의학석사 학위논문

Functional Characteristics of Peripheral CD4/CD8 Double Positive T cells as a Specific Biomarker for Graft Rejection in Nonhuman Primate Transplantation Model

영장류 이식 모델에서 거부반응의 바이오마커로서 CD4/CD8 DP 림프구의 기능적 특성

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최윤정

A thesis of the Degree of Master of Science

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The Department of Medicine,

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by

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(Directed by Prof. Jae Il Lee)

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최윤정

최윤정의 의학석사 학위논문을 인준함

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ABSTRACT

Previous observation has shown that there were substantial presences of CD4/CD8 double positive (DP) T cells in peripheral blood and secondary lymphoid organs in human and animals. These extrathymic DP T cells have been described in multiple human diseases. Some studies reported that peripheral DP T cells have the cytotoxic potential in diseases such as LCMV, HIV, and cancer. Others reported that these cells have more helper functions in systemic sclerosis and rheumatoid arthritis. However, it is not yet clearly explained whether there are any correlations of DP T cells in peripheral blood with transplantation, and if so, the functional characteristics of peripheral DP T cells in nonhuman primates was examined. DP T cells were functionally equivalent to either conventional CD4 or CD8 T cells with respect to their helper or cytotoxic activity. DP T cells highly expressed CXCR5 and PD-1 levels, and showed equivalent secreting capacity of IFN-γ, IL-4, and IL-21 as compared to CD4 T cells. They also have highly producing capacity of granzyme B and perforin as compared to CD8 T cells. In addition, these cells expressed eomesodermin (Eomes) and promyelocytic leukemia zinc finger protein (PLZF) in steady status. In islet transplantation model, it turns out that absolute number of DP T cells were positively correlated with graft rejection, whereas this was not the case in long-term survival group. Effector memory T cell (TEM) subpopulations of DP T cells were significantly increased in only the graft rejection group, unlikely TEM in CD8 T cells which revealed increase both rejection and survival group. Taken together, peripheral DP T

cells have dual functions in helper and cytotoxic immune responses, and also have an innate-and memory-like phenotype. In that positive correlation with graft survival, it suggests that DP T cells may play a crucial role in graft rejection mechanism in organ transplantation.

Keywords: DP T cells, Innate T cells, Memory phenotype, Helper function, Cytotoxic activity, Transplantation, Rhesus monkey

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LIST OF ABBREVIATIONS

DP : Double positive

DN : Double negative

SP : Single positive

Eomes : Eomesodermin

PLZF : Promyelocytic leukemia zinc finger protein

IL : Interleukin

PBS : Phosphate buffer saline

FBS : Fetal bovine serum

IFN-γ : Interferon-gamma

TCR : T cell receptor

TN : Naïve T cell

TCM : Central memory T cell

TEM : Effector memory T cell

NK : Natural killer

INTRODUCTION

$CD4^+/CD8^+$ double positive (DP) T cells are well known as one of the T cell developmental stages within the thymus, before differentiation into either the CD4 single positive (SP) or CD8 single positive lineage. Lymphoid progenitor cells that enter the thymic cortex do not express both the T cell receptor (TCR) and the CD4 and CD8 co-receptors, so called double negative stage of T lymphocyte maturation. Once productive TCR α and β chain rearrangement is achieved, the affinity of TCR for the major histocompatibility complex (MHC) is sequentially tested [1]. DP T cells expressing TCR with appropriate affinity for self MHC–peptide complexes presented on cortical epithelial cell are positively selected, being differentiated into either $CD4^+$or $CD8^+$ SP cells and finally migrate into thymic medulla [1]. In this stage, the expression of CD4 and CD8 molecules is regulated by a very strict transcriptional program involving the transcription factors called Runx3 and ThPOK [2].

It is known that the expression of the CD4 and CD8 molecules by mature T lymphocytes is mutually exclusive [3]. Early 2000^{th}, the presence of a small proportion of DP T cells in human peripheral blood has been reported [4-6]. In addition, the majority of DP T cells were found in the peripheral blood and secondary lymphoid organs of swine, monkeys, and chickens [7]. These DP T cells in peripheral blood are presumed to be a subset from immature thymocytes or extrathymic events. There are two hypothesis in terms of DP T cells developmental pathway; one is that positive thymic selection fails to

induce extinction of both co-receptors and subsequently, DP T cells might easily pass through [1]. The other one is that under certain circumstances (i.e., diseases) mature SP T cells might be able to get another co-receptor, CD4 or CD8 molecule, whereas variety of inflammatory cytokines are secreted [8-12].

Molecular analysis of several innate T cell lineages has identified key transcription factors which regulate the signature functions of each cell subset [13-15]; both promyelocytic leukemia zinc finger protein (PLZF) and eomesodermin (Eomes) are most prominent transcription factors. iNKT cells and human peripheral MAIT cells are representative cell types which express PLZF [9, 11-16]. Recent reports demonstrated that PLZF positive CD4 T cells during the thymic development are responsible for generating Eomes positive thymic $CD8^+$ T cells [17-19]. It has been reported that this memory-like $CD8^+$ T cells expressing Eomes are additional subsets of innate T cells [20]. Therefore, whether DP T cells are able to express Eomes and PLZF transcription factor is one of the key findings in this study. If DP T cells have innate functions like $Eomes^+$ $CD8^+$ T cells, they might play an essential role in severe inflammatory condition such as organ transplantation.

As was well known, in a variety of auto-reactive conditions particularly thyroiditis [21], atopic dermatitis [22], systemic sclerosis [23], and rheumatoid arthritis (RA) [24], DP T cells seem to play a key role in disease progression, in terms of helper functions. On the other hand, this type of cells has another functional property which is the cytotoxic potential in viral infections and cancer diseases by expressing high level of FasL and secreting

of IFN-γ [2, 25]. In addition to these diseases, transplantation is also known to induce potent inflammatory conditions.

In this study, the characteristics of peripheral DP T cells including cytokines, expression markers, transcription factors, and enzymes were analyzed, and also their correlation with graft rejection in islet transplantation model was investigated.

