Effect of bacterial globin on survival of pancreatic beta cells

Summary / Zusammenfassung

Worldwide 15 million people are diagnosed with type 1 diabetes formerly also designated as insulin-dependent diabetes mellitus (IDDM). Current practiced therapies include insulin administration or whole pancreas transplantation. In the latter case, a clear discrepancy between the number of available organs and the number of Type 1 diabetes patient exist, which leads to a strong shortage of transplantable organs. Type 1 diabetes is characterized by an autoimmune and inflammatory process ultimately leading to the destruction of pancreatic b-cells. Nitric oxide induced by inflammatory cytokines, Interleukine1b (IL-1b), tumor necrosis factor a (TNF-a) and Interferon-g (IFN-g), is a possible mediator of b-cell destruction. Cytokines stimulate b-cell resident or intraislet macrophage resident inducible nitric oxide synthase (iNOS) expression with subsequent production of NO. The production of NO finally leads to the induction of apoptosis in the beta-cells and therefore to the destruction of islet tissue. However, alternative NO-independent pathways exist which lead to b-cell death by cytokines. The distinct stimuli that cause apoptosis converge on a common cell death machinery, which is driven and controlled by caspases.

Primary nonfunction of transplanted islets is a common event in human islet transplantation, which can be caused due to several factors, two of which are ischemia and non-specific inflammation. Proinflammatory cytokines, such as interleukin-1, TNFalpha and INFgamma are toxic to pancreatic islets. These toxic effects are mediated by NO and other factors. Various treatments have been evaluated to block the non-specific inflammation reactions, such as soluble receptors of cytokines or molecules inhibiting the formation of NO (N-monomethyl-arginine). In previous experiments we have shown that globin proteins protect a beta-cell line from NO induced damage. Bacterial globin proteins, which show high structural homology to globin proteins of mammals, possess a potent NO detoxifying activity. Unlike their mammalian counterparts, these proteins possess an altered protein conformation around the heme, which increases the catalytic NO-turnover. We expressed two bacterial globin proteins in the pancreatic b-cell line MIN6. MIN6 is mouse insulinoma derived cell line, which secretes insulin in response to physiological glucose levels. Constitutive expression of globin proteins increased resistance of MIN6 cells against nitrosative stress up to two fold after a 24-hours period. Furthermore, when the cells were exposed to NO, apoptosis was induced assessed as caspase activity. Caspase activity was increased by 55% in the parental MIN6 cells, whereas under the same conditions no significant induction of caspase activity was observed in globin expressing MIN6 cells. These data show that globin proteins due to their NO degrading activity can protect MIN6 cells from NO-induced apoptosis in vitro. In order to verify the potential effect of this gene therapy approach we will treat animals with Streptozotocin (STZ) to induce destruction of pancreatic beta-cells. This will lead to a Diabetes Typ-1 phenotype, which will be verified by a resting glucose level above 20 mmol/l. MIN6 cells and MIN6 cells expressing globin proteins will be transplanted under the capsule surrounding the kidney.

Project Leadership and Contacts / Projektleitung und Kontakte

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Funding Source(s) / Unterstützt durch

Foundation

In Collaboration with / In Zusammenarbeit mit
Duration of Project / Projektdauer
Jan 2005 to Dec 2007