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学位授与の要件	学位規則第4条第1項該当 医学系研究科未来医療開発専攻
学位論文名	Development of Donor-Specific Immunoregulatory T-Cells After Local *CTLA4Ig* Gene Transfer to Pancreatic Allograft （同種膵グラフトへのCTLA4Ig遺伝子導入によるドナー特異的免疫制御T細胞の誘導）
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論文内容の要旨

〔目的〕

Local CTLA4Ig gene transfer directly to the graft may have the potential to avoid the opportunistic infections and malignancies by systemic immunosuppression. In our previous studies using Bio-Breeding (BB) rats, local Adenovirus-mediated (Ad.) CTLA4Ig gene transfer could prevent the pancreas from autoimmune and alloimmune responses. This study investigated the potency of CD28/B7 costimulatory blockade by local Ad. CTLA4Ig gene transfer for induction of donor-specific tolerance and further examined the underlying mechanisms.

〔方法〕

Brown Norway (BN ; $RT1^n$)-pancreaticoduodenal grafts transfected with Ad. CTLA4Ig via intraarterial *ex vivo* perfusion were transplanted into streptozotocin-induced diabetic Lewis (LEW ; $RT1^l$) rats. FK506 was used as an immunosuppressive agent for 3 days at a dose of 1 mg/kg on the day before, the day of, and the day after pancreas transplantation (PTx). Expression of CTLA4Ig in the graft was detected by immunohistochemical staining. T lymphocytes were harvested and separated from spleen for mixed lymphocyte reaction (MLR) and cell-mediated lymphocytotoxicity (CML) assays. Flow cytometry was used for the analysis of splenic T-cell subpopulations from the tolerant recipients.

〔結果〕

Local Ad. CTLA4Ig gene transfer significantly prolonged graft survivals (>59.3 days). Further, the gene transfer indefinitely prolonged graft survivals (>156 days) when combined with a short course of FK506. CTLA4Ig was predominantly expressed in the grafts on day 4 posttransplantation. The expression was gradually diminished and was only slightly detectable at day >100. In the tolerant rats, proliferative

responses against BN antigens were inhibited up to the similar level which was comparable to those of naive rats. The T cells from tolerant recipients (>100 days) also showed poor cytotoxic responses. In the adoptive transfer experiment, the splenic T cells of tolerant recipients were able to suppress the rejection of BN, but not third-party Wistar Furth (WF ; $RT1^u$), cardiac grafts in irradiated (480 cGy) LEW recipients. The percentage of $CD4^+CD25^+$ splenic T-cells was significantly increased in tolerant recipients (13.53±4.06% vs. 6.06±0.56% in naive rats).

〔総括〕

1. CTLA4Ig gene tranfer to the pancreaticoduodenal allograft combined with a short course of FK506 induced an indefinite graft survival.
2. In MLR, proliferative responses of tolerant splenic T cells against BN antigen were inhibited up to the similar level which was comparable to those of naive rats ; In CML, splenic T cells from the tolerant recipients showed poor cytotoxic responses compared with those from naive or rejected recipients.
3. In the second cardiac graft challenge, a donor-specific tolerance was obtained.
4. Adoptive transfer experiments indicated the presence of immunoregulatory cells, $CD4^+CD25^+$ T cells, in the maintenance phase of tolerance.

We concluded that development of donor-specific immunoregulatory T cells might be responsible for the tolerance after local CTLA4Ig gene transfer to pancreatic allografts.

論文審査の結果の要旨

臓器移植における免疫抑制療法は急性期での免疫抑制剤の高容量全身投与による副作用やその後の日和見感染、悪性腫瘍の出現など様々な問題を有する。本研究では上記有害事象を回避する目的で、ラット移植膵に対する局所免疫抑制療法を行い、ドナー抗原特異的な免疫寛容の誘導を可能にした。免疫抑制の方法は T 細胞の活性化に不可欠な B7-CD28 分子間相互作用による共刺激（二次シグナル）を抑制する CTLA4Ig を移植膵へ直接遺伝子導入し、さらに術前後の３日間 FK506 を併用した。

寛容が成立したレシピエント脾 T 細胞を用いた MLR での増殖応答、ならびに CML での細胞傷害性は抗原特異的に抑制されており、さらに、同細胞の養子移入実験により、ドナー心の生着を認めた。この免疫寛容の機序は免疫制御 T 細胞の誘導によるものであると考えられた。

以上より、本研究は臓器移植における、より理想的な免疫抑制療法として意義深く、学位に値するものと考える。