MATERIALS AND METHODS

1. Subjects

Eight male and fifteen female adult rhesus macaques were used in this study. Their ages ranged from 48 to 72 months (60.3 ± 5.1) and body weights ranged from 3.72 to 5.7 kg (4.44 ± 0.7). After being imported from China, a quarantine process of 1 month was conducted with the subjects in a good condition. Each monkey was housed in a single cage and had daily access to biscuits (2050 Harlan, Teklad Diets, Madison WI, USA) with some fresh fruits and vegetables, and unlimited access to water. Their room was maintained at 24 ± 4℃ at a relative humidity of 50 ± 10%, with an artificial light-dark cycle of 12:12 (7:00 AM onset) and with 13 - 18 air changes per hour. All animals used in this study were cared in a strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2. Sample

Heparin- or EDTA-anticoagulated whole blood was obtained from the monkeys for a functional analysis and phenotyping. Peripheral blood mononuclear cells (PBMCs) were separated by the gradient method with Ficoll-Paque (GE healthcare, Uppsala, Sweden). The isolation of lymphocytes from mesenteric lymph node (MLN), thymus, spleen, and liver was performed

after autopsy (n =5). Then, the isolated tissues were minced into a single-cell suspension and then re-suspended in RPMI 1640 medium supplemented with 10% FBS at 4℃.

3. Cell sorting

Peripheral blood mononuclear cells (PBMCs) from the monkeys were stained with anti-CD4 and anti-CD8 antibody. PBMCs were re-suspended in PBS supplemented with 1% FBS. The sorting was performed with BD FACS Aria instrument (BD Bioscience, San Diego, CA, USA).

4. Flow cytometric analysis

Fluorochrome- or biotin-labeled human monoclonal antibodies against the following antigens: CD8α (SK1), CD4 (L200), CD3 (SP34-2), CD28 (CD28.2), CD95 (DX2), CD1b (SN13), CD8β (SIDI8BEE), HLA-DR (G46-6), CXCR5 (MU5UBEE), and PD-1 (EH12.2H7) were purchased from BD Bioscience (San Jose, CA, USA), eBioscience (San Diego, CA, USA), and BioLegend (San Diego, CA, USA). Single-cell suspensions were labeled with antibodies for 30 minutes at 4℃. For intracellular labeling, prepared cells were re-suspended in a mixture of fixation and permeabilization buffers from the Foxp3 staining buffer kit (eBioscience, San Diego, CA, USA). Labeling was performed using Eomes (BD Bioscience, San Jose, CA, USA) and PLZF (eBioscience, San Diego, CA, USA) antibody. Flow cytometry was performed

on a FACS Calibur (Becton Bioscience, Mountain View, CA, USA) and LSR Fortessa (Becton Bioscience, Mountain View, CA, USA). All data were analyzed using the FlowJo software (TreeStar, Ashland, OR, USA).

5. Staining for cytokine analysis

For the intracellular cytokine assay, peripheral CD4, CD8, and DP T cells from the monkeys' whole blood were sorted and stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (PMA), 1.5 μM ionomycin (Sigma-Aldrich, St Louis, MO, USA), and 6.7 μg/mL monensin (Sigma-Aldrich, St Louis, MO, USA) for 6 hours at 37℃ in a CO_2 incubator. After the cell culture, they were washed with complete media (RPMI and 10% FBS) and re-suspended with staining buffers (PBS, 0.5% BSA, and 0.5 mM EDTA). Then, these cells were fixed, permeabilized, and labeled with anti-IL-4 (BioLegend, San Diego, CA, USA), anti-IL-21 (BioLegend, San Diego, CA, USA), and anti-IFN-γ (BioLegend, San Diego, CA, USA).

6. Staining of granzyme B and perforin

Peripheral CD4, CD8, and DP T cells from the rhesus monkeys were sorted. These cells are stimulated with PMA (50 ng/ml), ionomycin (1.5 μM), and monensin (6.7 μg/ml) and cultured for 6 hours at 37℃ in a CO_2 incubator. After the cell culture, they were washed with complete media (RPMI and 10% FBS) and re-suspended with staining buffers (PBS, 0.5% BSA, and 0.5 mM

EDTA). These cells were fixed, permeabilized, and then stained with primary-conjugated anti-granzyme B (BioLegend, San Diego, CA, USA) and anti-perforin (Mabtech, Nacka Strand, Sweden).

7. DP T cells analysis in islet transplantation models

After type I diabetic induction by streptozotocin (STZ; Sigma -Aldrich, USA), xenogenic (n = 5) and allogenic (n = 3) islets were transplanted intraportally in 8 rhesus monkeys. The population of DP T cells in peripheral blood was monitored before transplantation, and on days, 3, 7, 14, 28 post-transplantation and monthly for the following 6 months, and then bimonthly after islet transplantation.

8. Statistical analysis

All data were analyzed using the Prism program (GraphPad Software, Inc., LaJolla, CA, USA). The difference was determined via *t*-testing in all cell analysis data. p value < 0.05 was considered significant.

RESULTS

DP T cells in peripheral blood are differ from thymic DP T cells

To determine that peripheral DP T cells are differ from the immature DP thymocytes, cell surface markers were observed in CD4 SP, CD8 SP, and DP T cells from the peripheral blood and the thymus of naive rhesus monkeys using flow cytometry (Fig. 1).

First, since there is a possibility that thymic precursors might be released from the thymus to peripheral blood without further differentiation, the expression of CD8 receptor chains on DP T cells in the peripheral blood and the thymus was identified. DP T cells in thymus expressed 85.0% of CD8β chain on their surface (Fig. 1A), whereas DP T cells in the peripheral blood expressed only 4.01% of CD8β chain (Fig. 1B). That is, DP T cells in thymus have heterodimer types expressing both CD8 α and β chains like CD8 T cells, while peripheral DP T cells have homodimer types with only CD8 α chain.

In addition, peripheral lymphocytes and thymocytes were stained with CD1b antibody, which is known to express only on the surface of thymocytes and dendritic cells (DC). 94.1% of DP thymocytes expressed CD1b molecule on their cell surface (Fig. 1A), but it was not expressed on peripheral DP T cells (Fig. 1B). These findings demonstrated that peripheral DP T cells are fundamentally differ from thymic DP T cells.

