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$CD4^+CD25^+$ T cells maintain homeostasis by promoting TER-119 cell development and inhibiting T cell activation

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ABSTRACT

$CD4^+CD25^+$ regulatory T cells involved in the regulation of self-tolerance and normality of homeostasis. CD122 deficient mice are model animals that have an abnormal immune system characteristically have a high number of activated T cells and TER-119 cell decreased. Here we showed evidence that the transfer of $CD4^+CD25^+$ regulatory T cells derived from normal mice to CD122-defficient neonates prevent the development of activated memory T cells and elicit TER-119 differentiation. Bone marrow reconstitution derived from $CD122^{-/-}$ mice to normal mice resulting tolerance to individual that genetically different. Importantly, $CD4^+CD25^+$ regulatory T cells derived from normal mice can replace $CD4^+CD25^+$ cells derived from $CD122^{-/-}$ mice. The results of this experiment suggest that regulatory T cells from normal mice exert a critical role in maintaining peripheral tolerance and controlling hematopoietic disorder.

Keywords: *$CD4^+CD25^+$, CD122-defficient, tolerance, homeostasis*

INTRODUCTION

Study focused on $CD4^+CD25^+$ regulatory T cells becomes one attention in immunological field. This important cells is known as 'naturally occurring '. Many evidences explain the ability of $CD4^+CD25^+$ regulator T cells to prevent the development of autoreactive cells [1-5]. CD4 is a coreceptor molecule on T cells and it also expressed in limited population of macrophages. CD4 molecule is also known to become MHC class II ligand. On the other hand, CD25 molecule is known as important receptor for IL-2. CD25 is a receptor sub unit alpha of IL-2 (IL-2Rα). IL-2 receptor has three kinds of chains namely alpha, beta, and gamma. Attention of scientists against IL-2 receptor alpha (IL2-Rα) has had a very broad impact on the medical fields, including the discovery of new concepts in bone marrow transplantation (BMT).

Loss of CD25 expression due to mutation or genetic engineering lead to T cells become anergic, a condition in which T cells are not able to respond anti-CD3 stimulation. The loss expression of CD25 (IL-2Rα) on cell surface is known to cause autoimmune diseases [6-16]. $CD4^+CD25^+$ regulatory T cells serve as important component to maintain normal homeostasis.

To determine whether $CD4^+CD25^+$ regulatory T cells can be used to overcome hematopoietic disorder we transfuse $CD4^+CD25^+$ regulatory T cells to $CD122^{-/-}$ mice. In this study we found evidence that $CD4^+CD25^+$ regulatory T cells have an ability to normalize the number of TER-119 and B220 cells. $CD4^+CD25^+$ regulatory T cells are also important for maintaining self tolerance. In bone marrow transplantation $CD4^+CD25^+$ regulatory T cells have important role to mediate donor acceptance by host.

MATERIALS AND METHODS

Animal

In this experiment we used $CD122^{-/-}$ and $C57BL/6^{CD45.1/CD45.1}$ mice as animal models. They were maintained in pathogen free facility. $CD4^+CD25^+$ regulatory T cells were isolated from $C57BL/6^{CD45.1/CD45.1}$ mice, while the hosts were $CD122^{-/-CD45.2/CD45.2}$ mice. In another experiment we transferred bone marrow cells

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derived from CD122$^{-/-}$ mice to C57BL/6$^{CD45.1/CD45.1}$ mice. Shortly, 5 x 10^6 bone marrow cells derived from CD122$^{-/-}$ mice were intravenously injected to C57BL/6$^{CD45.1/CD45.1}$ mice that had been treated with lethal dose radiation (850 rad).

