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添付資料： **研究報告書**

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2．研 究 テ ー マ Effect of FTY720 on donor and recipient T cells in the tolerant rats to GVHD after small bowel transplantation

3．成 果 の 概 要 (100字程度)

FTY720 altered lymphocytes recirculation and prevented donor-derived T cell into the target organs, resulting in prolong the recipient survival in the acute GVHD of small bowel transplantation(SBTx). And also, FTY720 induced lengthy tolerance of GVHD in SBTx. The possibility is continually through mediating the donor and recipient T cell subpopulation and associated with down-regulated Th1 and Th2 immune response.

4．研 究 業 績

(1) 学会における発表 無 ・ 有 (学会名・演題)

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(2) 発表した論文 無 ・ 有 (雑誌名・題名)

—日中医学協会助成事業—

小腸移植におけるＧＶＨＤの寛容期に対するFTY720の効果

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ABSTRACT

In the small bowel transplantation (SBTx), graft-versus-host disease (GVHD) of mediated by donor-derived T cells recognizing host major histocompatibility complex (MHC) alloantigen is an important immunological event influencing the life in the experimental and clinical situation. Donor T cells are crucial for target organ injury in GVHD. Our previous study determined that FTY720 altered lymphocytes recirculation and prevented donor-derived T cell into the target organs, resulting in prolong the recipient survival in the acute GVHD of small bowel transplantation. In this study we further report a long-term effect of FTY720 on the tolerant recipient of GVHD following SBTx. Heterotopic total SBTx was performed from Wistar-Furth (WF) rat to (WF x ACI) F1 rats. Recipients was administered by a low dose FTY720 (0.5 mg/kg/day) for 14 days after SBTx. The subset and subpopulation of donor and recipient derived T cells and the expression of cytokine production were evaluated. On POD150 the donor-derived $CD4^+$ and $CD8^+$ T cell subpopulation were retained in the low level and almost were similar to the level of POD14 in the target tissues of host. However, the recipient derived T cells were increased from the acute phase to long-term phase of GVHD in peripheral blood. In contrast, the $CD4^+$ and $CD8^+$ T cell subpopulation in mesenteric lymph nodes and Peryer's patch were recovered in a low level on POD150. The production of Th1 cytokine (IFN-γ, IL-2) and Th2 cytokine (IL-4, IL-10) were significantly lower level in the chronic phase of GVHD in target tissues by FTY720 treatment. FTY720 induced lengthy tolerance of GVHD of SBTx. That possibility is continually through mediating the donor and recipient T cell subpopulation and associated with down-regulated Th1 and Th2 immune response.

Key word: Small bowel transplantation, GVHD, FTY720, donor-derived T cells, IFN-γ.

Introduction The donor-specific tolerance induction has the potential to render the host susceptible to GVHD because of an unopposed reaction of the graft against the host [1]. Previous studies have determined donor T cells are crucial for the development of GVHD [2]. It is seem to very important to understand lymphocytes trafficking in the target organ for the immune responds of recipients. The unique mechanism of FTY720 associated with altered lymphocytes recirculation has been determined [3, 4,]. During GVHD of SBTx, FTY720 caused a significantly prevention in the subpopulation of donor-derived $CD4^+$ and $CD8^+$ T cell into the target organs and prolong the survival of recipients. However, the long-term influence and results had not been investigated. To address this question, we examined the migration of donor and recipient T cells, the pathological changes and cytokine production of GVHD on the mesenteric lymph nodes (MLN), Peryer's patch (PP) and lamina propria (LP) of host intestinal in the different phase, using the parent-into-F1 model of GVHD in SBTx. The patterns of migration of both $CD4^+$ and $CD8^+$ T-cell were recorded and evaluated in relation to acute and tolerance phase of GVHD.

MATERIALS AND METHODS

Animals and Experimental Design

Heterotopic SBT in rat was performed by interposing the graft using the cuff technique, as previously described [5]. Male

Wistar-Furth (WF) rats were used donors, and male F_1 (WF x ACI) hybrid rats were used recipients. The protocol comprised three groups: (1) the syngeneic group(group 1, n=6); (2)untreated GVHD control group (group 2, n=8);(3) F1 recipient rats were treated orally with 0.5mg/kg FTY720 by way of gastric tube daily from days 0 to 13 after SBTx (group 3, n=8). FTY was donated by Novartis Pharma AG (Basel, Switzerland) as dry powder.