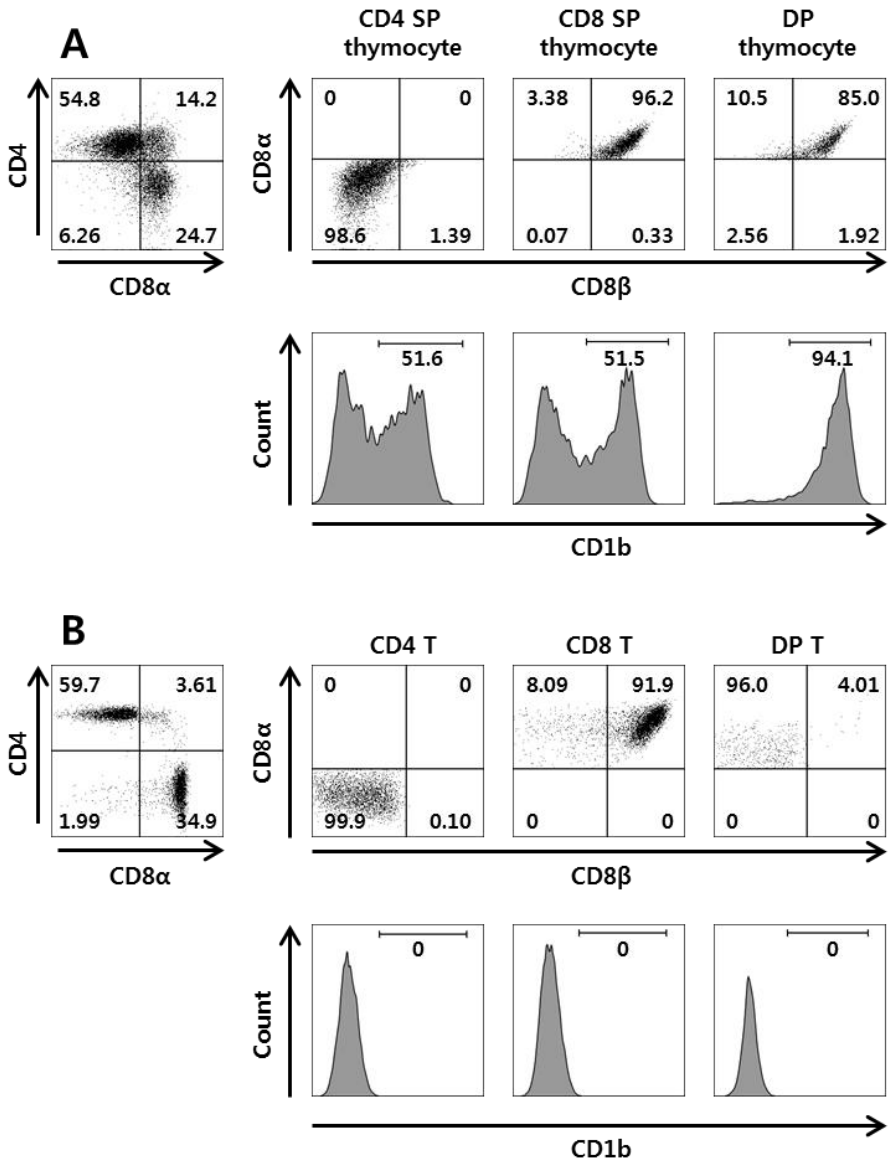

Figure 1.The peripheral DP T cells are differ from thymic DP T cells. (A) CD4 SP, CD8 SP, and DP thymocytes in thymus and (B) CD4, CD8, and DP T cells in peripheral blood were stained with CD4, CD8α, and CD8β antibodies. Peripheral and thymic cells were also stained with CD1b antibody, respectively (A, B). (SP : Single positive, DP : Double positive)

The peripheral DP T cells expressed Eomes and PLZF

Eomesodermin (Eomes) and promyelocytic leukaemia zinc finger protein (PLZF) transcription factors were stained on CD4, CD8, and DP T cells, respectively (Fig. 2). 16.83% of CD8 T cells expressed Eomes positive, followed by DP T cells which expressed at 7.18%. CD4 T cells showed only 1.22% of Eomes positive cells (Fig. 2A). In PLZF expression levels, DP T cells showed very high PLZF positive at 14.68%, whereas CD8 and CD4 T cells showed 4.78% and 1.57%, respectively (Fig. 2B).