Antibodies

FITC-conjugated anti-mouse CD25 (clone PC61.5), Phycoerythrin (PE) or allophycocyanin (APCn)-conjugated anti-mouse CD4 (clone GK1.5), FITC- or biotin-conjugated anti-mouse CD45.1 (clone A20), anti-TER-119, anti-CD62L, anti-Gr-1 (clone RB6-8C5), Fluorescein isothiocyanate (FITC)-conjugated anti-mouse B220, anti-mouse CD62L, anti-mouse CD44, and biotin-conjugated anti-mouse CD122 (clone 5H4). When we used biotin-conjugated antibodies, we visualized the antibodies by streptavidin-PE-Cy5 (eBioscience, San Diego, CA).

Flow cytometry and cell sorting.

We performed cell sorting by using FACS Vantage cell sorter (BD-Biosciences, San Jose, CA) to obtain highly purified CD4$^+$CD25$^+$ regulatory T cells. Analytical flow cytometry was performed by using FACS CaliburTM flow cytometer (BD-Biosciences, San Jose, CA).

Adoptive cell transfer.

Mice which are known as CD122-defficient (CD122$^{-/-}$) in this study have C57BL/ 6$^{CD45.2/CD45.2}$ lineage. CD122-defficient mice (CD122$^{-/-}$) is also often called the IL-2Rβ knockout. Mice with genotype CD122$^{-/-}$ obtained by a breading between CD122$^{+/-}$ with CD122$^{+/-}$. The result of this breading produces individuals that follows Mendellian laws with genotype of CD122$^{+/+}$, CD122$^{+/-}$, and CD122$^{-/-}$. Mice with genotype of CD122$^{-/-}$ can be examined by DNA fingerprinting (DNA typing). Mice with genotype CD122$^{-/-}$ (2-day-old) were adoptively transfused with 2x10^5 CD4$^+$CD25$^+$ regulatory T cells derived from normal mice that has a genotype of CD45.1/CD45.1. CD4$^+$CD25$^+$ regulatory T cells are sorted out by FACS Vantage sorting machine (BD Biosciences). Analysis of cell surface marker was done by FACS machine CaliburTM flow cytometer (BD-Biosciences, San Jose, CA). Results of transfusion were observed 12 weeks after treatment. To determine the presence of activated T cells we observed CD44 and CD62L molecule expressions on T cells. Profiles of B cells were observed by looking at the expression of B220, while the determination of an occurrence anemia we measured hematocrit from peripheral blood aspiration. B220 monoclonal antibody was used to determine the existence of B cells, whereas TER-119 was used to examine erythroblast lineage.

Statistical analysis

One-way ANOVA was used to analyze the data. The differences between groups were considered significant at $P<0.01$. All results were presented as the mean of ± SD values of 6 mice in each group.

RESULTS AND DISCUSSION

It is known that CD4$^+$CD25$^+$ regulatory T cells generally have an ability to regulate homeo stasis in healthy individuals. In this study CD4$^+$CD25$^+$ regulatory T cells were tested for their ability to suppress uncontrolled expanded Gr1 and B cells in CD122 deficient mice (Figure 1). The results of the analysis indicate that Gr1 and B cells level could be normalized in CD122 deficient mice after receiving CD4$^+$CD25$^+$ regulatory T cells (2 x 10^5) derived from normal mice. In general, this study describes the important of CD4$^+$CD25$^+$ regulatory T cells to normalize hematopoietic cells in CD122 deficient mice. Infusion of CD4$^+$CD25$^+$ regulatory T cells in CD122 deficient mice in the age of 2-days allowed the mice to develop normally. On the other hand, CD4$^+$ CD25$^-$ transfusion with the same number had no effect in improving survival of CD122 deficient mice and the mice will die after 10-12 weeks. From these results it is clear that the population of T cells that have properties with regulatory activity existed in CD4$^+$CD25$^+$ cell population. One of the causes of death in CD122 deficient mice that were not manipulated with regulatory T cells is the appearance of high number of Gr1 and also the decrease of B cells [17-22]. In many reports, activated and memory cells were also contribute to the worse condition in CD122 deficient mice. It was also reported that naive cells in CD122 deficient mice decrease dramatically. Exami nation of T cell absolute number in CD122 deficient mice showed that the mice have higher

number of the cells compare to normal mice. In this study could not be explained why the memory cells and activated cells could accumulate in CD122 deficient mice. Logically, if the T cells lose expression of CD122 molecule, then T cell number will decrease because CD122 molecule is a component of IL-2 receptor. But, it is unexpected T cells in CD122 deficient mice accumulated in the peripheral lymphoid organ even though the mice did not respond to the proliferation factor (IL-2). According to Abbas and the other group [23] T cells in CD122 deficient mice resistant again apoptosis .

