated release of Fas ligand, but also counteract cytokine-induced production of NO. In the first study to pursue this line of reasoning, insulin producing cells were transfected with an insulin/IL-1ra hybrid gene construct to

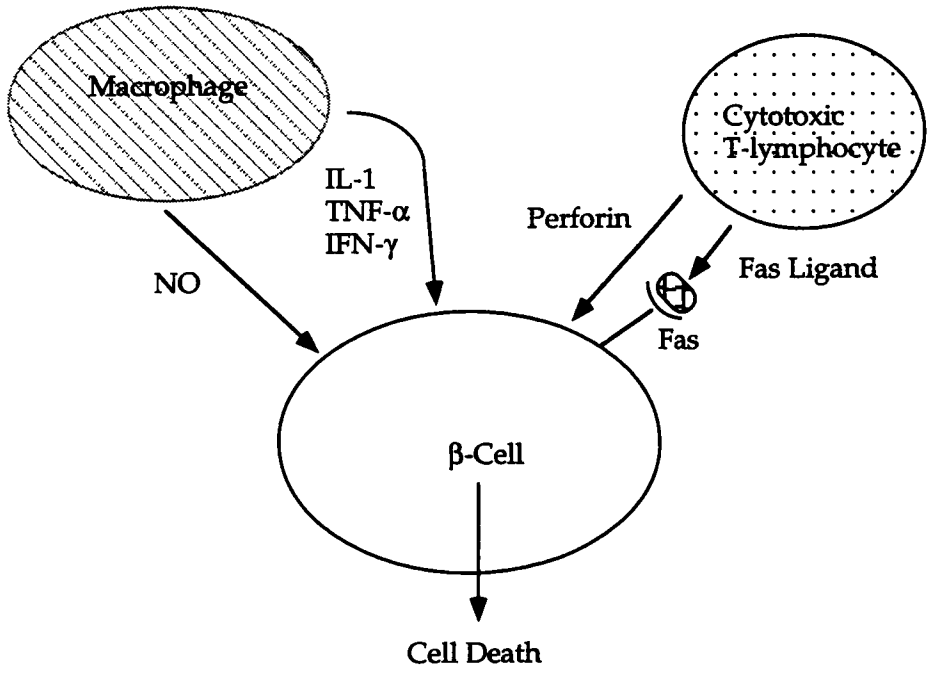

Fig. 2. Tentative view of factors involved in β-cell destruction caused by macrophages and cytotoxic T-cells during the course of type 1 diabetes. The macrophage kills the β-cell via direct NO release and cytokine induced damage. The cytotoxic T-lymphocyte induces β-cell necrosis by perforin release and induces apoptosis via a Fas-Fas ligand interaction.

make the cells produce and release the human IL-1ra (95). It was found that human IL-1ra was released from the transfected cells, which led to a marked desensitization to IL-1β thereby demonstrating the feasibility of the concept. Systemic administration of IL-1ra has also been found to counteract so-called recurrence of disease in syngeneically islets transplanted into diabetic NOD mice (79).

In the second category, we find the modulators of cytokine signaling, including TGF-β, the calcitonin gene-related peptide (CGRP), the adenoviral E3 gene products, IL-4 and IL-12p40. TGF-β and CGRP have recently been demonstrated to prevent diabetes in the NOD mouse (50,70). The transgenic mouse model was used in both studies, but whereas CGRP was expressed in the islet β-cells, expression of TGF-β was directed to the surrounding β-cells. In another transgenic model, β-cells were manipulated to express the adenoviral *E3* genes (92). This resulted in protection against both allograft rejection and autoimmune destruction of β-cells in a mouse model of virus induced type 1 diabetes. In addition, lentiviral directed production of IL-4 resulted in protection of the transduced islets against autoimmune destruction (47). Finally, adenoviral mediated islet expression of the IL-12 antagonist IL-12p40 prolonged syngeneic islet graft survival in diabetic NOD mice (102). Thus, enhanced b-cell expression of factors that promote a Th2 response or that