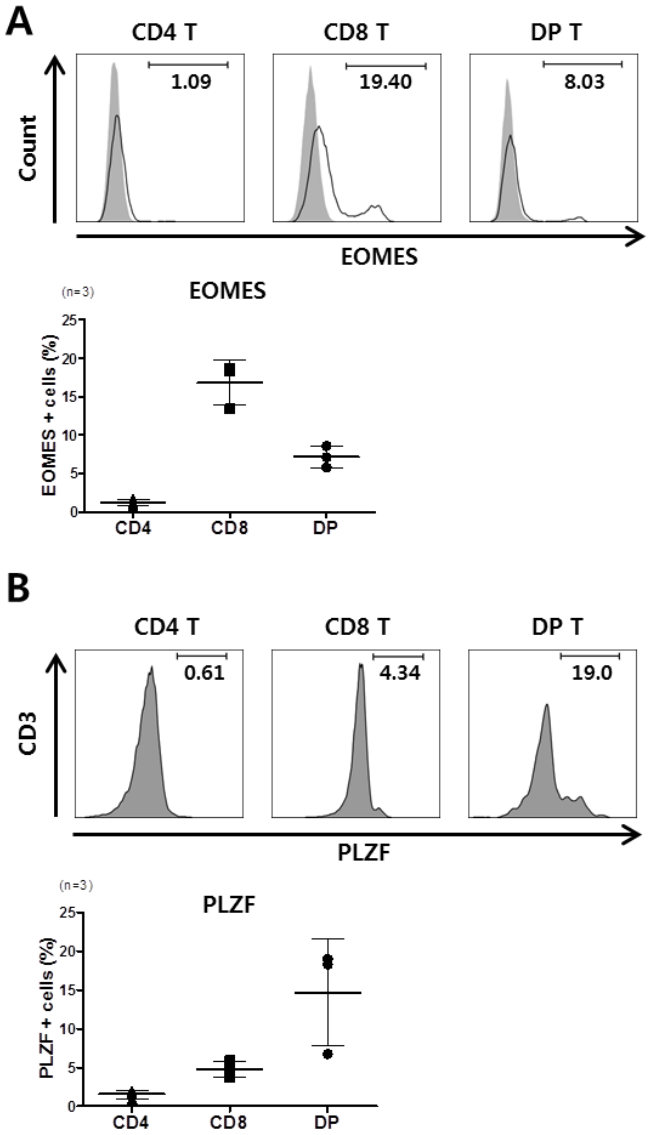

Figure 2. Phenotypic characterization of DP T cells. CD4, CD8, and DP T cells were stained with (A) Eomes and (B) PLZF transcription factors (n = 3; age, 48 - 54 months). Representative data from one independent experiment (*top panel*) and cumulative data (*bottom panel*) are shown; bars indicate means ± SD. (Eomes : Eomesodermin, PLZF : promyelocytic leukaemia zinc finger protein)

The proportion of DP T cells in peripheral blood and tissues

The proportion of DP T cells in peripheral blood, spleen, MLN, and liver tissue was analyzed in adult rhesus monkeys. In the peripheral blood, the proportion of CD4, CD8, and DP T cells was 51.27%, 40.81%, and 3.84%, respectively (Fig. 3A). These proportions were comparable to those of all tissues as follows; 3.31% in spleen, 3.13% in MLN, and 3.76% in liver, respectively (Fig. 3B).

For phenotyping of naïve and memory cell in peripheral blood, cell analysis was performed with CD28 and CD95 antibodies (Fig. 3C). The percentage of naïve cell ($CD28^{+}CD95^{-}$) in DP T cells was lower than that of CD4 and CD8 T cells. It was found that most of DP T cells have central memory ($CD28^{+}CD95^{+}$) and effector memory ($CD28^{-}CD95^{+}$) phenotypes (Fig. 3D). In spleen, the phenotype of DP T cells was mostly central/effector memory phenotype like liver tissue (Fig. 3D). However, DP T cells of MLN showed mostly central memory phenotype about 57%.

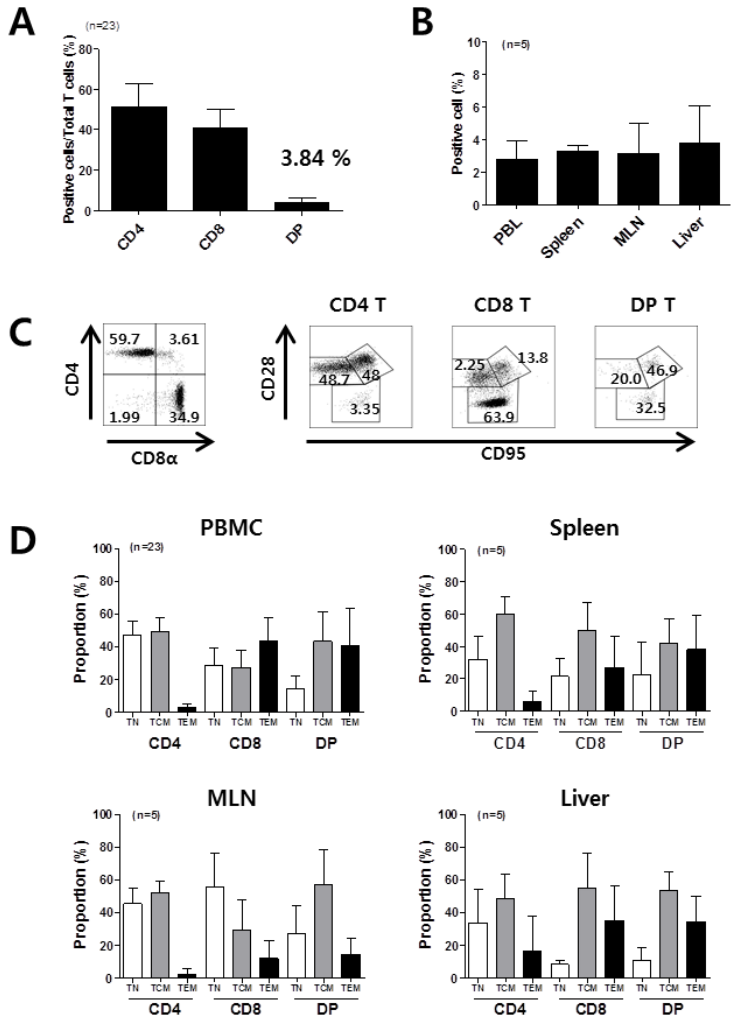

Figure 3. The proportion of DP T cells in peripheral blood and tissues. (A) The proportion of CD4, CD8, and DP T cells in peripheral blood (n = 23; age, 48 - 72 months), and (B) that of DP T cells in spleen, MLN, and liver (n = 5; age, 48 - 54 months) in rhesus monkeys were examined. The phenotypic characterization of T cell subsets in (D) peripheral blood and tissues was analyzed with CD28 and CD95 antibodies (C). The bars indicate means ± SD. (MLN : Mesenteric lymph node, TN : Naïve T cell, TCM : Central memory T cell, TEM : Effector memory T cell)

DP T cells have a helper function and cytotoxic activity *in vitro*

To determinate that DP T cells in peripheral blood have a helper function, CD4, CD8 and DP T cells were stimulated with PMA/ionomycin and stained with IFN-γ, IL-4, and IL-21. IFN-γ production of DP T cells was equivalent to CD8 T cells, but it was significantly higher than CD4 T cells ($p = 0.0234$; Fig. 4A). IL-4 production of DP T cells was 2.3% and it was as much as CD4 T cells (Fig. 4B). The level of IL-21 in DP T cells was similar to that of CD4 T cells (Fig. 4C). Moreover, the expression of HLA-DR, T cell activation marker [26], was twice as higher in DP T cells than that of CD4 and CD8 T cells (Fig 4D). CXCR5 and PD-1 known as biomarkers of T follicular helper cells were determined. The expression level of $CXCR5^{+}PD\text{-}1^{+}$ cells of DP T cells was higher than that of CD4 T cells (Fig. 4E).