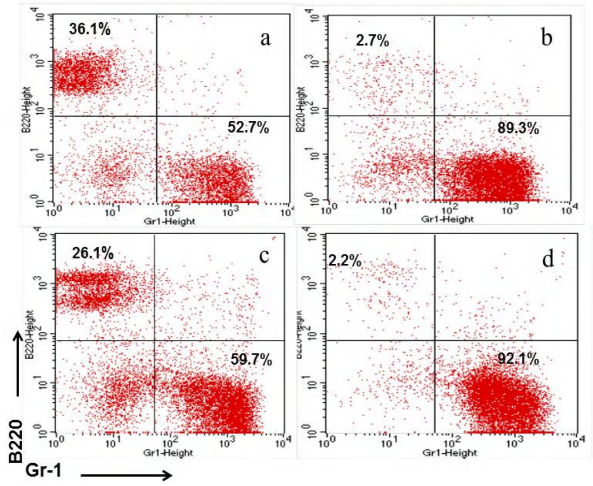

Figure 1. Adoptive transfer of CD4+CD25+ regulatory T cells derived from normal mice to CD122-/- neonate mice (2-day- old) increase B and Gr-1 cells. (a) B220 and Gr-1 profiles of normal mice. (b) B220 and Gr-1 profiles of CD122 deficient mice. (c) After 12 weeks from the transfer of CD4+CD25+ regulatory T cells into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-Gr-1, anti-TER-119, and anti-CD45.1 antibodies, then analyzed by flow cytometry. Percentages of Gr-1+ and B220 cells are shown for cells gated to CD45.1-. (d) After 12 weeks from the transfer of CD4+CD25- cells into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-Gr-1, anti-TER-119, and anti-CD45.1 antibodies, then analyzed by flow cytometry. Percentages of Gr-1+ and B220 cells are shown for cells gated to CD45.1-.

In this phenomenon Malek et al. [24-25] postulated that such condition resulting in accumulation of memory cells continued throughout life span. Although this opinion is not irrefutable, but still leaves the question why CD122 deficient mice cell culture were not survive compare to normal ones. In normal individuals the apoptotic program is necessary to maintain the biological balance. Resistance to apoptosis in CD122 deficient mice lead to accumulation of memory type cells, CD44+CD62-. In this study, we found that adoptively transferred of CD4+CD25+ regula- tory T cells to CD122-/- neonate mice can prevent the development of memory type (Figure 3). Anemia that occurs in CD122 deficient mice can be explained by looking at the low erythrocyte precursors (TER-119) in the bone marrow (data not shown). However, hematopoietic disorder can be seen in spleen. TER-119 expression in spleen decrease in CD122-/- mice. This can be overcome by transferring CD4+CD25+ regulatory T cells from normal mice (Figure 2).

