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－在留中国人研究者研究助成－

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1．研究テーマ

膵グラフトへの CTLA4Ig 遺伝子導入による拒絶反応の制御

2．本年度の研究業績

(1) 学会・研究会等における発表 有 ・ 無 （学会名・演題）

第 39 回日本移植学会：Development of allorective T suppressor cells after local CTLA4Ig gene transfer to pancreatic allograft.

(2) 学会誌等に発表した論文 有 ・ 無 （雑誌名・論文名）

Transplantation 2004 (in press)： Development of donor-specific immunoregulatory T cells after local CTLA4Ig gene transfer to pancreatic allograft.

3．今後の研究計画

Pancreas transplantation (PTx) is the most effective method of normalizing glucose control. In terms of temporal relationship between PTx and reversal of diabetic complications, it has been extremely difficult but quite important to determine a 'point of no return'. Thus, it is of great clinical interest to evaluate the efficacy of PTx on diabetic secondary complications. PTx is not commonly used in type 2 diabetes, however, experience is accumulating of transplantation in type 2 diabetic patients with end-stage renal disease. Outcomes for these individuals are as good as for type 1 diabetes. Therefore, whether PTx could protect islet from glucose toxicity (one of the etiologies in type 2 diabetes) remains to be clarified as an interesting hypothesis. The purpose of this research are 1) to evaluate the effectiveness of PTx in preventing the progression of diabetic nephropathy and ocular complications, and to further investigate the reversibility of diabetic secondary complications in SDT rat after successful PTx. 2) To evaluate the potency of PTx in protecting islets from glucose toxicity in type 2 diabetic SDT rat.

4．指導責任者の意見

鐘剛君は平成13年4月大阪大学大学院医学系研究科（臓器制御外科学）に入学し、膵島移植、特に膵膵移植における研究に従事してきた。研究態度はまじめですでに2編の英文論文（Transplantation）にacceptされております。又、1編の論文を作成中です。研究内容は、糖尿病合併症に対する膵移植の効果と、移植膵に対する遺伝子導入による免疫抑制療法に関するものであり、臨床応用可能なものとして高く評価するものである。

指導責任者氏名 伊藤壽記 ㊞

5．研究報告書

別紙「研究報告書の作成について」に倣い、指定の用紙で作成して下さい。

研究発表または研究状況を記録した写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

※発表に当っては、*日中医学協会助成金*による旨を明記して下さい。

-日中医科協会助成事業-

膵グラフトへのCTLA4Ig遺伝子導入による拒絶反応の制御

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Abstract

CTLA4Ig gene transfer directly to graft tissue might have the potential to avoid the need for systemic immunosuppression. In our previous studies of BB rats, local adenovirus-mediated CTLA4Ig gene transfer protected the pancreas from autoimmune and alloimmune responses. This study investigated the potency of local CD28/B7 costimulatory blockade for induction of donor-specific tolerance and further examined the existing mechanisms. Methods. Brown Norway (BN; $RT1^n$)-pancreaticoduodenal grafts transfected with Ad.CTLA4Ig via intra-arterial *ex vivo* perfusion were transplanted into streptozotocin-induced diabetic Lewis (LEW; $RT1^l$) rats. Results. Ad.CTLA4Ig transduced grafts combined with a short course of FK506 resulted in indefinitely prolonged survival (>156 days vs. 19.5 days with FK506 alone). CTLA4Ig was predominantly expressed in grafts on day 4. The expression was gradually diminished, and was only slightly detectable at day >100. The proliferative responses against BN antigen were remarkably enhanced among recipients with rejected grafts, but the T cells from tolerant recipients (>100 days) showed poor cyto toxic responses. On adoptive transfer assay, the splenic T cells of tolerant recipients were able to suppress the rejection of BN, but not third-party Wistar Furth (WF; $RT1^u$) heart 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 naïve rats). Conclusions. CTLA4Ig gene transfer to the pancreaticoduodenal allograft combined with a short course of FK506 induces donor-specific tolerance. The mechanism of maintaining tolerance could be explained by development of splenic T suppressor cells.

Key Words　Pancreas transplantation; Gene transfer; CTLA4Ig; T suppressor cell;

Introduction:

To prevent immunological rejection of allografts, it is necessar y to administer systemic immunosuppressants to the recipients. However, these immunosuppressants have several side effects, including toxicity, an increased incidence of malignancy and susceptibility to opportunistic infections. However, CTLA4Ig gene transfer directly to the allografts has been found to inhibit immune responses in several organ transplantations by allowing production of immunomodulatory proteins in the donor grafts resulting in local rather than systemic immunosuppression (1-4). In the present study, we demonstrate that CTLA4Ig gene transfer combined with a short course of FK506 therapy consistently induced donor-specific tolerance in a rat pancreas allotransplant model. Furthermore. we document that immunoregulatory cells could be involved in the maintenance phase of tolerance induced by this strategy.