Granzyme B and perforin that are known to be predominant in cytotoxic T lymphocytes were observed in each T cell subset (Fig. 5). When they were stimulated with PMA/ionomycin, a significant amount of granzyme B and perforin were produced in DP T cells as much as in CD8 T cells ($p = 0.0104$ and $p < 0.0001$, respectively; Fig. 5A, B).

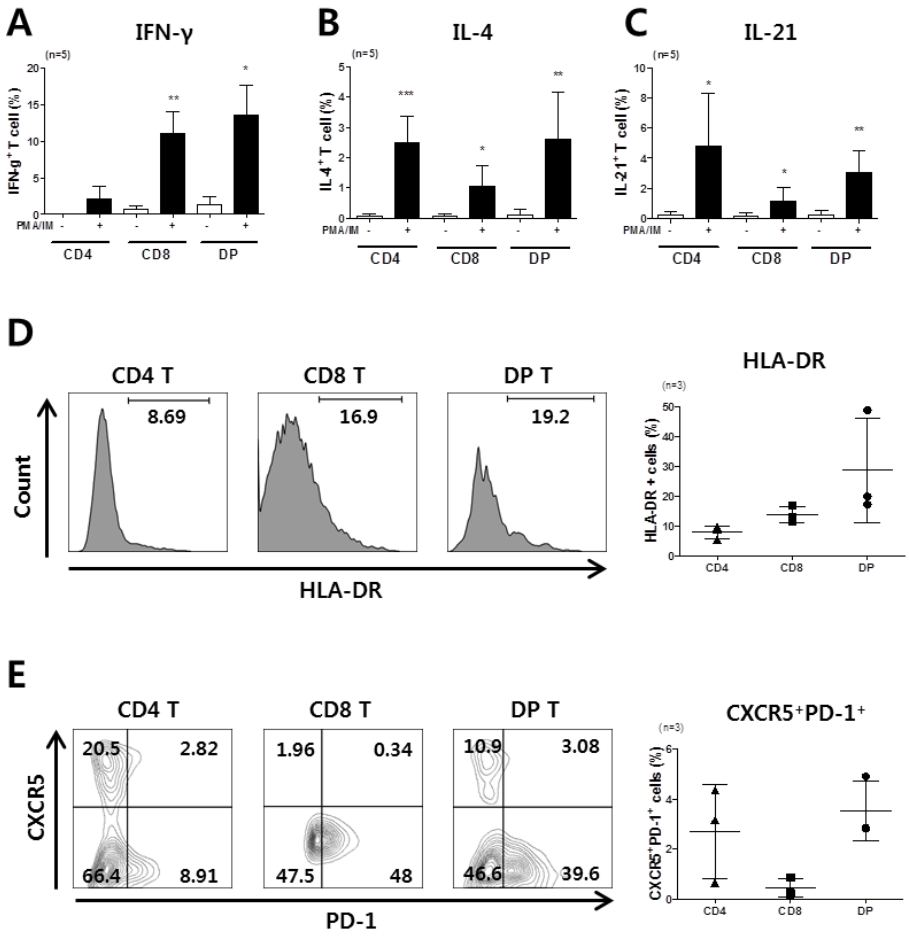

Figure 4. DP T cells in peripheral blood have a helper function *in vitro*. After sorting of T cells, CD4, CD8, and DP T cells were stimulated with PMA/Ionomycin and stained with cytokine antibodies including (A) IFN-γ, (B) IL-4, and (C) IL-21 (n = 5; age, 48 - 54 months). The percentage of T cells positive for (D) HLA-DR and (E) CXCR5/PD-1 in T cell populations is indicated (n = 3; age, 48 - 54 months). Representative data from one independent experiment (*left panel*) and cumulative data (*right panel*) are shown; bars indicate means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

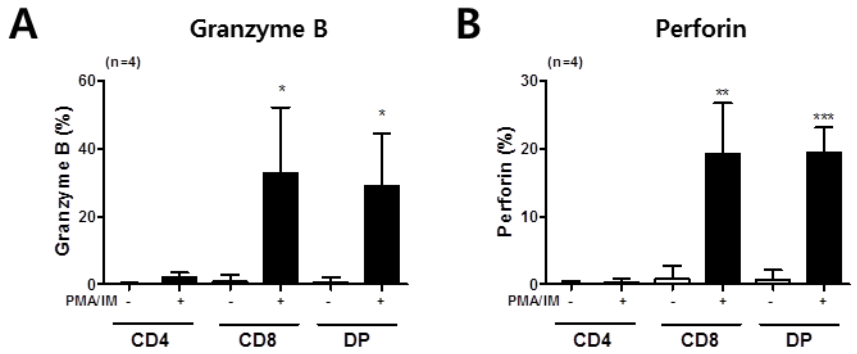

Figure 5. DP T cells in peripheral blood have cytotoxic activity *in vitro*. After sorting of T cells, CD4, CD8, and DP T cells were stimulated with PMA/Ionomycin and stained with (A) granzyme B and (B) perforin (n = 4; age, 48 - 54 months). The bars indicate means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Peripheral DP T cells of the monkeys that failed to graft survival markedly increased in islet transplantation models

The population of DP T cells in peripheral blood significantly increased in a rejected group which showed an increase of blood glucose levels and lack of C-peptide levels (Fig. 6B). On the contrary, the monkeys of long term graft survival group showed no change or suppression in the population of DP T cells during the period of survival (Fig. 6A).

The subpopulations of CD4, CD8, and DP T cells were analyzed before and after the islet transplantation. When the graft was rejected, CD8 T cells as well as DP T cells were significantly increased ($p = 0.0001$; Fig. 7). Especially, the increased population was effector memory T cells both CD8 T cells and DP T cells. However, effector memory CD8 T cells increased in long term survival group, unlike DP T cells (Fig. 7). There were no distinctive changes in CD4 T cell subsets in both groups (Fig. 7).