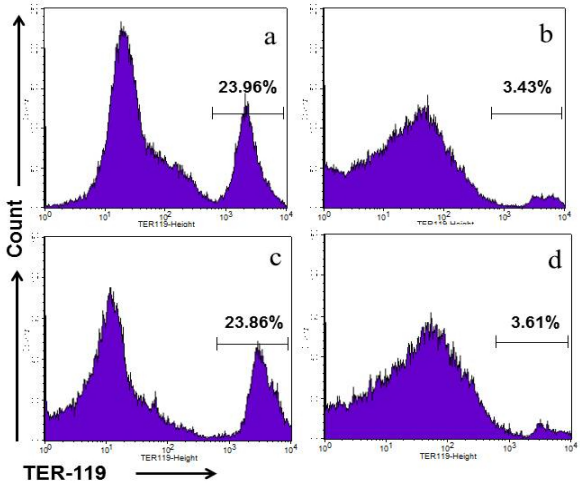

Figure 2. Adoptive transfer of CD4+CD25+ regulatory T cells derived from normal mice to CD122-/- neonate mice normalized TER-119 cell generation. (a) TER-119 profile of normal mice. (b) B220 and Gr-1 profiles of CD122 deficient mice. (c) After 12 weeks from the transfer of CD4+CD25+ regulatory T cells into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-TER-119 and anti-CD45.1 antibodies, then analyzed by flow cytometry. Percentages of TER-119 cells are shown for cells gated to CD45.1-. (d) After 12 weeks from the transfer of CD4+CD25- cells into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-TER-119 and anti-CD45.1 antibodies, and analyzed by flow cytometry. Percentages of TER-119 cells are shown for cells gated to CD45.1-.

B cells (B220) which has the potential to generate antibodies also significantly reduced in CD122 deficient mice, so that they were more

susceptible then normal ones [26-27]. In this study we show that transfusion of $CD4^+CD25^+$ regulatory T cells could stimulate the development of erythrocyte precursors in the bone marrow. Therefore, transfusion of $CD4^+CD25^+$ regulatory T cells will give opportunity to CD122 deficient mice to life normally. The examination of hematocrit showed that CD122 deficient mice increase the cell ratio to serum after receiving $CD4^+CD25^+$ regulatory T cells derived from normal mice. Hematocrit in normal mice reached about 55 whereas in CD122 deficient mice dropped dramatically up to 30. The low hematocrit in CD122 deficient mice is closely related to the ability of bone marrow to generate erythrocytes precursor. In observations with marker of TER-119, proved that CD122 deficient mice were not able to produce such precursors (Figure 2).

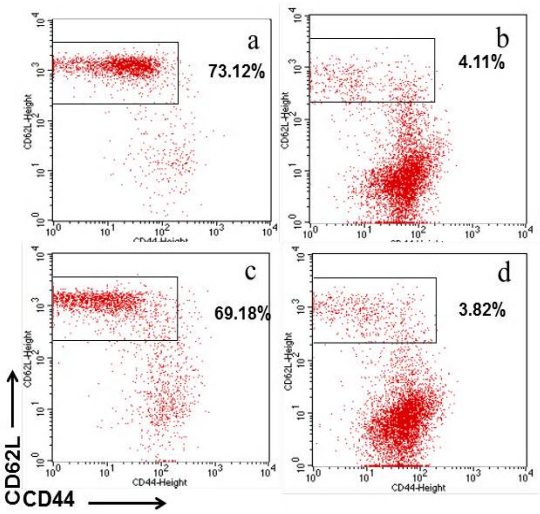

Figure 3. Adoptive transfer of $CD4^+CD25^+$ regulatory T cells derived from normal mice to $CD122^{-/-}$ neonate mice increase naïve type cells. (a) Profile naïve T cell of normal mice. (b) Profile of naïve T cells in CD122 deficient mice. (c) After 12 weeks from the transfer of $CD4^+CD25^+$ regulatory T cells into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-CD4, anti-CD62L, anti-CD44, and anti-CD45.1 antibodies, and then analyzed by flow cytometry. Percentages of naïve T cells ($CD62L^+CD44^-$) are shown for cells gated to CD45.1-. (d) After 12 weeks from the transfer of $CD4^+CD25^-$ T cells into CD122-deficient neonates, spleen cells obtained from the mice were stained with anti-CD4, anti-CD62L, anti-CD44, and anti-CD45.1 antibodies, and then analyzed by flow cytometry. Percentages of naïve T cells ($CD62L^+CD44^-$) are shown for cells gated to $CD45.1^-$.