Lymphocytes Isolation and Flow Cytometry

Lymphocytes were isolated from MLN, PP, and LP of host and PB. PB was spun on density separation medium (Ficoll-Paque Plus, Uppsala, Sweden). PP and LP lymphocytes were prepared by an enzymatic dissociation method, using collagenase as described [6]. FITC-conjugated anti-rat $RT1A^{ab}$ (C3) were purchased from Pharmingen (San Diego, CA), PE-conjugated anti-rat CD4 (W3/25). CD8 (OX-8), were obtained from Serotec (Oxford, UK). The stained cells were analyzed using FACScan and Cell quest software (Becton Dickinson, CA).

Cytokine Production in Culture Supernatants

MLN, PP and LP lymphocytes were culture for 48 hr in 24well plates that had been coated with carbonate buffer (pH 9.6) containing 10 μ g/ml mouse anti-rat CD3 mAb (clone 1F4; Serocet, Oxford, UK). Supernatants from culture plates were assayed for interleukin (IL)-2, IL-4, IL-10 and interferon (IFN)-γ by solid phase sandwich enzyme-linked immunosorbent assays (ELISA) kit (Biosource International, Camarillo, CA).

Statistical Analysis

Results are expressed as mean±SD; Group mean values were compared by two-tailed Student's t-test. A value of $P < 0.05$ was considered significant.

RESULTS

FTY 720 Reduces Target Organ Injuries Caused by GVHD

The recipients survival rate were significant prolonged over 150 days by FTY720-treatment in a low dose (0.5mg/kg) and progressive gained the body weight, in contrast, the GVHD control recipients resulted in weight loss and death. In POD14 and POD150, FTY 720 treated group inhabited histopathology changes in the small bowel, such as surface erosion, villous blunting, and cellular infiltration in the lamina propria the architecture remained intact.

Effect of FTY720 on Donor and Recipient Derived T Cells

On the tolerant recipients of GVHD (POD150 group), the donor-derived $CD4^+$ and $CD8^+$ T cell subpopulation were retained in the low level in MLN, PP and PB of host. But in LP, the increase tendency was observed compare with POD14. Very interestingly in PB, the percentage of recipient-derived $CD4^+$ and $CD8^+$ were significantly decreased in acute phase and steadily increased in the tolerant phase of GVHD compared with GVHD control group (Fig 1A). In contrast, in MLN and PP of recipient, the subpopulation of $CD4^+$ and $CD8^+$ T cell was from a steady increase to decrease in this two phase of GVHD. (Fig 1 C, D). However, in LP of host, the subpopulation of $CD4^+$ and $CD8^+$ T cell of were reduced gradually from acute phase to the tolerant phase in the FTY-treatment group. (Fig1B).

Effect of FTY720 on Cytokine Production in Target Tissue

Further analyzed cytokine level on POD150 (Figure 2), we found the production of cytokine were generality reduced in MLN, PP and LP of recipient, and almost the same as the level of naive rats. The IFN-γ level in all site ($P<0.01$) and the IL-2 level in PP and LP ($P<0.05$) were obviously lower than untreated GVHD recipient. Th2-type cytokine analysis show the IL-4 level in PP ($P<0.05$), MLN ($P<0.01$) and IL-10 level in PP and MLN ($P<0.01$).

DISCUSSION

Small bowel transplantation had limited success presumably because the immunogenicity of graft elicits such a vigorous response that a more intense regimen of immunosuppression is required. Our previously experiment has shown FTY720 in

