

Proteome Analysis used in the Discovery of Molecular Mechanisms Involved in the Pathogenesis of Type 1 Diabetes Mellitus

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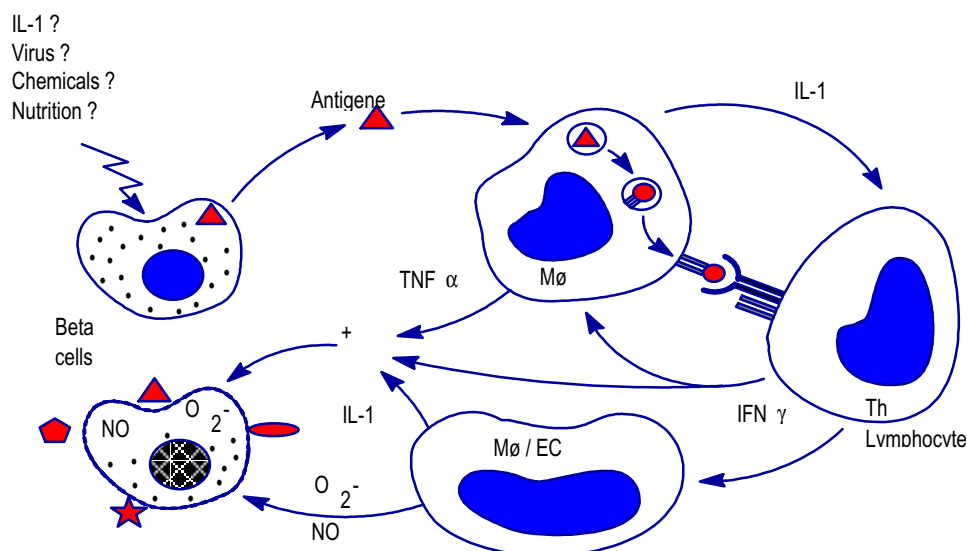
Background

Type 1 Diabetes Mellitus (T1DM) is a multifactorial polygenetic autoimmune disease characterized by mononuclear cell infiltration in the islets of Langerhans (insulinitis) and selective destruction of the insulin producing β -cells (1-3). It is generally accepted that the destruction of the β -cells results from interactions between various environmental factors and immune mechanisms in genetically susceptible individuals (3). The very first events initiating the destructive process have not yet been described in details. Cytokines, in particular interleukin-1 β (IL-1 β), are known to be released within the islets in sufficient quantities to modulate and inhibit the β -cell function *in vitro* (4). Furthermore, IL-1 β influences many important cellular functions such as reducing DNA content, decreasing protein synthesis and intracellular energy production and induce β -cell apoptosis. During the destructive process IL-1 β , tumor necrosis factor (TNF) α and interferon (IFN) γ are released in the islets resulting in production of free radicals (e.g. nitric oxide (NO \cdot), super oxide (O $_2^{\cdot-}$) and hydroxyl (OH \cdot)) in the β -cells (3) (Fig. 1, The Copenhagen Model).

Free radicals are normally scavenged by protective proteins (e.g. haeme oxygenases and manganese superoxide dismutase) (5, 6). Many IL-1 β effects are mediated through induction of the inducible NO synthase (iNOS) and its product, NO (7). We hypothesize that the β -cell, when exposed to IL-1 β initiates a protective response in competition with a series of deleterious events, and that in β -cells the deleterious events prevail (3). In support of this, over-expression in islet cells of scavengers of free radicals such as catalase and glutathione peroxidase reduces the deleterious effects of cytokines on β -cells (8).

T1DM is characterized by absolute insulin deficiency, abrupt onset of symptoms, proneness to ketosis and dependency on exogenous insulin to sustain life. It is the most common form of diabetes among children and young adults in populations of Caucasoid origin, where the prevalence is approximately 0.4%. T1DM is traditionally regarded as a disease of the childhood and adolescent period.

