学位論文内容の要旨
（Summary of Dissertation）

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（Dissertation Title）

Anti-inflammatory Effects of *ex-vivo* Generated Donor Antigen-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）

【Background and Objectives】

Pancreatic islet transplantation (PITx) is an effective treatment option for patients with type 1 diabetes mellitus. This minimally invasive procedure involving the infusion of islets into the portal vein enables β-cell replacement, offering the potential for a cure for diabetes. However, several challenges must be addressed before this procedure can become a mainstream strategy for treating insulin deficiency.

Non-specific lifelong immunosuppression has numerous adverse effects and often fails to ensure long-term islet acceptance. Therefore, inducing donor-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), was shown to induce tolerance with a 70% success rate following clinical 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, primary non-function of transplanted islets is driven by the non-specific innate immunity, resulting in substantial early islet loss. Macrophages, as key effectors of the innate immune response, play a central role in this process. Transient macrophage inhibition and/or depletion prolongs pancreatic islet graft survival, highlighting the importance of ameliorating inflammatory responses after PITx to improve graft survival.

The purpose of this study was to investigate the anti-inflammatory potential of IMCs to modulate macrophage activation, specifically addressing the challenge of inflammation-mediated islet damage following PITx. Findings from this research may support the development of IMC therapy for clinical application in 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, and the immunosuppressive effects of IMCs were studied in a proliferation assay. To investigate the efficacy of IMCs to modulate macrophage polarization, RAW264, a murine macrophage cell line, 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 M1 macrophages, was assessed using the Griess assay. 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. Lastly, 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 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. The cell phenotype during induction was assessed by flow cytometry, and the immunosuppressive effects of human IMCs were studied in a proliferation assay. We investigated whether human IMCs exhibited similar anti-inflammatory effects as murine IMCs. THP-1, a human monocytic cell line, 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 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. Pig 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-specific immunosuppressive effects in the proliferation assays. In the macrophage polarization assays, IMCs suppressed M1 phenotype conversion, 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-1β in the recipient liver, while reducing the number of $F4/80^{+}$ macrophages at the transplantation site.

(2) Human IMCs exhibited immunosuppressive effects in the proliferation assays. IMCs suppressed the conversion to the M1 phenotype in THP-1 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 increase in M2 macrophage markers, CD163 and CD206 were observed. Higher doses of IMCs increased the secretion of pro-inflammatory cytokines, TNF-α, IL-6, and IL-1β.

(3) Human IMCs effectively protected pig pancreatic islets from LPS-stimulated THP-1 macrophage-mediated damage in an in vitro xenogeneic PITx model.

【Discussion】

The present study demonstrated that IMCs can promote a shift from the M1 to the M2 macrophage phenotype through direct cell-cell interactions, which was associated with decreased pro-inflammatory cytokine secretion, reduced NO production by activated macrophages, and increased IL-10 production by M2 macrophages. Consistent with previous reports showing that the suppression of macrophage activation prevents islet death, treatment with IMCs favored pancreatic islet survival in both murine syngeneic and xenogeneic human/pig macrophage-islet co-culture assays. 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 driving the subsequent adaptive immune response that leads to allograft rejection, attenuating the inflammatory response following PITx is critical for improving long-term graft function and survival. Further in vivo studies on allogeneic PITx are necessary to gain a deeper understanding of this therapy before its clinical application in PITx.

【Conclusion】

IMCs modulate macrophage polarization, promoting a shift towards the M2 anti-inflammatory phenotype and protecting pancreatic islets from macrophage-mediated damage. Combined with its intrinsic donor antigen-specific immunosuppressive capacity, IMC therapy is a promising strategy for improving the outcomes after PITx.