a low dose (0.5 mg/kg) treatment for 14 days inhibited the acute GVHD immune responds and prolong the survival of recipients over 100 days. In the tolerant recipient, the lasting effect also is shown in POD150, the donor-derived $CD4^+$ and $CD8^+$ T cell subpopulation were retained in the low level in MLN, PP and PB of host. However, the changes of recipient-derived T cells were differently. In the tolerant recipients, the percentage of recipient-derived $CD4^+$ and $CD8^+$ were increased in PB compared with the acute phase. In contrast, in MLN and PP of recipient, the subpopulation of $CD4^+$ and $CD8^+$ T cell was from a steady increase to decrease compared with untreated group. Our results suggest that the effect of homing donor-derived T lymphocytes into the secondary lymph tissues were possible ongoing by FTY720 treatment, but failed on the recipient-derived T cells in the long-term phase. Further investigation found that the Th1 cytokine (IFN-γ, IL-2) and Th2-type cytokine (IL-4, IL-10) production level were obviously lower in MLN, PP and LP of recipient than untreated GVHD recipient, and different with the acute phase that only inhibited the Th1 cytokine production. Thus, FTY720 induced lengthy tolerance of GVHD in rat SBTx model, by steady redistributed the donor and recipient $CD4^+$ and $CD8^+$ T cell subpopulation and progressively inhibited Th1 and Th2 immune response. These results may possibility offer some helps for evaluate the GVHD of solid organ transplantation.

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Figure 1

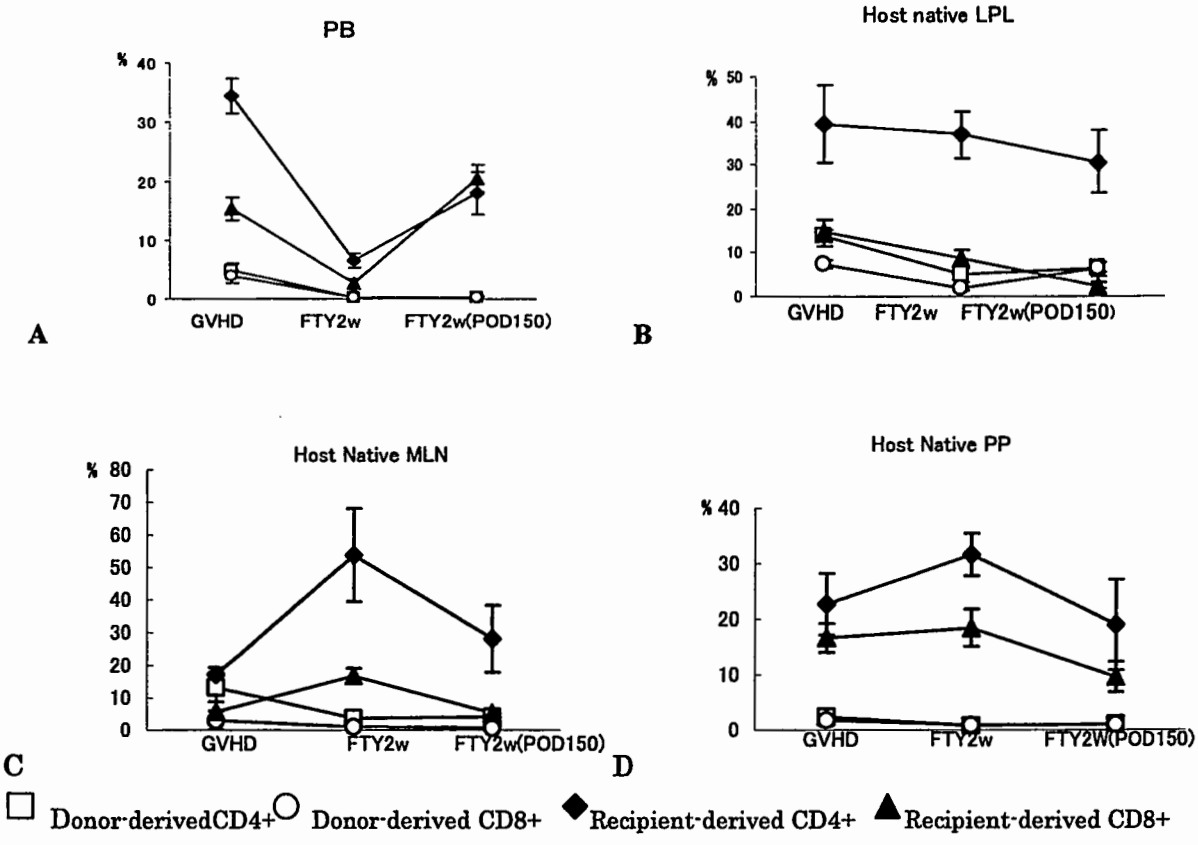

Figure 2