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学 位 論 文 (要約)

ASKP1240, a Fully Human Anti-CD40 Monoclonal Antibody,
Prolongs Pancreatic Islet Allograft Survival in Nonhuman Primates
(カニクイザル臍島移植モデルにおける完全ヒト抗 CD40 抗体、
ASKP1240 のアロ臍島生着期間延長効果)

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【Background and Objectives】

The primary outcomes and safety of pancreatic islet transplantation (PITx) have improved steadily over the decades. However, the toxicity associated with immunosuppression is still a major obstacle that prevents long-term allogeneic islet acceptance. Although steroid avoidance has been shown to be feasible, calcineurin inhibitors have significant concomitant side effects, including nephrotoxicity and diabetogenicity. Furthermore, sirolimus also negatively affects β cell viability and regeneration as well as the engraftment and function of transplanted islet grafts. A new immunosuppressive strategy that avoids the use of these conventional immunosuppressants and introduces a suitable, less toxic agent is essential for improvement of clinical PITx. The CD40-CD154 pathway is representative of various co-stimulatory signaling pathways that play a critical role in alloimmune responses. The blockade of this pathway is a key therapeutic strategy to induce donor-specific immunosuppression and/or tolerance. However, the clinical application of anti-CD154 mAbs is not feasible, mainly because of thromboembolic complications. Alternatively, inhibition of the counter receptor CD40 has recently received attention for the blockade of CD40-CD154 costimulation. Previously, we demonstrated that a fully human anti-CD40 mAb, ASKP1240, markedly prolonged renal and hepatic allograft survival in cynomolgus monkeys without causing apparent adverse effects. In this study, we evaluated the effect of ASKP1240 on allogeneic PITx in cynomolgus monkeys.

【Materials and Methods】

Purpose-bred male cynomolgus monkeys (*Macaca fascicularis*) aged 5.01 ± 0.74 years and with a body weight of 5.26 ± 0.97 kg were used in the study. Diabetes was induced by a total pancreatectomy. Two weeks later, pancreatic islets were isolated from a donor monkey, and allogeneic islets, 8,201-12,438 IEQ/kg, were transplanted into the recipient liver via the portal vein. PITx was performed across ABO blood type compatible but MHC class II DRB mismatched monkey pairs that was also confirmed by MLR (stimulation index (S.I.) >4.6). Three animals receiving no treatment served as the control ($n = 3$). For the induction treatment group ($n = 5$), ASKP1240 (10 mg/kg) was given intravenously on days 0, 4, 7, 11, and 14. For the maintenance treatment group ($n = 4$), weekly ASKP1240 (5 mg/kg) administration was subsequently continued for up to 6 months after PITx. Graft rejection was considered when the fasting blood glucose (FBG) level exceeded 250 mg/dl for 3 consecutive days. Humoral and cellular immune response was assessed by examining serum anti-donor Ab level and IFN- γ ELISpot assay. Graft histopathology of the liver was performed at the time of the animal death or sacrifice. Intra venous glucose tolerance test (IVGTT) was examined sequentially to assess graft function.

【Results】

Following PITx, FBG levels were normalized promptly in all animals. In the control animals, transplanted islet allografts were rejected within 9 days, and fasting serum C-peptide levels were reduced to an undetectable level on the next day of rejection. In contrast, treatment with ASKP1240 maintained normoglycemia in the long term, and serum C-peptide was detected during the period of normoglycemia. The ASKP1240 induction treatment prolonged allograft survival to 210 (I-#3), 250