To investigate which subpopulation increases at the rejection point, DP T cells were divided into $CD4^{hi}CD8^{lo}$ and $CD4^{lo}CD8^{hi}$ subpopulations (Fig. 8A). As a result, only $CD4^{hi}CD8^{lo}$ DP T cells were markedly increased at the rejection point (Fig. 8B, D), but $CD4^{lo}CD8^{hi}$ DP T cell populations were not changed (Fig. 8C).

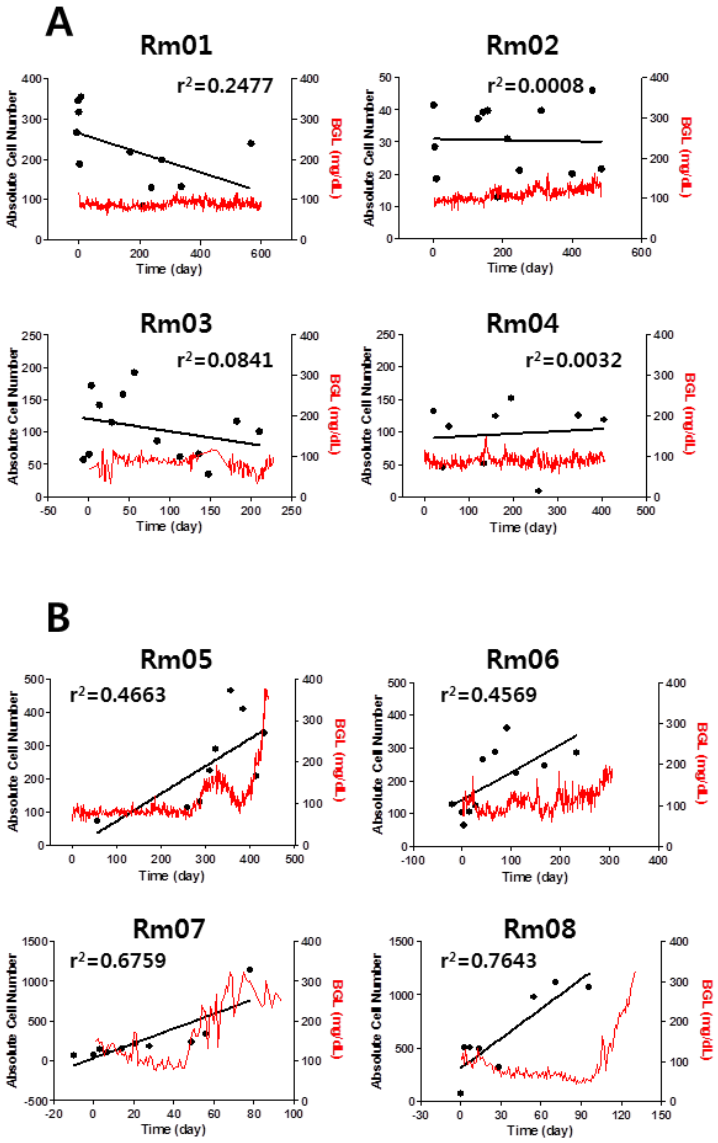

Figure 6. Peripheral DP T cells increased in the monkeys that failed to long term graft survival in islet transplantation models. (A) The population of DP T cells in peripheral blood has no change or suppression in long term survival group (n = 4; age, 54 - 72 months). (B) In graft rejection group, the population of DP T cells in peripheral blood significantly increased (n = 4; age, 54 - 72 months). (BGL: Blood glucose level)

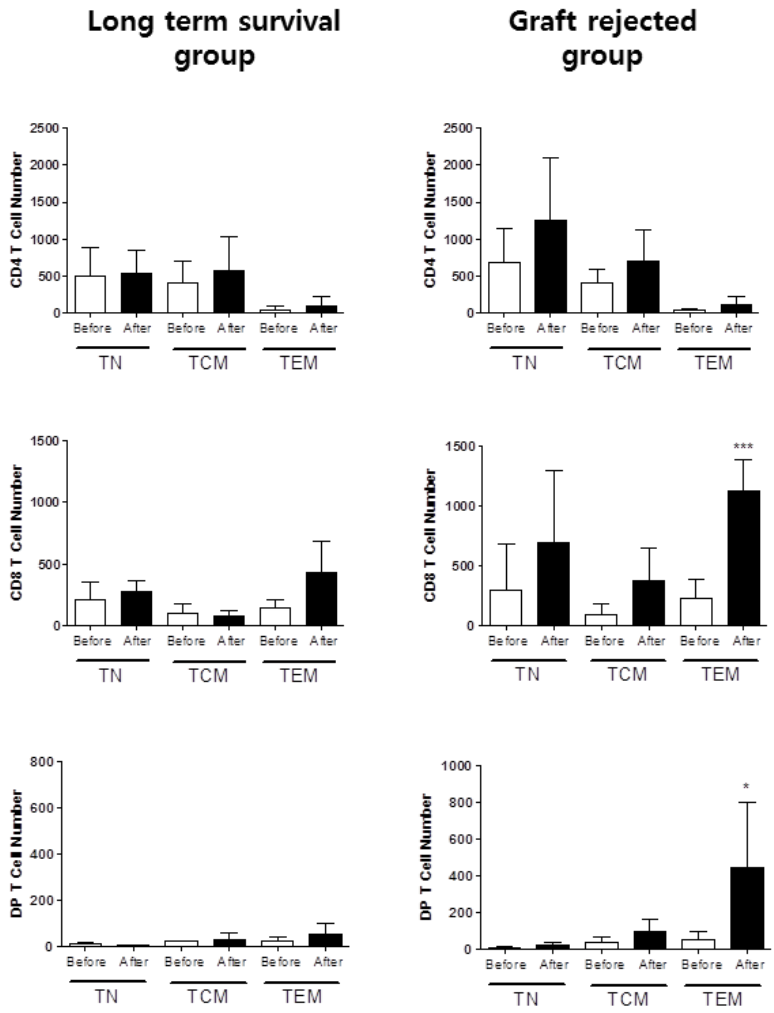

Figure 7. TEM of DP T cells were equivalently increased in graft rejection group compared to TEM of CD8. The subpopulations of CD4, CD8, and DP T cells were investigated in long term survival group (n = 4; age, 54 - 72 months), and graft rejection group (n = 4; age, 54 - 72 months) . The bars indicate means ± SD. * $p < 0.05$, *** $p < 0.001$. (TN: Naïve T cell, TCM: Central memory T cell, TEM: Effector memory T cell)

