

Inflammatory response and its impact on outcome of islet transplantation

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ABSTRACT

Inflammation plays a detrimental role in islet transplantation leading to poor clinical outcomes. One of the major contributors of islet loss is IBMIR leading to 50% decrease in transplanted islet mass immediately post-transplantation. The detrimental effect of IBMIR was initially noted in Xenogenic and Allogenic islet transplantation, and later we reported that IBMIR also causes significant islet loss in auto-islet transplantation. Our group has mainly focused on anti-inflammatory strategies to dampen the effect of IBMIR. Consequently, we introduced the clinical use of Interleukin-1 beta blocker (Anakinra) along with Tumor Necrosis Factor alpha inhibitor (Etanercept) in islet transplantation. This protocol has shown improved outcomes compared to just Etanercept alone. We also explored the use of Nuclear Factor Kappa B inhibitor (Withaferin A) to prevent the early damage caused by IBMIR and obtained excellent results *in vitro*. Here, we have summarized our clinical and research findings pertaining to factors that affect the functional islet mass post-transplantation.

Total Pancreatectomy with Islet Autotransplantation (TPIAT) is currently the most favorable option for patients who are debilitated by chronic pancreatitis. Total pancreatectomy relieves the pain and auto-islet transplantation prevents the development of brittle diabetes. Allogenic islet transplantation is a procedure that is used to restore normoglycemia and prevent occurrence of hypoglycemic episodes in Type-1 diabetic patients. Despite the success of islet transplantation being improved significantly in the last couple of decades, several factors still need

to be addressed that lead to unfavorable outcomes. Some of the factors that affect the functional islet mass post-transplantation have been described (Figure 1). Mechanical and enzymatic stress

cause islet damage during isolation which may diminish islet quality resulting in poor transplant outcomes. We have previously investigated the islet damage that occurs in the various stages of isolation including transportation, perfusion, digestion, recombination, purification and bagging. Islet damage was determined by measuring the amount of miRNA375 released in the medium at the different stages. Islet damage was highest during digestion, which is reasonable because of the mechanical and enzymatic activity-taking place at this stage. Elevated level of miRNA 375 in the media during digestion was correlated with higher 6 month HbA1C percentage and increased insulin requirement in TPIAT patients¹. Therefore, optimization of isolation conditions is needed to reduce islet damage due to enzymatic and mechanical stress without affecting the yield and quality of the islets.

Instant Blood Mediated Inflammatory Reaction (IBMIR) during the peri-transplant period results in substantial loss of islet mass². IBMIR is an innate immune response that occurs when islets come in direct contact with the blood. It is characterized by coagulation, complement activation, proinflammatory cytokine/chemokine production and immune cell infiltration. Isolated islets express increased levels of Macrophage Chemotactic Protein 1 (MCP-1), Interleukin 8 (IL-8) and Tissue Factor (TF). TF presents in islets may instigate the activation of coagulation pathway, whereas, chemokines MCP-1 and IL-8 might be involved in the recruitment of innate immune cells into the islets causing significant damage. IBMIR results in loss of almost 50% of the transplanted islet mass before