(I-#4), and more than 608 (I-#5) days; 2 animals with functional islet grafts died on days 15 (I-#1) and 23 (I-#2) because of surgical complications. The ASKP1240 maintenance treatment also markedly prolonged islet allograft survival time to 523 (M-#4) and more than 96 (M-#1), 115 (M-#2), and 607 days (M-#3). Two animals in the ASKP1240 maintenance treatment group developed gastric dysmobility and were sacrificed at 96 (M-#1) and 115 (M-#2) days after PITx without apparent rejection. In animals rejecting islet allografts (I-#3, I-#4, and M-#3), The frequency of donor-antigen reactive T cells in the periphery increased gradually after PITx. Anti-donor IgG antibodies were evident at 127, 126, and 383 days after PITx, and the islet allograft was rejected thereafter in animals I-#3, I-#4, and M-#3, respectively. After rejection, blood glucose levels in response to IVGTT showed a diabetic pattern, and C-peptide and insulin levels did not respond to glucose injection. In contrast, in the animals accepting allografts over the long term (I-#5 and M-#4), both direct and indirect responses against donor antigens were strongly suppressed. In addition, formation of antidor IgM and IgG antibodies was also completely abolished throughout the study period. At the time of sacrifice due to the termination of the study, durable islet allografts also were confirmed by insulin staining, without evidence of cellular infiltration of CD3⁺, CD4⁺, and CD8⁺ cells into or surrounding the islet allografts as determined by immunohistochemistry. Blood glucose, C-peptide, and insulin levels in response to IVGTT remained normal even at the end of the study. The number of peripheral CD4⁺ cells increased especially in accepted animals (I-#5 and M-#4). The number of peripheral CD20⁺ cells slightly decreased during the period of ASKP1240 administration, and it increased after the discontinuation of ASKP1240 administration in some animals (I-#4, I-#5, and M-#4). The number of peripheral CD4⁺CD25⁺Foxp3⁺ T-cells did not increase, even in accepted animals (I-#5 and M-#4), and there was no clear evidence of regulatory T cell induction by ASKP1240. No serious side effects, including thromboemboli, were observed in animals receiving the ASKP1240 treatment.

【Discussion】

In this study, we demonstrate that ASKP1240 monotherapy markedly prolonged the survival of pancreatic islet allografts. Notably, allografts survived for more than 600 days in 2 animals, even though the ASKP1240 treatment was terminated shortly after PITx, and the function of the transplanted islets in these animals was confirmed by C-peptide production and IVGTT evaluated at the time of euthanasia. The result was different from that in our previous ASKP1240 trial in kidney transplantation, in which the grafts became positive for C4d and IgG, and all of the renal allografts underwent chronic nephropathy. Additionally, the identical ASKP1240 regimen resulted in chronic graft rejection after drug cessation in our previous liver transplantation study in cynomolgus monkeys. In the current PITx study, we found that both cellular and humoral alloimmune responses were not elicited, even after ASKP1240 cessation in the 2 animals that accepted islet allografts (I-#5 and M-#4). The discrepancy regarding the efficacy of ASKP1240 in the current PITx and in Tx models in other organs is likely caused by different immunological characteristics of the transplants. Pancreatic islets mainly consist of β cells that only express major histocompatibility complex (MHC)

class I, although low expression of MHC class II may exist because of contaminating leukocytes.

Our IFN- γ ELIspot assay revealed that the cellular immune response against third-party but not donor antigens recovered after ASKP1240 cessation in animals that did not show signs of rejection (I-#5 and M-#4). These findings suggest that ASKP1240 treatment induced donor-specific tolerance in the 2 animals that accepted islet allografts. Alongside, the number of peripheral CD4 $^{+}$ T cells and CD20 $^{+}$ B cells increased in these animals (I-#5 and M-#4). Previous studies demonstrated that higher expression of CD4 $^{+}$ CD25 $^{+}$ Foxp3 $^{+}$ and $\gamma\delta$ TCR $^{+}$ T-cells or an increased number of peripheral B cells was associated with a state of clinical operational tolerance. In our flow cytometric analyses, however, the number of peripheral CD4 $^{+}$ CD25 $^{+}$ Foxp3 $^{+}$ T-cells did not increase, and there was no clear evidence of regulatory T cell induction by the ASKP1240 treatment. With regard to B cell expansion, we have previously reported that suppression of germinal center formation in the spleen and lymph nodes was noted in some ASKP1240-treated animals, and we speculated that this suppression caused a rebound increase in the number of B cells in the periphery after ASKP1240 cessation in kidney transplant recipients; however, such an event was not reported for other anti-CD40 mAbs. The potential role of B cells in the tolerant state remains unclear, and further studies are necessary to define the role of B cell expansion after PITx.

