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specific Immunomodulatory Cells on Pancreatic Islet
Transplantation

(ドナー抗原特異的免疫抑制性細胞の膵島移植における
抗炎症効果)

2025 年 3 月

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Background and Purpose

Pancreatic islet transplantation (PITx) is an effective treatment option for patients with type 1 diabetes mellitus. This minimally invasive procedure involves the infusion of islets into the portal vein, providing an effective means of β -cell replacement and offering the potential for a cure for diabetes. Despite its advantages, PITx faces multiple challenges that must be addressed to enhance its clinical efficacy and accessibility.

One of the most significant obstacles is the requirement for lifelong, non-specific immunosuppression to prevent allograft rejection. These treatments have numerous adverse effects including an increased risk of infections, malignancies, and metabolic complications, while also failing to consistently ensure long-term islet acceptance. Therefore, inducing donor antigen-specific immune tolerance has emerged as the ultimate goal in transplantation medicine. Cell therapy with alloantigen-specific immunomodulatory cells (IMCs), generated *ex-vivo* in the presence of anti-CD80 and CD86 monoclonal antibodies (mAbs), has been shown to induce tolerance with a 70% success rate following clinical living-donor liver transplantation. Before extending this promising IMC therapy to the field of PITx, it is crucial to address the strong inflammatory responses that occur immediately after PITx.

Unlike in the case of solid organ transplants, where adaptive immunity plays a dominant role in graft rejection, PITx is significantly impacted by non-specific innate immune responses that lead to early islet loss. Macrophages, as key effectors of the innate immune response, play a central role in this process. Studies have demonstrated that transient macrophage inhibition or depletion can significantly prolong pancreatic islet graft survival, highlighting the importance of mitigating inflammatory responses to improve PITx outcomes. Additionally, emerging evidence suggests that the predominant macrophage phenotype within the transplant microenvironment is a critical determinant of graft fate, with a shift toward an anti-inflammatory M2 phenotype being associated with improved graft survival and immune tolerance.

This study aimed to investigate the potential of IMCs to modulate macrophage activation and ameliorate inflammation-mediated islet damage following PITx. By assessing the effects of IMCs on macrophage polarization, cytokine production, and pancreatic islet survival, this research provides critical insights into the therapeutic potential of IMCs in the context of PITx.

Materials and Methods

(1) Murine IMCs were induced using mouse splenocytes in the presence of anti-mouse anti-CD80 (RM80) and anti-CD86 (GL-1) mAbs. The cell phenotype during induction was assessed by flow cytometry. To evaluate the immunosuppressive effects of IMCs, proliferation assays were conducted using mixed lymphocyte reactions (MLRs). To investigate the efficacy of IMCs to modulate macrophage polarization, the murine macrophage cell line RAW264 was stimulated with LPS and co-cultured with IMCs in a cell-cell contact or separate culture setting. After 6 hours of culture, the M1 macrophage markers, CD80 and CD86, as well as the M2 markers, CD163 and CD206, were studied by flow cytometry, and the cytokine profile in the culture supernatants was

studied using enzyme-linked immunosorbent assay (ELISA) kits. Nitric oxide (NO) production, a hallmark of pro-inflammatory M1 macrophages, was quantified using the Griess assay. Additionally, to determine whether IMCs could mitigate inflammatory damage to pancreatic islets, BALB/c islets were co-cultured with activated macrophages and IMCs. A macrophage migration assay using a transwell system was used to study the effects of IMCs on macrophage migration towards allogeneic C57BL/6 islets. Finally, to study the effects of IMCs on the inflammatory response in the liver after PITx, IMCs were infused together with C57BL/6 islets via the portal vein in a syngeneic PITx mouse model. The mRNA expression of pro-inflammatory cytokines in the recipient liver was determined by polymerase chain reaction, and the liver samples were stained by immunohistochemistry to assess the number of F4/80⁺ macrophages at the transplantation site.