Figure 1



The age-specific incidence pattern is similar worldwide within this age segment, although the worldwide incidence differs markedly for the age group up to 15 years. The disease is rare before 6 months of age, but the incidence increases up to puberty, and thereafter declines. The overall age-adjusted incidence of T1DM varies from 0.1/100.000 per year in China and Venezuela to 36/100.000 per year in Sardinia and Finland (9). An increasing incidence of T1DM for individuals < 15 years of age has been observed in many countries. Studies have suggested that more than 50% of all T1DM cases develop after the age of 21 years, and that the cumulative incidence (up to age 80 years) may reach approximately 1% (10, 11). As detailed above, it is believed that T1DM is associated with the destructive effects of cytokines released in the islets during the insulinitis process occurring before clinical onset of the disease. The Copenhagen model (Fig. 1) details this process, and is a testable model, which can be approached by using proteome analysis as follows: Pancreatic islets of Langerhans are isolated, kept in culture *in vitro* and exposed to the different cytokines for fixed periods of time. Extensive research has documented that the metabolic function of such isolated islets (from either rat, mouse and humans) may be severely impaired by the co-culture with cytokines, which dependent on combination and concentration, may also induce cell death (7). Using this *in vitro* culture system with rat islets allowed us to make the first proteome analysis of cytokine-exposed islets (12-15).

Aim

The aims of our studies is to identify proteins and protein-modifications and pathways responsible for or is involved in cytokine mediated β -cell destruction.

Methods

In our studies we have used isolated rat islets of Langerhans, cell-lines and transplanted islets. Neonatal islets were isolated from Wistar Furth (WF) and diabetes prone BioBreeding (BB-DP) rats and were either exposed to recombinant human IL-1 β or BB-DP rat islets were syngeneically transplanted under the kidney capsule of 30 days old rats and excised at different time-points after transplantation or at onset of diabetes. The cell-lines used were two different cell-types, which dependent upon cell-culture maturates from a glucagons-producing pre- β -cell phenotype (NHI-glu) to an insulin-producing β -cell phenotype (NHI-ins). Islet, transplants and cells were labelled for 4 hours with [35S]-methionine immediately after exposure to cytokines or graft retrieval and snap frozen. The frozen samples were resuspended in DNaseI/RNaseA and lysed by freeze-thawing or lysis-buffer. First dimension gels contained 4% acrylamide, 0.25% bisacrylamide and ampholytes and second dimension gels contained 12.5% acrylamide and 0.063% bisacrylamide. The gels were placed in contact with X-ray films or phosphorimager screens. The fluorographs of the gels were analysed on the BioImage 2-D Analyzer version 6.1 programme. Expression levels from corresponding time points or conditions were compared and considered as changed if the p-value was below 0.01 (Students t-test). Significantly changed protein spots were cut out of preparative gels and sought identified by MALDI-MS. The final step in the experimental design is to characterize the selected proteins/protein modifications for the putative involvement in cytokine mediated β -cell destruction and T1DM by functional and further genetic analysis. This involves cloning and recombinant expression of the protein in β -cell lines to elucidate if the protein influences the known effect of cytokines.

Results

At present, mass spectrometric analysis of the protein spots with statistically significant altered expression level in response to IL-1 β or spontaneous diabetes development in BB-DP rats have identified more than two hundred proteins from islets, transplants and cell-lines. Several of these proteins have been found in more than one spot, suggesting that they are present in posttranslational modified or immature forms as well. The proteins identified have been grouped in the following groups: a) energy transduction and redox potentials, b) glycolytic and Krebs cycle enzymes, c) protein, DNA and RNA synthesis, chaperoning and protein folding, d) signal transduction, regulation, differentiation and apoptosis, e) cellular defence and f) other functions. The proteins identified *in*

vitro can be re-identified *in vivo* in the transplants. Several of the re-identified proteins in the transplants changes expression level in the same direction both *in vitro* and *in vivo*.