Although the exact mechanisms responsible for islet allograft rejection remain unclear, previous studies have shown that cellular immune responses play a primary role in islet graft rejection. A histologic examination of islet allografts in the liver revealed that the cellular infiltrate surrounding the rejected islets was strongly positive for CD3 $^{+}$ T cells but not for CD20 $^{+}$ B cells, C4d, or neutrophils. Indeed, immunodepletion or modulation of T cells has emerged as a valuable adjunct treatment after PITx. Among several co-stimulatory signals, the CD40-CD154 pathway is a representative cascade that has been shown to play a central role in the activation of immune cells, including T cells, and in the priming of alloimmune responses. Blockade of this signaling pathway by various approaches has been shown to induce potent immunosuppression against cellular immunity and tolerance to allografts in experimental organ transplantation. It has been shown that other anti-CD40 mAbs, i.e., 2C10 and 3A8, are also effective for preventing rejection of islet allografts in a non-human primate PITx model. The efficacy of the blockade of CD40 signaling with regard to cellular immune responses was comparable to our results, in which both direct and indirect cellular alloimmune responses were suppressed by ASKP1240 monotherapy during the treatment period; the effect continued to some degree even after ASKP1240 became absent. With regard to the prolongation of islet allograft survival time, ASKP1240 appeared to have a stronger effect than other anti-CD40 mAbs, e.g., Chi220, 2C10, or 3A8, but was equivalent to or less potent than anti-CD154 mAbs.

Transplanted islets have not been considered to be at risk for antibody-mediated injury because pancreatic islets are cells and not a vascularized organ. However, it is increasingly being recognized that B cells play a substantial role in graft damage, not only through their derived antibodies but also through interactions between T cells and other immune cells. Previous studies have shown that the

presence of anti-HLA Abs before transplantation was associated with a significant loss of C-peptide after clinical PITx and that flow cross match alone was unable to predict outcome. Furthermore, pancreatic islet transplant recipients developed *de novo* DSA after clinical and non-human primate PITx. We observed a similar trend in our non-human primate PITx model, as all animals (I-#3, I-#4, and M-#3) that showed islet rejection had developed DSA before graft rejection, although further studies are necessary to define the role of DSA in islet allograft rejection.

Because CD40 is constitutively expressed on dendritic cells, macrophages, and B cells, signaling via CD40 has been shown to be crucial for B cell activation and immunoglobulin class switching. Indeed, we have demonstrated in the present study that ASKP1240 abrogated the formation of DSA, both IgM and IgG isoforms, and anti-ASKP1240 antibodies, at least during the treatment course. These results were consistent with our previous findings for liver and kidney transplantation using ASKP1240. In contrast, 60% of PITx monkeys receiving the 3A8-based regimen had developed DSA at the time of rejection. Considering that addition of CTLA4Ig to this 3A8-based regimen strongly prevented generation of DSA and markedly prolonged allograft survival, it seems likely that DSA is mainly T cell-dependent, and that controlling donor-reactive helper T-cell responses in addition to DSA formation is a key issue to achieve long-term engraftment of allogeneic islet grafts. Because ASKP1240 can potently inhibit both cellular and humoral immune responses, CD40 blockade by ASKP1240 may be advantageous for immunosuppression after PITx.

Clinical trials on anti-CD154 mAbs were terminated because of thromboembolic complications; therefore, side effects following ASKP1240 treatment have always been a major concern. In the present study, no histopathological evidence of thromboembolism was observed in any animal during ASKP1240 treatment, after drug cessation, or at the time of autopsy. In addition, no abnormal findings were seen in peripheral blood hematology and chemistry or in tissues such as the brain, heart, lung, intestine, or kidneys.

【Conclusion】

We conclude that the fully human anti-CD40 mAb, ASKP1240, induced potent immunosuppressive effects for pancreatic islet allografts without causing serious side effects. CD40 blockade by ASKP1240 suppressed cellular and humoral alloimmune responses and prevented rejection for the duration of therapy; furthermore, it allowed long-term acceptance of islet allografts in 2 cynomolgus monkeys. ASKP1240 seems to be a promising agent for immunosuppression after PITx.