Materials and methods:

Male Brown Norway (BN; $RT1^n$), Wistar Furth (WF; $RT1^u$), and Lewis (LEW; $RT1^l$) rats, 8-10 weeks of age, were utilized. BN and LEW rats were purchased from Charles River Japan, Inc. (Kanagawa, Japan). WF rats were bred and maintained at Osaka University Animal Facilities. LEW rats were used as recipients, BN rats as donors, and WF rats as third-party donors. Pancreaticoduodenal grafts were transplanted into streptozotocin (65mg/kg; Sigma, St. Louis, MO) –induced diabetic LEW recipients as described previously (5). Graft function was monitored by daily measurement of blood glucose during the first 2 weeks and at least twice a week thereafter. Rejection was considered when the blood glucose level was over 200mg/dl for two consecutive days. Graft rejection was further assessed by histological examination.

Results:

Ad.CTLA4Ig transduced grafts combined with a short course of FK506 resulted in indefinitely prolonged survival (>156 days vs. 19.5 days with FK506 alone). CTLA4Ig was predominantly expressed in grafts on day 4. The expression was gradually diminished, and was only slightly detectable at day >100. The proliferative responses against BN antigen were remarkably enhanced among recipients with rejected grafts, but the T cells from tolerant recipients (>100 days) showed poor cytotoxic responses. On adoptive transfer assay, the splenic T cells of tolerant recipients were able to suppress the rejection of BN, but not third-party Wistar Furth (WF; $RT1^u$) heart in irradiated (480 cGy) LEW recipients. The percentage of $CD4^+CD25^+$ splenic T cells was significantly increased in tolerant recipients ($13.53 \pm 4.06\%$ vs. $6.06 \pm 0.56\%$ in

naïve rats).

Discussion:

In this study, we first demonstrated that CTLA4Ig gene transfer to the pancreaticoduodenal allograft combined with a short course of FK506 could induce donor-specific tolerance related to an active suppression mechanism. CTLA4Ig, a recombinant fusion protein, binds to murine B7 with high avidity and blocks pancreatic islet rejection by directly affecting T cell recognition of $B7^+$ antigen-presenting cells (APCs) (6). Indeed, CTLA4Ig gene therapy has been effectively applied in various transplantation models. The strategy of transferring ge nes that encode CTLA4Ig protein capable of suppressing immune responses within the local microenvironment of the graft is of advantage to avoid systemic immunosuppression, because B7 molecules expressed on APCs are more effectively blocked within the graft (7). Recently, Grohmann et al. (8) reported that CTLA4Ig induces indoleamine 2,3-dioxygenase (IDO) expression in $CD11c^+$ dendritic cells (DCs) from the murine spleen. In a model of T cell adoptive transfer, Mellor et al. (9) showed that CTLA4Ig completely blocks $CD8^+$ T cell clonal expansion in an IDO-dependent manner. This evidence suggest that CTLA4Ig can reasonably be expected to function in tolerance induction in which the IDO mechanism might be involved.

As an additional important finding, a mild lymphocytic infiltration was found in the long-term accepted pancreatic graft. In the CTLA4Ig gene transfer to cardiac allografts from LEW.1W to LEW.1A rats, Guillot et al. (7) reported that local expression of CTLA4Ig could modulate the expression of activation markers associated with graft rejection. In this study, the persistent mononuclear infiltrate without parenchymal damage indicates the persistence of cellular responses against the grafted tissue, most likely reflecting the establishment of tolerogenic mechanisms. We hypothesize that a portion of the alloreative clones might have been deleted from these graft-infiltrating cells, and an additional aspect of the mechanism may be interference with the cytokine cascade. Moreover, it is possible that suppressor cells are not only generated in the spleen but also in the graft itself and that their late appearance in the spleens of tolerant animals is due to delayed migration from the graft to the spleen. To clarify these points, further studies, especially a detailed analysis of immune responses of graft-infiltrating cells and cytokine expression, or phenotypic and functional analysis of the lymphocyte subpopulation in the grafts are needed.

In conclusion, CTLA4Ig gene transfer to the pancreaticoduodenal allograft combined with a short course of FK506 induces donor-specific tolerance. CTLA4Ig, the

immunoregulatory reagent produced locally, protects pancreatic grafts from alloimmune destruction during the early stage of acceptance. Subsequently, the appearance of splenic T cells with donor-specific suppressive capacity plays a pivotal role in maintaining tolerance. This strategy may have clinical applications for tolerance induction in pancreatic transplantation.

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注：本研究は、2003年10月27日、「第39回日本移植学会」にて口演発表。
2004年5月16日、「American Transplant Congress」にて発表。
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