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Timing of anti-thymocyte globulin infusion affects islet engraftment after intraportal allotransplantation in patients with T1DM

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Background: Anti-thymocyte globulin (ATG) is a potent immunosuppression agent associated with superior long-term islet graft survival compared to anti-IL2R monoclonal antibodies. However, ATG infusion often leads to a vibrant inflammatory response compromising islet engraftment after intraportal transplantation. Therefore, we analyzed islet engraftment in relation to the timing of anti-thymocyte globulin infusion and islet transplantation (ITx).

Material: In this retrospective single-center study, we assessed islet engraftment after a single intraportal allogeneic islet transplantation in the following groups: concurrent ITx and ATG infusion (group "ITx/ATG" N=10), ITx 1-3 months after ATG induction (group "delayed ITx" N=6), and ITx more than 9 months after ATG or no ATG (group "late ITx" N=8). Groups did not differ regarding patient characteristics. Tacrolimus and myfortic were initiated at the time of ATG infusion. Additional basiliximab was given for induction in "delayed ITx" and "late ITx" groups. Islet engraftment (IEngraftment) was assessed based on the area under the curve (AUC) for c-peptide/AUC for glucose/islet mass transplanted calculated in 10^-6 pmol/mg/IEQ from the mixed meal tolerance test on post-transplant day 75. Patients received anti-inflammatory peri-transplant therapy with etanercept, reparixin, or placebo.

Results:

ATG/ITx Group.: ATG in a total dose of 6mg/kg given in two patients daily as 1.5mg/kg 6-hour infusion in the ITx/ATG group resulted in poor islet engraftment and early islet graft failure despite the use of reparixin or etanercept. In the subsequent 8 patients, the same total dose of ATG was infused slowly over 7 days. IEngratfment of 16 was recorded in 2/3 of patients receiving placebo anti-inflammatory therapy, but it improved to 30 (20-40) when reparixin was used instead of placebo in 5 patients.

Delayed ITx Group: Delaying ITx for 1-3 months after ATG infusion eliminated early islet graft failure, but did not improve overall IEngraftment, which was 10 when using placebo, 12 (9-14) in patients treated with etanercept, and 19 (17-21) with reparixin.

Late ITx Group: For patients receiving etanercept and ITx over 9 months after ATG infusion, IEngraftment improved to 32 (15- 62) compared to 13 (9-17) when ITx was closer to ATG infusion (p=0.004 Mann-Whitney).

Conclusions: Delaying ITx 1- 3 months after ATG prevented the incidence of poor islet engraftment and early graft failure after intraportal islet transplantation; yet, it did not improve overall islet engraftment compared to concurrent ITX and ATG infusion. However, ITx 9 months after ATG infusion was associated with significantly improved islet engraftment after islet intraportal transplantation.

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Overexpression of CTRP9 mediated by AAV prevents graft loss after islet transplantation

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Introduction: As a promising clinic therapy of type 1 diabetes, islet transplantation was limited by graft failure. The primary nonimmune obstructive factor was reactive oxygen species (ROS), which causing by intraperitoneal hypoxia environment. The generation of ROS leads sets of downstream reactions, such as Damage-associated molecular patterns (DAMPs) release, oxidative stress (OS), mitochondrial dysfunction, endoplasmic reticulum stress (ERS), inflammation, even apoptosis. C1q/tumor necrosis factor related protein 9 (CTRP9) is a recently discovered adipokine, which has diverse physiological functions, including mitigate OS, alleviate inflammation and suppress apoptosis in various organs. In this study, an islet cells overexpressing CTRP9 gene were established, and the engineering islets are aiming to prolong the graft survival.

Methods: Mouse islets were isolated by Liberase RT and the adeno-associated virus (AAV) mediated murine CTRP9 gene was transferred to the syngeneic suboptimal islets. The expression of CTRP9 at mRNA and protein level in engineering islets were measured by RT-qPCR and western blot (WB). In vitro, the engineering islets were incubated at hypoxia environment (1% O2) for 72 hours. The ERS markers, mitochondrial function, anti-oxidative enzymes, ROS generation, and the islets survival rate were detected. Then 200 engineering islets were transplanted to each diabetic mouse under the left kidney capsule, and the blood-glucose level were detected every other day.

Results: The CTRP9 mRNA and protein level of the engineering islet were significantly higher than control islets. After 72h hypoxia treatment, the overexpression of CTRP9 significantly decreased the hypoxia-induced ERS markers (GRP78, CHOP, IRE1α), and the ROS generation (ROS, NOS, NO). While it improved the mitochondrial function of islets, the expression of anti-oxidative enzymes (SOD2, Trx1, Catalase), and survival rate. In vivo, engineering islet transplanted mice achieved euglycemia at earlier time. The TUNEL staining and insulin immunohistochemical (IHC) staining of the transplanted grafts showed that the islets transferred with CTRP9 had less apoptosis and more insulin positive cells than control ones.

Conclusion: AAV mediated CTRP9 enhances the isolated murine islet anti-hypoxia ability and extended the islet graft survival time. And this study provided a clinic approach for islet homeo-transplantation.

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