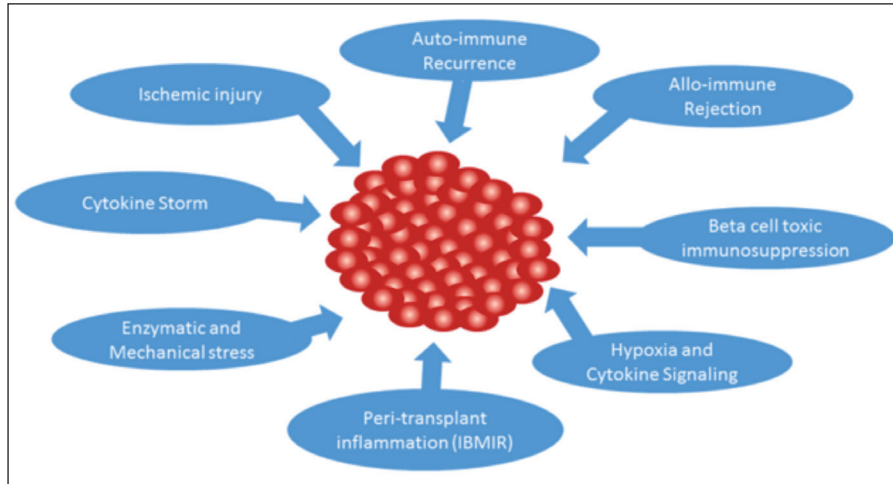


Figure 1. Factors affecting functional islet mass post-transplantation.

islet engraftment³. IBMIR is observed in allogenic, xenogenic and autologous islet transplantation. TF expression is increased within 15 mins after contact of islets with autologous blood. Leucocyte infiltration and platelet adhesion also occur within 3 hours of mixing of islets with autologous blood. Complement activation was absent in autologous islet transplant model but present in allogenic and xenogenic islet transplant models⁴. Reducing inflammation during the early peri-transplant period may be crucial for improving long term islet transplant outcomes. Interleukin 1 β (IL-1 β) and tumor necrosis factor α (TNF α) can cause islet damage by activating Nuclear Factor Kappa B (NF κ B) mediated apoptosis. Simultaneous blocking of IL-1 β using anakinra and TNF α with etanercept has shown improvement in islet transplant outcomes

compared to etanercept alone, and anakinra alone in pre-clinical models⁵. Similarly, in our clinical TPIAT cases, we have shown that double blockade of TNF α and IL-1 β results in lower levels of cytokines and chemokines in serum during the early peri-transplant period. Furthermore, islet damage was significantly reduced when both IL-1 β and TNF α were blocked. This was further supported by the lower HbA1c percentage and increased basal c-peptide levels at 6 month post-transplantation in the double blockade group⁶.

The central player regulating the molecules of inflammation is the transcription factor nuclear factor Kappa B (NF κ B). Previously, others and we have shown that activation of NF κ B by cytokines results in beta cell apoptosis. Thus, blocking NF κ B in the early stages of islet transplantation may protect beta

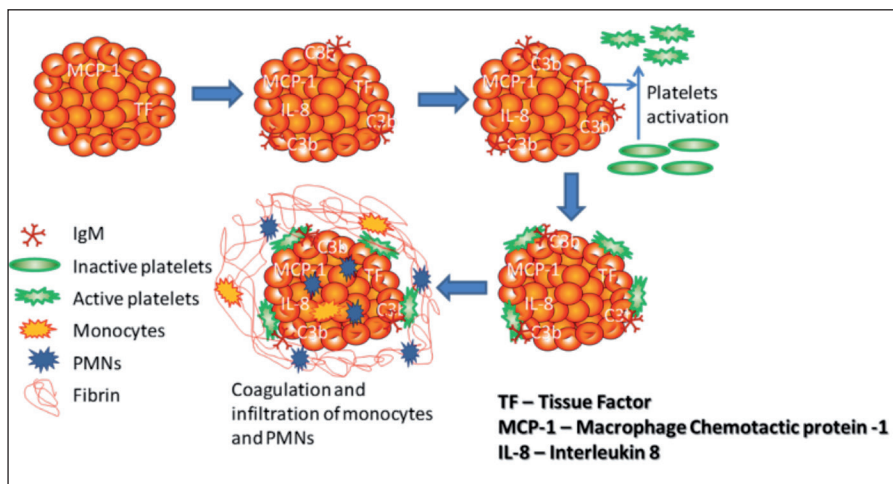


Figure 2. Molecular events postulated for IBMIR.

