



Prevention of type 1 diabetes: from the view point of β cell damage

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Received 30 August 2003; accepted 27 September 2003

Abstract

The hallmark of immune-mediated type 1 diabetes is T cell-mediated destruction of the insulin-producing β cells in the islets, which results from an imbalance between disease promoting factors and protective elements. The precise mechanisms of β cell destruction leading to diabetes remain unclear. There are many molecules, including Fas ligand (FasL) and cytokines, such as IL-1, TNF- α and IFN- γ that cause release of other cytokine-mediators that have potential to damage the β cells. The β cell-death appears to ultimately be caused by receptor (Fas/FasL)-mediated mechanisms and/or by secretion of cytotoxic molecules (e.g., granzymes, perforin). FasL-mediated β cell damage might play a role in promoting insulinitis and β cell destruction in autoimmune diabetes in addition to toxic molecules, such as reactive oxygen species (superoxide, hydroxy radical, nitric oxide) or perforin. Furthermore, DNA damage in β cells leads to poly (ADP-ribose) polymerase-activation which will increase NAD consumption and rapid depletion of NAD compromise ATP production in the cells. Nicotinamide inhibits poly (ADP-ribose) polymerase and reduces nitric oxide accumulation in the NOD pancreas and protect β cells against radical-induced necrosis. Transgenic mice with β cell specific overexpression of copper, zinc superoxide dismutase, or thioredoxin are resistant to autoimmune and STZ-induced diabetes. It is apparent that a number of different mechanisms of β cell destruction are operative in type 1 diabetes. Blockage of multiple pathways, rather than a single pathway, of β cell-death may, therefore be necessary to fully protect β cells from destruction and thereby prevent type 1 diabetes.

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Keywords: Type 1 diabetes; Prevention; Apoptosis; Free radical; Cytokine

1. Introduction

Type 1 diabetes results from selective destruction of the insulin-producing β cells in the pancreatic islets and is primarily a T cell-mediated autoimmune disease directed against one or more β cell autoantigens [1]. T cells specific for islet β cell autoantigens, may exist normally but are restrained by immunoregulatory

Abbreviations: NO, nitric oxide; APC, antigen presenting cell; SOD, superoxide dismutase; HSP70, heat shock protein 70; FADD, Fas-associated death domain-containing protein; FLICE, FADD-like IL-1 β -converting enzyme; FLIP, FLICE-inhibitory protein; iNOS, inducible nitric oxide synthase

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mechanisms (the self-tolerant state) and that type 1 diabetes develops when one or another immunoregulatory mechanism fails, allowing β cell autoreactive T cells to become activated, expand clonally and entrain a cascade of immune and inflammatory processes in the islets, culminating in β cell destruction. Strategies to prevent or reverse the development of immune-mediated type 1 diabetes can be divided into three categories, depending on whether they focus on β cell protection, β cell regeneration, or β cell replacement. β cell regeneration includes the islet from stem cells in the pancreatic ductal epithelium or the islets of Langerhans, using the β cell growth factors and stem cell technologies [2,3]. β cell replacement includes human whole pancreas or islet transplantation, genetically engineered insulin-secreting cells, automated insulin delivery devices and bioartificial pancreas. In this review, we will focus on the β cell protection from the view point of β cell damage, which are cytokine antagonists, modulators of cytokine signaling, inhibitors of Fas ligand and perforin, anti-apoptotic factors and anti-oxidative stress.

2. The mechanisms of β cell destruction

In the non-obese diabetic (NOD) mouse, a mixed lymphocytic infiltration termed “insulinitis”, develops in the pancreatic islets, which is followed by β cell destruction, insulin deficiency and hyperglycemia. Antigen presenting cells, such as macrophages and

dendritic cells appear early, followed by CD4 and CD8 T cells and B cells [4]. This benign insulinitis is usually located around the islets. Then non-destructive insulinitis invades the islet and becomes destructive. T cell-mediated cellular destruction is mediated by the release of cytotoxic molecules, including cytokines, granzyme B or perforin, or by direct delivery of cell-death signals via the Fas pathway (Fig. 1). Activated CD4 and CD8 T cells act in unison to activate β cell-death via a process known as apoptosis. Apoptosis is effected by activation of the caspase pathway, which is in turn activated by a number of alternative mechanisms including, Fas interaction with Fas ligand, action of nitric oxide and oxygen-derived free radicals and membrane disruption by perforin and granzyme B produced by cytotoxic T cells. T cell cytokines, including IL-1, IFN- γ and TNF- α exacerbate β cell-death by up-regulation of Fas and Fas ligand and stimulation of NO and free radical production [5].