(2) Subsequently, human IMCs were induced by co-culturing responder peripheral blood mononuclear cells (PBMCs) with irradiated stimulator PBMCs in the presence of anti-human anti-CD80 (2D10.4) and anti-CD86 (IT2.2) mAbs. Flow cytometry was used to monitor cell phenotype changes, while MLR assays were performed to evaluate the immunosuppressive effects of the generated cells. To determine whether human IMCs exhibited similar anti-inflammatory properties as murine IMCs, the human monocytic cell line THP-1 was differentiated into resting macrophages and used to study human macrophage polarization *in vitro*. THP-1 macrophages were stimulated with LPS and co-cultured with human IMCs. The M1 and M2 macrophage markers were assessed by flow cytometry, and the cytokine profile in the cell culture supernatants was examined using ELISA kits.

(3) To determine whether human IMCs could prevent human macrophage-mediated destruction of islets, IMCs were challenged in a xenogeneic PITx *in vitro* model. Porcine pancreatic islets were isolated using Liberase enzyme and co-cultured with LPS-activated THP-1 macrophages and human IMCs. The number of remaining islets was assessed after 12 hours of co-culture.

Results

(1) Murine IMCs exhibited donor antigen-specific immunosuppressive effects in the MLR assays. In the macrophage polarization assays, IMCs suppressed the M1 phenotype, as shown by reduced CD80 and CD86 marker expression, while promoting a shift towards the M2 phenotype, with increased CD163 and CD206 markers, particularly under direct cell-cell contact conditions. This was associated with reduced secretion of pro-inflammatory cytokines, tumoral necrosis factor (TNF)- α and interleukin (IL)-6, increased IL-10 secretion, and reduced NO production by activated macrophages. Murine IMCs also prevented macrophage-mediated islet death and inhibited macrophage migration towards allogeneic islets *in vitro*. Intraportal co-infusion of IMCs with syngeneic islets in a mouse PITx model suppressed mRNA expression of TNF- α and IL-1 β in the recipient liver, while reducing the number of F4/80⁺ macrophages at the transplantation site.

(2) Human IMCs demonstrated immunosuppressive effects in the MLR assays. IMCs suppressed the conversion to the M1 phenotype in THP-1 human macrophages as evidenced by the reduction of CD80 and CD86 marker expression, along with an increased secretion of anti-inflammatory cytokines, transforming growth factor (TGF)- β and IL-10. However, no significant increase in M2 macrophage markers, CD163 and CD206 was observed. Interestingly, higher doses of IMCs were associated with an increased secretion of pro-inflammatory cytokines, TNF- α , IL-6,

and IL-1 β .

(3) Human IMCs effectively protected porcine pancreatic islets from LPS-stimulated THP-1 macrophage-mediated damage in an *in vitro* xenogeneic PITx model, preserving islet viability compared with untreated controls.

Discussion

The present study demonstrated that IMCs can effectively promote a shift from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype through direct cell-cell interactions. This effect was associated with reduced secretion of pro-inflammatory cytokines, decreased NO production by activated macrophages, and increased IL-10 secretion by M2 macrophages. Consistent with previous reports showing that the suppression of macrophage activation protects pancreatic islets from destruction, treatment with IMCs significantly improved pancreatic islet survival in both murine syngeneic and xenogeneic human/porcine macrophage-islet co-culture models. Moreover, intraportal co-infusion of IMCs with syngeneic pancreatic islets downregulated the mRNA expression of pro-inflammatory cytokines and reduced macrophage infiltration in the liver of recipient mice. Given the crucial role of the early non-specific immune response in triggering the subsequent adaptive immune response that leads to allograft rejection, mitigating the inflammatory response following PITx is critical for improving long-term graft function and survival. Further research involving the use of *in vivo* models of allogeneic PITx are necessary to gain a deeper understanding of this IMC therapy before its clinical application in PITx.

Conclusion

IMCs modulate macrophage polarization, promoting an anti-inflammatory M2 phenotype while preserving pancreatic islets from macrophage-mediated damage. Combined with its intrinsic donor antigen-specific immunosuppressive capacity, IMCs represent a promising strategy for improving the outcomes after PITx.