cells from pro-inflammatory cytokine mediated damage. Hence, we used a plant-derived molecule called Withaferin A (WA), which is a potent inhibitor of the NF κ B activity. WA improved graft survival and islet transplant outcomes in mice receiving marginal islet dose. Moreover, WA also prevented apoptosis in islets exposed to pro-inflammatory cytokine stimulation⁷. Using *in vitro* model for IBMIR, we identified that pro-inflammatory cytokine levels in serum was significantly reduced and neutrophil infiltration into islets was efficiently blocked by WA⁸. Thus, blocking inflammation by inhibiting NF κ B activity is an excellent strategy to prevent islet cell damage during peritransplant period. Serum levels of chemokine interferon gamma inducible protein 10 (IP-10) were highly elevated immediately post-infusion of islets in clinical samples. High IP-10 levels in serum was correlated with poor graft function. Using IP-10 knockout mice, we found that islet derived IP-10 was responsible for poor islet transplant outcomes *in vivo*. Interestingly, blocking IP-10 using a monoclonal antibody resulted in significantly improved islet graft survival *in vivo*⁹. Blocking inflammatory mediators during islet transplantation seems to improve graft survival.

CONCLUSIONS

Our findings in both clinical and pre-clinical studies support the notion that inflammation during the early period of islet transplantation can be detrimental and can cause poor outcomes. We have focused our research towards developing anti-inflammatory strategies to prevent islet damage during the peritransplant period and improve long-term outcomes.

CONFLICT OF INTEREST:

The Authors declare that they have no conflict of interests.

REFERENCES

1. Saravanan PB, Kanak MA, Chang CA, Darden C, Yoshimatsu G, Lawrence MC, Naziruddin B. Islet damage during isolation as assessed by miRNAs and the correlation of miRNA levels with posttransplantation outcome in islet autotransplantation. *Am J Transplant* 2018; 18: 982-989.
2. Kanak MA, Takita M, Kunnathodi F, Lawrence MC, Levy MF, Naziruddin B. Inflammatory response in islet transplantation. *Int J Endocrinol* 2014; 2014: 451035.
3. Kanak MA, Takita M, Shahbazov R, Lawrence MC, Chung WY, Dennison AR, Levy MF, Naziruddin B. Evaluation of MicroRNA375 as a novel biomarker for graft damage in clinical islet transplantation. *Transplantation* 2015; 99: 1568-1573.
4. Naziruddin B, Iwahashi S, Kanak MA, Takita M, Itoh T, Levy MF. Evidence for instant blood-mediated inflammatory reaction in clinical autologous islet transplantation. *Am J Transplant* 2014; 14: 428-437.
5. McCall M, Pawlick R, Kin T, Shapiro AM. Anakinra potentiates the protective effects of etanercept in transplantation of marginal mass human islets in immunodeficient mice. *Am J Transplant* 2012; 12: 322-329.
6. Naziruddin B, Kanak MA, Chang CA, Takita M, Lawrence MC, Dennison AR, Onaca N, Levy MF. Improved outcomes of islet autotransplant after total pancreatectomy by combined blockade of IL-1beta and TNFalpha. *Am J Transplant* 2018; 18: 2322-2329.
7. SoRelle JA, Itoh T, Peng H, Kanak MA, Sugimoto K, Matsumoto S, Levy MF, Lawrence MC, Naziruddin B. Withaferin A inhibits pro-inflammatory cytokine-induced damage to islets in culture and following transplantation. *Diabetologia* 2013; 56: 814-824.
8. Kanak MA, Takita M, Itoh T, SoRelle JA, Murali S, Kunnathodi F, Shahbazov R, Lawrence MC, Levy MF, Naziruddin B. Alleviation of instant blood-mediated inflammatory reaction in autologous conditions through treatment of human islets with NF-kappaB inhibitors. *Transplantation* 2014; 98: 578-584.
9. Yoshimatsu G, Kunnathodi F, Saravanan PB, Shahbazov R, Chang C, Darden CM, Zurawski S, Boyuk G, Kanak MA, Levy MF, Naziruddin B, Lawrence MC. Pancreatic beta-cell-derived IP-10/CXCL10 isletokine mediates early loss of graft function in islet cell transplantation. *Diabetes* 2017; 66: 2857-2867.