3. Prevention of β cell damage by modulation of cytokine signaling

A various cytokines are involved in the enhancement or inhibition of β cell damage in type 1 diabetes. β cell destruction is enhanced by the Th1 subset of CD4 T cells and the type 1 cytokines, such as INF- γ , TNF- α , IL-2, IL-12 and IL-18. In contrast, there is inhibition of β cell destruction by Th2 and Th3 cytokines, such as IL-4, IL-5, IL-10 and TGF- β [6].

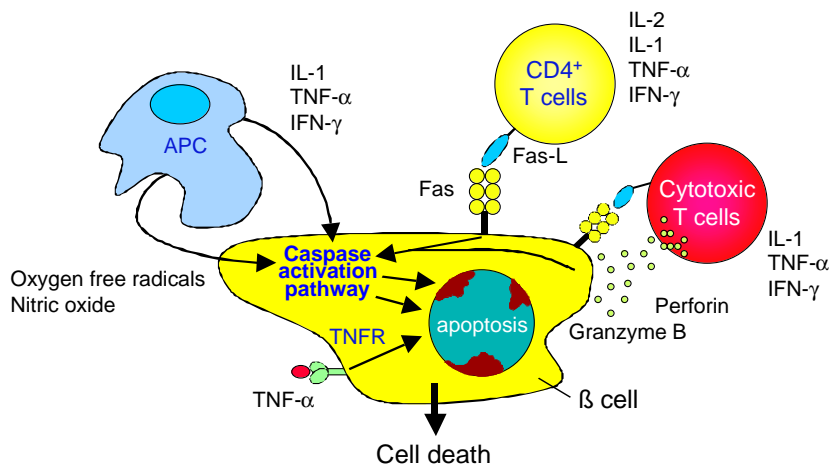


Fig. 1. Mechanisms of β cell destruction in type 1 diabetes.

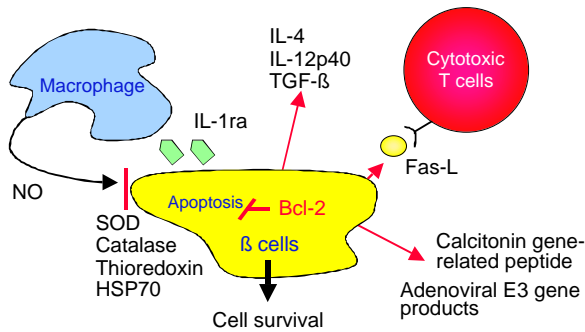


Fig. 2. Genetic manipulations of β cell to enhance the resistance against their apoptosis/necrosis.

These cytokines create a regulated immunological balance and generally type 1 diabetes is thought to be a Th1-dominance disease. In animal model of type 1 diabetes, a number of cytokines are tried to prevent the development of diabetes by injection and plasmid DNA vaccination [7–12]. There are several reports on the naturalization of Th1 cytokines by anti-cytokine antibody [13–15]. Furthermore, it was succeeded to prevent the β cell damage, using transgenic technique with calcitonin-gene-related peptide [16], TGF- β [17], IL-4 [18], vIL-10 [19] and adenoviral E3 gene [20] (Fig. 2).

4. Fas signal transduction pathway involved in cell-stress-induced apoptosis

As shown in Fig. 3, when cell surface receptor Fas binds its ligand FasL, the Fas clusters and signaling complex is assembled to FADD and first-level procaspases which is procaspase-8. Then pro-caspase-8 is cleaved to generate active caspase-8 and leads to apoptosis. The Fas signal is subject to regulation at the level of the Fas receptor and the caspase cascade. Recruitment of FLICE-inhibitory protein (FLIP) to the death-inducing signaling complex inhibits Fas signaling. The apoptosis pathway is inhibited by Bcl-2 and Bcl-x L.