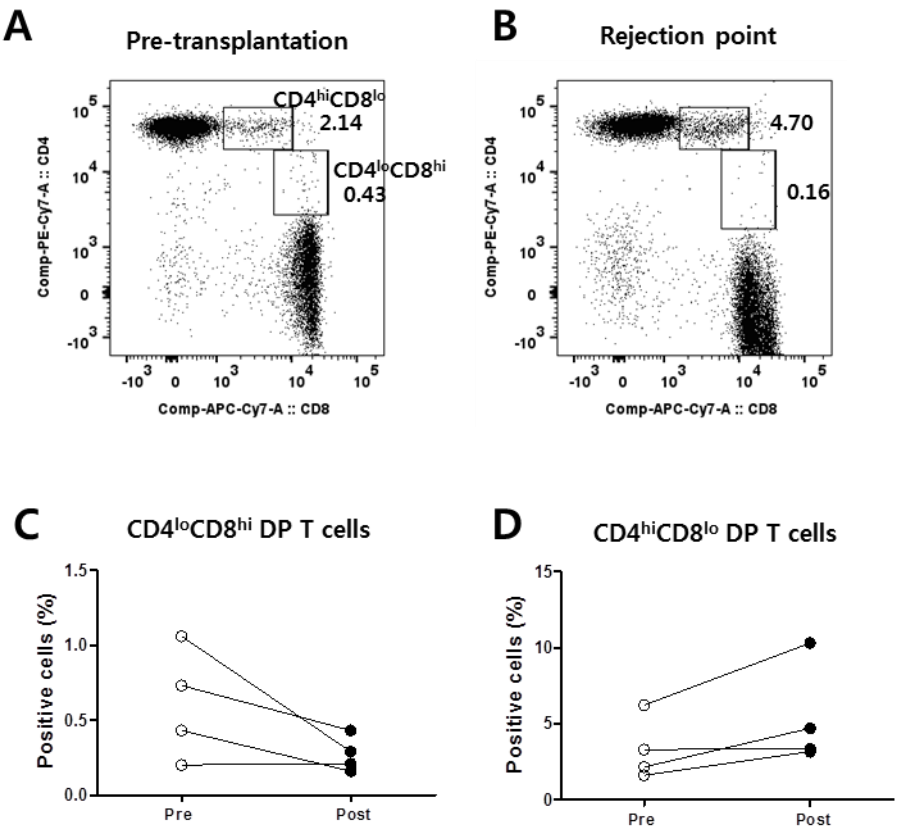

Figure 8. CD4^{hi}CD8^{lo} DP T cells are increased in graft rejection group.

The subpopulations of DP T cells at the rejection point (B) were compared to the point of pre-transplantation (A). The subpopulations of DP T cells were divided into (C) CD4^{lo}CD8^{hi} DP T cells and (D) CD4^{hi}CD8^{lo} DP T cells.

DISCUSSION

Immature DP T cells originating in the thymus have been well known and characterized. However, DP T cells' functions and roles in peripheral blood are not clearly known because of the contradicted view on their origin and function [27, 28]. In this study, cellular functions of DP T cells were determined via *in vitro* assay including cytokines, enzymes, and transcription factors. In addition, it was investigated how these cells are associated with graft rejection in islet transplantation model of nonhuman primate.

The CD1b molecules are similar to MHC class I, but rather than presenting peptide antigens to T cell receptor (TCR), they present lipid and glycolipid antigens which are derived from themselves as well as from mycobacteria. Thymic DP T cells expressed 95.1% of the CD1b molecule, which is normally expressed only on thymic cell-surface but not on extrathymic cell surface, but the peripheral DP T cells did not express. These results demonstrated that peripheral DP T cells did not express CD1b molecules in the rhesus monkeys, and it also matches with the result reported previously in cynomolgus monkeys [29].

In the expression of CD8 receptor chains on peripheral and thymic DP T cells, peripheral DP T cells have CD8αα homodimer on their surfaces, while thymic DP T cells have CD8αβ heterodimer. In terms of having different phenotypic makers, it suggests that the peripheral DP T cells are fundamentally differ from DP thymocytes.

Some T cells have a memory like phenotype in the thymus and immediately respond to antigen stimulation [30]. These T cells, called innate CD8 SP T cells, include NKT cells, T-T CD4 (or T-CD4) T cells, H2-M3 specific T cells, mucosal-associated invariant T (MAIT) cells, CD8αα$^+$ intraepithelial T cells, and innate CD8 T cells expressing Eomes [30]. Jacomet *et al*. recently, reported that there is a strong evidence of the existence of innate-like CD8 T cells in human, and they are Eomes positive cells [31]. Interestingly, around 7.18% of peripheral DP T cells in rhesus monkeys were highly existed as Eomes positive cells, even though it was less than 16.83% of the Eomes expressing CD8 T cells. PLZF$^+$ innate T cells are known to allow both effector and regulatory T cells to be activated in the thymus prior to their exit to the periphery [17-19]. In this study, a larger portion of PLZF$^+$ innate T cells was found in peripheral DP T cells than CD4 or CD8 T cells. Based on these results, it was confirmed that peripheral DP T cells in rhesus monkeys have properties such as memory like innate T cells.

As previously described [1], a very high proportion of DP T cells in peripheral blood is found in neonate rhesus macaques. In cell analysis of adult monkey tissue, it was found that a good number of DP T cells present in the secondary lymphoid organs such as spleen and MLN. Moreover, 3.76% of DP T cells are in the liver tissue as well. Out of the 3.76% of DP T cells in the liver tissue, 53.20% of DP T cells showed central memory phenotype.

