



**Karolinska
Institutet**

Department of Laboratory Medicine

EXPERIMENTAL ISLET TRANSPLANTATION

THESIS FOR DOCTORAL DEGREE (Ph.D.)

AKADEMISK AVHANDLING

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ABSTRACT

Pancreatic islet transplantation is a promising treatment modality for patients with insulin-dependent diabetes. Besides whole pancreas transplantation, it is the only treatment that can make patients normoglycemic without risking episodes of hypoglycemia. It can also prevent, slow down and even reverse the development of secondary complications to diabetes. Compared to whole pancreas transplantation, islet transplantation is much less invasive and may also be used in patients with a high surgical risk profile, but clinical outcome data are so far better for whole pancreas transplantation. However, graft survival necessitates life-long immunosuppression and for islet transplantation more than one donor is usually needed. There is therefore a need for more specific immunosuppression with less side effects as well as methods by which the donor pool can be expanded.

In this project we have assessed the capability of costimulation blockade, *i.e.* blocking the second signal of T lymphocyte activation, to prevent rejection of allogeneic (between individuals) and xenogeneic (between species) islet grafts, in particular when transplanted to recipients already sensitized to the graft. We have shown that a triple costimulation blockade regimen with anti-CD154 antibodies, CTLA4Ig and anti-LFA-1 antibodies could not prolong survival of islet allografts when transplanted under the kidney capsule of sensitized C57BL/6 mice. Either induced antibodies or memory T cells may be responsible for this inability of conventional costimulation blockade to prolong graft survival in sensitized animals. We tried to resolve this question in a rat-to-mouse xenotransplantation model, in which immune or naïve serum was injected intraperitoneal at the time of islet transplantation. Again, the recipient animals were given costimulation blockade. The immune serum had no negative impact on the grafts immediately (within 96 hours) post-transplantation or on the graft survival long-term in mice receiving costimulation blockade. These results suggest that preformed antibodies are not the main cause for graft rejection in sensitized recipients treated by costimulation blockade.

In the animal transplantation models used, streptozotocin or alloxan is used to induce diabetes through their toxic effects on pancreatic β -cells. It has been reported that these drugs are also toxic for other cells and tissues, including cells of the immune system. Therefore, we compared recipients given streptozotocin or alloxan for diabetes induction with regard to graft survival times, spleen size and toxic effects on leukemic cells *in vitro*. We conclude that streptozotocin is more toxic on immune cells than alloxan, and may therefore not be a suitable agent for diabetes induction in transplantation models assessing different immunosuppressive protocols. Further, we showed that the erythropoietin analogue, pyroglutamate helix B surface peptide (ARA 290) could protect islets from apoptosis when exposed to pro-inflammatory cytokines *in vitro*, while no clear effect was seen on graft survival when injected into the recipients. Further studies are needed on this potential islet-protective agent.

In conclusion, islet transplantation holds great promise for the future as a treatment modality for insulin-dependent diabetes. However, further research is needed in order to find optimal immunosuppressive protocols with acceptable side effects that can promote long term graft survival. Costimulation blockade may be such a modality provided memory T cell activation can be perturbed and tolerance induced also in sensitized recipients.