Fas is expressed on islet β cells and can be induced normal β cells by stimulation with IFN- γ and IL-1 [21,22]. β cells are also capable of expressing FasL. It has been reported that T cell release of FasL and cytokine-induced upregulation of the Fas receptor on

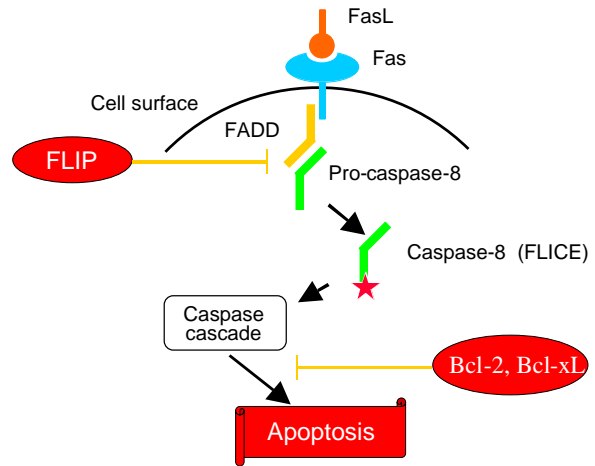


Fig. 3. Fas signal transduction pathway and its regulation.

β cells promotes β cell apoptosis in the NOD mouse [23,24]. Indeed, NOD mice with a defective Fas receptor (NOD/lpr mice) do not develop diabetes. 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 [25]. Cottet et al. reported the results of the study to evaluate the effects of caspase inhibitor FLIP on cytokine-induced apoptosis in mouse β cell line CDM3D [26]. They reported that overexpression of FLIP completely abolished cytokine-dependent activation of caspase-8 and protected the cells against apoptosis. This strategy may not be applied directly to the prevention of diabetes development in type 1 diabetes, but may be important for successful islet transplantation therapy of type 1 diabetes. Furthermore, anti-apoptotic factors, such as bcl-2, bcl-xL and crmA may prevent apoptosis in response to a multitude of factors including FasL, cytokines, toxins and viruses. It has been reported that overexpression of bcl-2 prevents β cell-death in response to cytokines in vitro [27,28].

5. Prevention of β cell damage by anti-oxidative stress

Evidence that β cell damage is mediated by nitric oxide and oxygen-derived free radicals, includes the

following observations:

- β cells express very low levels of free radical scavenging enzyme, indicating highly susceptible to free radical-induced β cell damage [29,30];
- IL-1 alone or in combination with IFN- γ induces β cell production of nitric oxide (NO) that increases β cell apoptosis [31];
- cytokine-induced β cell apoptosis is mediated through raised expression of NO synthase (iNOS) and consequent production of NO [32,33];
- iNOS-deficient islets exhibit less cytokine-induced cell-death than wild type islets [34,35].

Recent studies reported the evidence that anti-oxidative stress can prevent β cell damage in animal model of diabetes (Fig. 2). Kubisch et al. reported that the overexpression of copper/zinc superoxide dismutase (SOD) in β cells enhanced their resistance to oxidative stress induced by alloxan and STZ [36]. Furthermore, overexpression of manganese SOD, copper/zinc SOD, catalase, or glutathione peroxidase in insulinoma cells prevents IL-1-induced cytotoxicity [37,38]. Hotta et al. reported that the β cell expression of thioredoxin in NOD mice led to a marked reduction in diabetes [39]. Recently, Piganelli et al. reported that administration of a metalloporphyrin-based SOD mimic prevent the onset of type 1 diabetes and inhibited the insulinitis in the NOD scid mice injected the SOD mimic before transfer the diabetogenic BDC2.5 T cell clone [40]. These results suggest that the SOD mimic can be used to inhibit ongoing T cell responses to autoantigens.

6. Prevention of human type 1 diabetes by β cell protection

Nicotinamide (niacinamide, vitamin B3), restores intracellular levels of NAD and helps prevent cytokine-mediated β cell damage by increasing protection against the cytotoxic effects of NO. Nicotinamide may also protect β cells by its action in inhibiting poly (ADP-ribose) polymerase, a nuclear enzyme that detects and binds DNA strand breaks [41]. Treatment with high doses of nicotinamide prevents or delays diabetes onset in several animal models of type 1 diabetes and protects islet cells against cytotoxic actions in vitro. Based on these

studies, nicotinamide has been used in many studies in new-onset type 1 diabetic patients with mixed results, but a meta-analysis suggested that there is improvement in residual β cell function with minimal adverse effects [42]. However, two large multicenter randomized, double-masked, controlled clinical trials in islet-autoantibody positive first-degree relatives, the Deutsche Nicotinamide Intervention Study (DENIS) and the European Nicotinamide Diabetes Intervention Trial (ENDIT) reported negative findings on protection of diabetes progression [43,44].

7. Conclusion

Pancreatic β cells are able to activate defense/resistance genes and proteins in response to toxic or immunologic assaults. Detailed knowledge on how these genes are regulated, may point towards novel approaches to prevent β cell destruction during the course of immune-mediated type 1 diabetes.

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