Young Porcine Pancreatic Islets Cultured in the Presence of ECM Cues Show Improved Resistance towards Mediators of the Human Immune System

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Introduction

Type 1 and type 2 diabetes together constitute a major burden for health systems worldwide. In Canada alone, almost 7 % of the population are diagnosed with diabetes with increasing prevalence in both types. Whilst diabetes type 2 in early stages can still be treated with various medications, weight loss and change of life style, autoimmune type 1 diabetes is insulin-dependent upon diagnosis. In the past few years, islet transplantation rather than exogenous insulin replacement therapy has moved into focus of intensive clinical and academic research. This project aims to develop and validate a strategy to better protect the islet graft against the host's immune response. As a first step, we validated our carboxymethyl-dextran (CMD)-surfaces bearing covalently grafted fibronectin, CDPGYIGSR- and RGD using rat insulinoma cells (INS-1), a cell line secreting insulin in response to glucose. Pancreatic islets, encased in various three-dimensional (3D) systems functionalized with cues from the extracellular matrix (ECM), have shown improved survival and function before and after transplantation. Based on these findings, we embedded young porcine pancreatic islets in fibrin gels and exposed them to hydrogen peroxide (H_2O_2) to verify a possibly protective effect of fibrin. Consequently, we evaluated this protective function of fibrin by co-culturing pancreatic islets with human blood-derived immune cells.

Materials and Methods

For the first study, CMD was grafted on amine-functionalized tissue culture polystyrene plates (TCPS) plates. Then, fibronectin, CDPGYIGSR- and RGD were grafted covalently to the CMD and INS-1 cells seeded onto the plates. RGE, CMD and TCPS served as controls. After a 7-day culture, a glucose-stimulated insulin secretion (GSIS) assay was performed, as well as staining for Ki-67, Pdx-1, E-cadherin, insulin, glucagon and $\alpha_V ß_3$ and α_5 integrins. Proliferation was measured using a CyQUANT® assay.

For the second study, young porcine pancreatic islets were embedded in fibrin (2 mg fibrinogen/ml with 1 U/ml thrombin) and exposed to 10 and 100 µM H_2O_2 for 12 h. Islets cultured on TCPS served as control. After a GSIS assay, islets were stained for insulin, glucagon, E-cadherin

and integrins on paraffin sections. A TUNEL assay was used to evaluate apoptosis in response to H_2O_2.

Consequently, for the third study, porcine pancreatic islet preparations were embedded in fibrin or in 3% clinical-grade liquid alginate in presence of isolated human immune cells (monocytes/macrophages) from healthy or diabetic individuals. The RGD-bearing surfaces were included in both systems to study their possible effect on islet function. Macrophage migration through both fibrin and alginate was evaluated as well as cytokine secretion (TNF-α, IF-γ, IL-1β, IL-10, IGF-β). Islet viability and functionality was tested with an insulin secretion assay and a TUNEL apoptosis staining.

Results and Discussion

When cultured on fibronectin, RGD and CDPGYIGSR, INS-1 cells showed higher insulin secretion, when stimulated with glucose, compared to the controls. The INS-1 cell number increased in all conditions with the least cell number on CMD and the highest on fibronectin. Immunostaining for E-cadherin and the integrins αvß3 and α5 showed no convincing differences between the conditions but proved that cell-cell contacts supported cell proliferation in the non-adherent CMD and RGE-CMD systems, and confirmed the integrin-mediated cell binding to the ECM-covered surfaces. Overall, INS-1 cells exposed to fibronectin-, CDPGYIGSR-, and RGD-modified CMD surfaces showed elevated insulin secretion and increased cell numbers and therefore better function.

Young porcine pancreatic islets cultured in fibrin seemed to be better protected against H_2O_2 compared to those on TCPS. Significantly less islet cells of islets embedded in fibrin were apoptotic following a TUNEL assay. Islets cultured on TCPS have diminished ability to secrete insulin in response to glucose whereas islets embedded in fibrin secreted between 2 and 5 times more insulin in response to high glucose concentration compared to low glucose. Results from the macrophage experiments will also be presented.

Based on these results, we conclude that fibrin has a protective effect on porcine pancreatic islets during exposure to mediators of the human immune system. These results suggest possible applications in terms of islet transplantation. Porcine pancreatic islets could be an almost limitless alternative tissue source. If fibrin could reduce or even diminish the exorbitant host's immune response towards allo- or xenogenic grafts, causing islet cell loss within the graft, islet transplantation could be more successful and therefore could constitute a valuable and more accessible treatment for type 1 diabetes mellitus.