One of the identified proteins, galectin-3, involved in early pancreas development, cell growth and has anti-apoptotic properties, has been cloned and functionally characterized by over expression in a β -cell line. Galectin-3 has, when over expressed, a partial protective effect against the toxic effect of IL-1 β .

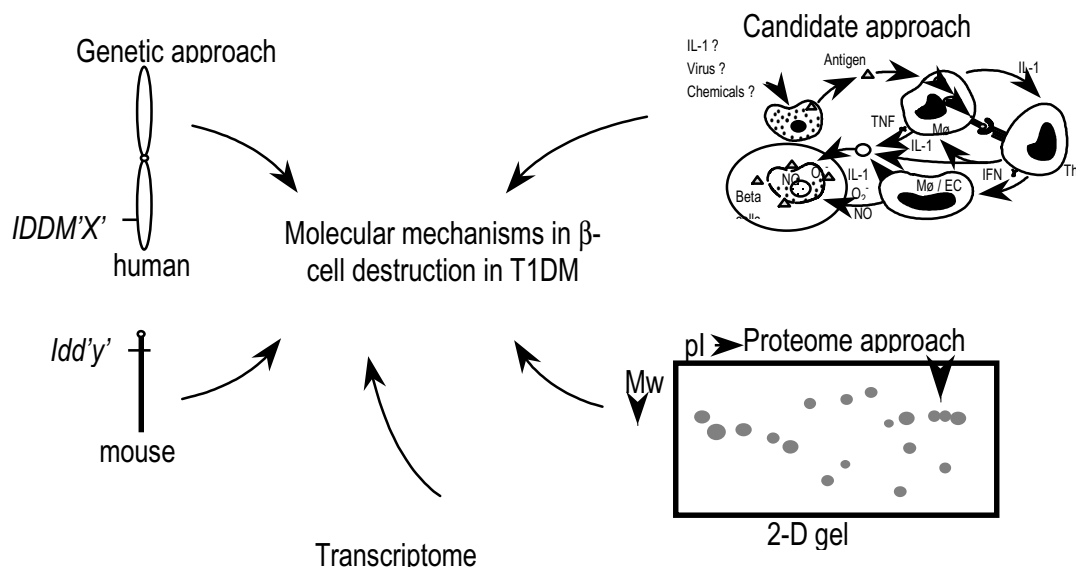
Conclusion

Combining information from these *in vitro* studies with data from our proteome analysis of beta-cell differentiation and islets transplanted to diabetic animals, reveals a complex picture of the β -cell response to cytokines and immune system. The use of proteome analysis may elucidate relevant mechanisms for β -cell death after cytokine exposure. Whether IL-1 β induced changes in protein expression levels in rat islets *in vitro* will also reflect pathogenically important changes in β -cells in rats spontaneously developing T1DM remains to be determined. Preliminary observations using 2D-gel studies of excised syngeneic islet transplants from different time points post-transplantation in BB-DP rats (16) suggest that the approach will be useful for studies of T1DM pathogenesis *in vivo*. Interestingly, the exquisite β -cell sensitivity to cytokine toxicity may be an acquired trait developed during β -cell maturation (17).

We believe that data obtained by the methodology presented here alone or in combination with e.g. genetic studies, transcriptome analyses and studies of candidate genes e.g. identified through the Copenhagen model (Fig. 1) will lead us to a detailed and complex picture of the molecular processes producing β -cell destruction *in vitro* (Fig. 2).

Although the picture is complicated and far from complete we are looking at the ailing β -cell through a new window and the challenge now is to learn to fully understand what we see. Further proteome and transcriptome analyses may eventually complete the picture. The long-term perspective of this is the development of new and specific intervention modalities in β -cell destruction in T1DM. Making the β -cell more resistant to immunological mediators may increase the survival time of transplanted islets or engineered β -cells with reduced immunosuppressive modalities in treatment of T1DM patients, and potentially prevent the ongoing β -cell destruction in predisposed individuals.

Figure 2



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