In RA patients, peripheral DP T cells have helper functions and promote the inflammatory process through their capacity to produce a variety of

cytokines including IL-4, IL-21, and IFN-γ [10]. On the other hand, Nam *et al.* reported that the cytotoxic activity of DP T cells was similar to that of CD8 T cells, although the helper activity was lower than that of CD4 T cells [32]. Therefore, in this study, it was found that DP T cells in peripheral blood have a producing capacity of various cytokines in response to mitogen stimulation. DP T cells in peripheral blood produced IFN-γ (16.07%) much more than CD8 T cells (9.91%) and CD4 T cells (1.30%) did. They also produce IL-4 (3.32%) and IL-21 (7.67%) as much as CD4 T cells (2.73% and 3.85%, respectively) did. There is one more important thing that it seems peripheral DP T cells have helper functions. CXCR5 and PD-1 are known to help antibody producing of B cells [10, 33-35]. On chemokine and receptor analysis, it was found that peripheral DP T cells substantially expressed 3.52% of CXCR and PD-1 positive cells. In addition, they also expressed a high amount of granzyme B (29.05%) and perforin (19.38%) enzyme as much as CD8 T cells (32.90% and 19.27%, respectively) in response to mitogen stimulation. Consequently, it suggests that peripheral DP T cells have both functions *in vitro*; helper/inducer functions like CD4 T cells and cytotoxic/cytolysis activity like CD8 T cells. Based on these properties of cytokine and transcription factors, it is found that peripheral DP T cells are associated with helper and cytotoxic immune response, and also have a role of innate immunity.

In various diseases including LCMV, HIV, and cancer, it was reported that because peripheral DP T cells express a high level of FasL and secret of IFN-γ, they have cytotoxic potential [2, 25]. On the other hand, it was reported that

DP T cells have helper functions through the high secretion of IL-4 in some diseases such as systemic sclerosis and rheumatoid arthritis [10, 23].

In islet transplantation model of rhesus monkeys, significant differences between long-term graft survival group and rejected group were observed in peripheral DP T cell population. That is, DP T cells in peripheral blood were markedly increased in the graft rejected group, but not in the long-term survival group. TEM subpopulation of CD8 T cells that contributed prominently in organ graft rejection, was significantly increased during the rejection period. In parallel, TEM subpopulation of DP T cells was highly increased at the same period as well. However, interestingly, TEM population of DP T cells was increased only in graft rejected monkeys, unlike CD8 TEM showed an increase in both long-term survival and rejected group. Therefore, DP T cells are very important biomarker in islet transplantation model with CD8 T cell subpopulations.

Several authors have proposed that DP T cells might be originated extrathymically from CD4 SP T cells and acquired the ability to express CD8 receptor [36-39]. On the contrary, DP T cells have also been proposed to arise from the activated CD8 SP T cells that have up-regulated the surface expression of CD4 receptor [7, 40, 41]. In this study, $CD4^{hi}CD8^{lo}$ and $CD4^{lo}CD8^{hi}$ sub-population of DP T cells were examined to identify the major role population in islet transplantation. It showed that $CD4^{hi}CD8^{lo}$ DP T cells were increased in graft rejection group, but $CD4^{lo}CD8^{hi}$ DP T cells were not changed. This data indicates that $CD4^{hi}CD8^{lo}$ DP T cells are the major sub-

population in islet graft rejection. But it needs further study for the rejection mechanism related with these cell sub-population.

In conclusion, peripheral DP T cells in rhesus monkeys have dual functions such as helper and cytotoxic activity *in vitro* analysis. In addition, it is confirmed that these cells play a crucial role in graft rejection in islet transplantation model.

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국문 초록

이중 양성 (DP) T 세포는 가슴샘에서 T 세포의 성숙 과정 단계 중 하나로만 생각되어 왔다. 하지만 이전 연구들에서 이중 양성 T 세포가 사람뿐만 아니라, 쥐, 닭, 돼지, 그리고 원숭이와 같은 동물들의 말초 혈액이나 이차 면역 기관에도 존재하는 것이 확인되었다. 말초 혈액에 존재하는 이 세포의 유래와 명확한 그 역할에 대해서는 아직 다양한 의견들이 있다. 본 연구에서는 사람과 비슷한 영장류를 실험 모델로 하여 이러한 이중 양성 T 세포의 기능적 특성을 알아보았고, 장기 이식에서의 이식 거부 반응과의 연관성에 대해 확인하였다. 말초 혈액에 있는 이중 양성 T 세포가 가슴샘에 있는 이중 양성 T 세포와 근본적으로 구분되었으며, 일부 선천 면역 세포에 속할 가능성이 있음을 확인하였다. 원숭이의 말초 혈액 내의 이중 양성 T 세포는 주로 기억 표현형을 가지며 이차 면역 기관뿐만 아니라, 간에도 존재함을 확인하였다. 또한 이중 양성 T 세포는 CD4 T 세포만큼의 IL-4 와 IL-21 와 많은 양의 IFN-γ 를 분비하고 $CXCR5^{+}PD-1^{+}$ 을 가지고 있으며, 높은 HLA-DR 발현 정도를 통해 도움 기능을 가지는 것을 확인하였다. 뿐만 아니라, CD8 T 세포만큼의 많은 양의 granzyme B 와 perforin 을 발현하는 것으로 보아 세포 독성 활성의 기능도 가지고 있음을 확인하였다. 마지막으로 장기 이식 실험에서 이식체가 오랜

기간 살아남은 그룹과 이식 거부 반응이 생긴 그룹으로 나누어 이중 양성 T 세포의 변화를 비교하였다. 이식 거부 반응이 일어나지 않은 그룹에서는 이중 양성 T 세포의 수가 변하지 않거나 감소하는 경향을 보였으나, 이식 거부 반응이 일어난 그룹에서는 모두 유의성 있게 증가하였다. 따라서 이중 양성 T 세포가 장기 이식에서 주목해야 할 요소 중 하나라고 생각된다.

주요어 : 이중 양성 T 세포, 선천 T 세포, 기억 표현형, 도움 기능, 세포 독성 활성, 장기 이식, 붉은 털 원숭이

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