移植グループ

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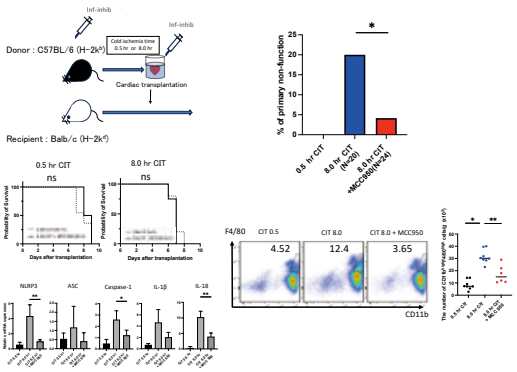
冷阻血中のインフラマソーム阻害が拒絶反応に与える影響とその基礎的機序解明

【背景】冷阻血時間（CIT）の延長は虚血再灌流障害や拒絶反応を増悪させることが知られている【目的】CIT中インフラマソーム阻害がグラフト生存期間延長効果を検討する。【方法】B6ドナー、Balb/cレシピエントとしたallo心移植モデルを用いた。グラフトはCIT中ヘパリン（1%）生食に保存した。インフラマソーム阻害（Inf-inhib）はNLRP阻害薬を灌流液中およびCIT中保存液に混じて使用した。【結果】CIT中のインフラマソーム阻害はグラフトサバイバルへ影響を与えなかった。しかしCIT30分では観察されなかった再灌流直後にグラフト機能不全を示す割合がCIT8時間では20%（n=20）あり、CIT8時間＋ Inf-inhibでは4%と減少傾向を示した（n=24、P<0.00010 vs. CIT 8時間）。CIT 30分の心グラフトへ移植後2日目に浸潤するCD11b^{high}F4/80^{high}細胞数は8.45x103個であり（n=8）、CIT8時間では同細胞数は31.5x10^{3}個であった（n=8、P<0.0001 vs. CIT 30分）。これらの細胞はCIT8時間 ＋ Inf-inhib群では21.7x10^{3}個と抑制された（n=6、P=0.46 vs. CIT 8時間）。【結語】CIT中インフラマソーム阻害は再灌流直後のグラフトに保護的な作用を示し、移植後早期のグラフト浸潤自然免疫細胞も抑制した。CIT中のグラフト内炎症制御による新しい治療戦略をさらに検証する。



膵島移植における効果的な免疫抑制法の開発

Introduction: Pancreatic islet transplantation (PITx) is a promising treatment option for patients with type 1 diabetes mellitus. Controlling the innate immune response is essential not only to prevent the rapid destruction of transplanted islets, but also for the long-term acceptance of allogeneic islet grafts. We have previously demonstrated that the adoptive transfer of donor antigen-specific immunomodulatory cells (IMCs) could induce tolerance in recipient patients after living-donor liver transplantation. We hypothesize that the same beneficial effects could be applied to PITx.
Methods: Based on our clinical trial, IMCs were generated by co-culturing C57BL/6 (H-2^b) mouse splenocytes with irradiated BALB/c (H-2^d) splenocytes in the presence of anti-CD80/86 monoclonal antibodies (mAbs) . The phenotypes of IMCs were assessed by flow cytometry. The immunosuppressive effect was evaluated by mixed lymphocyte reaction (MLR) . C57BL/6 splenocytes were stained with CellTrace Violet (CTV) and stimulated with irradiated donor (BALB/c) or 3rd party (C3H/HeJ, H-2k) splenocytes, and the proliferation of CTV-labelled cells was measured by flow cytometry. To study the effect of IMCs over macrophage polarization, RAW264 cells (0.5x106 cells/well) were cultured with IMCs (1x10^6 cells/well) and stimulated with lipopolysaccharide (LPS, 100 ng/mL) . The cells and supernatant were collected after 6 hours of stimulation, and the expressions of M1 and M2 markers were assessed by flow cytometry. The mRNA expression of pro-inflammatory cytokines was assessed by qPCR. Nitric oxide production by LPS-stimulated macrophages was also measured as a marker of macrophage activation using the Griess assay. Isolated pancreatic islets from BALB/c mice (50 islets) were cultured together with macrophages with or without IMCs, and LPS was added to the culture to evaluate the IMCs islet-protective effects. Islet count was performed at 12 hours after culture, and cells were collected to measure the mRNA expression of pro-inflammatory cytokines. Finally, a separate co-culture model was used to study the mechanisms behind IMCs anti-inflammatory effects. Transwell inserts with 0.4 μm porous membrane were placed into 12 well-plates. Macrophages were seeded in the lower chamber, and IMCs were added to the upper chamber. M1 and M2 markers were assessed by flow cytometry after 6 hours of LPS-stimulation.
Results: During the generation of IMCs, the cell number decreased from 80x106 to 8.8±2.8x10^6 (mean±SD, n=8) . CD4$^+$ and CD8$^+$ T cells were increased after culture. IMCs effectively impeded the proliferative response of C57BL/6 mouse splenocytes against BALB/c antigen in an IMCs-dose-dependent fashion. The treatment of RAW264 cells with IMCs at the time of LPS stimulation suppressed the expression of M1 marker CD86, and M2 marker CD163 was increased. M1/M2 ratio was lower in the treatment group compared to controls. Treatment groups showed a trend toward reduced mRNA expression of pro-inflammatory cytokines TNF-α and IL-1β, but IL-10 expression was not increased. The addition of IMCs reduced nitric oxide production compared to control groups. The islet counts in groups treated with IMCs was higher than untreated controls. CD86 expression was suppressed in the separate culture model, but CD163 was not increased.
Conclusion: IMCs showed both immunosuppressive and anti-inflammatory properties in vitro, and islet-protective effects were also observed. The strategy using IMCs may be a promising therapeutic approach to improve the outcome of PITx