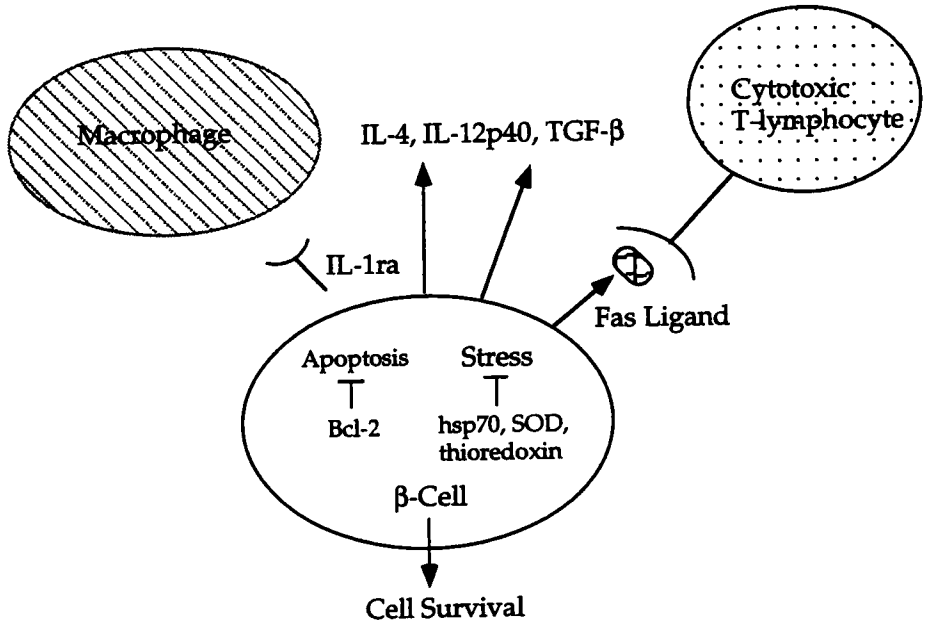

Fig. 3. The genetically manipulated β-cell can be made to release a cytokine antagonist (IL-1ra), modulators of cytokine signaling (IL-4, IL-12p40, TGF-β) or Fas ligand with the aim of antagonizing macrophage or cyt ivity. β-cell survival might also be achieved by intracellular overexpre ptotic (Bcl-2) or anti-stress factors (hsp70, SOD, thioredoxin). An enhan gainst β-cell death can be obtained if one or more of these factors is success

decrease antigen presentation (adenoviral *E3* genes) seems to have a beneficial influence on β-cell survival.

The third category, Fas ligand and perforin mediated cytotoxicity, is currently attracting much attention. It has been suggested that T-cell release of Fas ligand and cytokine-induced upregulation of the Fas receptor on β-cells promotes β-cell apoptosis in the NOD mouse (13,45). Indeed, NOD mice with a defective Fas receptor (NOD/lpr mice) do not develop diabetes. Moreover, adoptive transfer of diabetogenic splenocytes or a CD8+ T-cell clone specific for β-cells to NOD mice lacking the Fas receptor did not result in diabetes (13,45). In addition, analysis of pancreatic biopsies taken from newly diagnosed type 1 diabetic patients has revealed that a high percentage of the islet infiltrating T-cells are Fas ligand positive and that Fas expression could be observed in β-cells (63). However, the proposed role of Fas ligand as the major effector molecule in the autoimmune killing of β-cells has been challenged (3), and other investigators have instead proposed a major role of CD8+ T-cell-mediated perforin release (52). Nevertheless, Fas ligand and/or perforin inhibitors remain attractive β-cell survival candidates in strategies to create a resistant β-cell. Examples of Fas ligand signaling inhibitors are the caspase-8 inhibitor I-FLICE and Fas-antisense constructs.

The killing properties of Fas ligand could possibly be used as a weapon against the cytotoxic T-lymphocyte. If the β-cells are made to express Fas ligand on their surface, it is conceivable that attacking T-lymphocytes are killed before they can kill the β-cell. It has been observed that allograft rejection of β-cells was prevented by mixing the β-cells with myoblasts expressing Fas ligand (53). The β-cell expression of Fas ligand probably needs to be combined with a Fas ligand signaling inhibitor so that β-cells do not kill themselves or each other.

In the fourth category are the anti-apoptotic factors such as bcl-2, bcl-xL and crmA, which are potent downstream inhibitors of apoptosis. This means that they can prevent apoptosis in response to a multitude of factors including Fas ligand, cytokines, toxins and viruses. It has been observed that overexpression of bcl-2 prevents β-cell death in response to cytokines *in vitro* (57,74,77).

The last category includes antioxidative stress enzymes such as SOD, catalase, thioredoxin and the stress protein hsp70. Recent studies report that adenoviral mediated expression of SOD and catalase in islet cells protects against the deleterious effects of IL-1 and oxidative stress (8,41,91). β-cell expression of thioredoxin in NOD mice led to a marked reduction in diabetes (43). Finally, we have observed that increased levels of hsp70 protect against IL-1 *in vitro* (62).

To summarize, examples of putative β-cell survival factors that could prevent β-cell destruction in type 1 diabetes might be considered for β-cell transfection (Fig. 3). However, this does not exclude that other factors may become interesting in this context. It should also be kept in mind that factors of importance in mouse models are not necessarily relevant to the human situation.

CONCLUDING REMARKS

We and others have shown that the pancreatic β-cells are able to activate defence/resistance genes and proteins in response to toxic or immunologic assaults. Detailed knowledge on how these genes are regulated may point towards novel appropaches to prevent β-cell destruction during the course of autoimmune type 1 diabetes. The methodology applied can either be β-cell targeted gene transfection or administration of compounds mediating up-regulation of the β-cell defence mechanisms. This strategy can also be adopted to β-cells transplanted to type 1 diabetic patients in order to prevent recurrence of the the autoimmune disease in the grafted β-cells and/or to prevent β-cell rejection.

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