The loss of the ability to produce precursors of erythrocytes in CD122 deficient mice is suspected of granulocytes penetration into bone marrow compartment in beside abnormality of CD8 responses [17, 19, 28-29].

Examination of spleen cells in $CD122^{-/-}$ mice showed an increase in memory ($CD44^+CD62L^-$) type compared to normal ones. In neonate $CD122^{-/-}$ mice the abnormalities could be overcome by adoptive transfer (transfusion) of $CD4^+CD25^+$ regulatory T cells as much as 2 x 10^5. $CD4^+CD25^+$ regulatory T cell transfusions might increase naïve cells ($CD44^-CD62L^+$). $CD4^+CD25^-$ cells as much as 2 x 10^5 did not have a preventive effect and the status of T cells in $CD122^{-/-}$ mice remain in an activated state and a memory type (Figure 3). $CD122^{-/-}$ mice severely anemic due to a combination of autoimmune haemolysis and the defect of erythropoiesis. Examination of bone marrow showed that the erythrocyte precursor cell with the marker of TER-199 dramatically decreased in CD122 deficient mice.

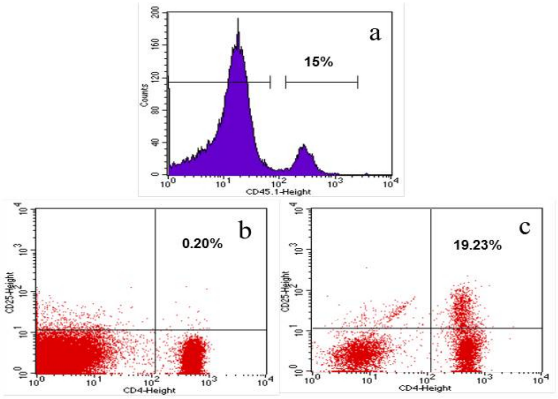

Figure 4. Bone marrow transplantation derived from $CD122^{-/-}$ neonate mice to normal mice resulting a tolerance chimeric mice. (a) After 12 weeks from the transfer of bone marrow cells from CD122-deficient neonates to normal mice, spleen cells obtained from the mice were stained with anti-CD4, anti-CD25, and anti-CD45.1 antibodies, and then analyzed by flow cytometry. Percentage of host remaining cells (15%) are shown in upper panel. Percentages of $CD4^+CD25^+$ regulatory T cells are shown for cells gated to $CD45.1^-$ in panel (b) and gated to $CD45.1^+$ in panel (c).

Erythropoiesis that occurs in CD122 deficient mice can be normalized by adoptive transfer (transfusion) of $CD4^+CD25^+$ regulatory T cells in neonate mice as much as 2 x 10^5, whereas

transfusion of $CD4^+CD25^-$ T cells with the same number cannot cure erythropoiesis characterized low number of cells that express the TER-119 (Figure 2). Normalization of TER-119 in spleen is also in tune match with the changes in the peripheral blood. Hematocrit increased in mice $CD122^{-/-}$ T cells that acquire transfusion $CD4^+CD25^+$ as much as 2 x 10^5, whereas transfusion of $CD4^+CD25^-$ in the same number cannot increase the hematocrit. To determine whether $CD4^+CD25^+$ regulatory T cells can develop in CD122 deficient mice we examined with a distinctive profile CD45.1. We found that transfusions of $CD4^+CD25^+$ regulatory T cells from normal mice allowed $CD4^+CD25^+$ regulatory T cells from CD122 deficient mice can develop in early step but immediately replaced by $CD4^+CD25^+$ regulatory T cells derived from normal ones (data not shown).

CONCLUSIONS

$CD4^+CD25^+$ regulatory T cells could control homeostasis normality in which Gr-1 and B220 were maintained in appropriate numbers. $CD4^+CD25^+$ regulatory T cells could suppress the development of activated memory type and allow hemotopoietic cells remain in the status of naïve type. $CD4^+CD25^+$ regulatory T cells maintain hematocrit by promoting the development of TER-119 cells.

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