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In Vivo Treatment of Class II MHC-Deficient Mice with Anti-TCR Antibody Restores the Generation of Circulating CD4 T Cells and Optimal Architecture of Thymic Medulla

Mariam Nasreen,* Tomoo Ueno,† Fumi Saito,† and Yousuke Takahama2*†

TCR ligation by the self-peptide-associated MHC molecules is essential for T cell development in the thymus, so that class II MHC-deficient mice do not generate CD4⁺CD8⁺ T cells. The present results show that the administration of anti-TCR mAb into class II MHC-deficient mice restores the generation of CD4⁺CD8⁻ T cells. Unlike peripheral CD4 T cells that disappeared within 5 wk after the treatment, CD4⁺CD8⁻ thymocytes remained undiminished even after 5 wk, suggesting that CD4 T cells in the thymus are maintained separately from circulating CD4 T cells and even without class II MHC molecules. It was also found that the mass of medullary region in the thymus, which was reduced in class II MHC-deficient mice, was restored by the anti-TCR administration, suggesting that the medulla for CD4⁺CD8⁻ thymocytes is formed independently of the medulla for CD4⁺CD8⁺ thymocytes. These results indicate that in vivo anti-TCR treatment in class II MHC-deficient mice restores the generation of circulating CD4 T cells and optimal formation of the medulla in the thymus, suggesting that anti-TCR Ab may be useful for clinical treatment of class II MHC deficiencies. The Journal of Immunology, 2003, 171: 3394–3400.

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differentiation of immature CD4⁺CD8⁻ double-positive (DP)³ thymocytes into mature CD4⁺CD8⁻ or CD4⁺CD8⁺ single-positive (SP) cells is an obligatory step for intrathymic development of conventional T cells, referred to as positive selection. Thymocytes that are tolerant to self-Ags but are potentially reactive to foreign Ags are positively selected by TCR interaction with self-peptide-associated MHC molecules expressed in the thymus (1–3). DP thymocytes that recognize class I MHC ligands become CD4⁺CD8⁻ cells (4–6), whereas thymocytes bearing class II MHC specificity differentiate into CD4⁺CD8⁻ cells (7, 8). Disruption of class I or class II MHC genes by gene-targeting technology has shown their essential role in the development of CD4⁺CD8⁻ or CD4⁺CD8⁺ T cells, respectively (9–13).

Previously, we have reported that the treatment with anti-TCRβ mAb of the fetal thymus organ culture (FTOC) from class II MHC-deficient mice restores the generation of a distinct population of CD4⁺CD8⁻ thymocytes, indicating that Ab-mediated TCR ligation could mimic TCR signals that induce positive selection of CD4⁺CD8⁻ thymocytes (14). Other investigators have also reported that anti-TCR Ab restores the generation of CD4⁺CD8⁻ thymocytes in class II MHC-deficient FTOC (15, 16). However, the in vitro culture system of FTOC did not allow us to examine whether the newly generated CD4⁺CD8⁻ thymocytes could emigrate the thymus to supply CD4 T cells in the periphery. It was also unclear whether the formation of the medullary region might be affected upon the Ab treatment that restores the generation of CD4⁺CD8⁻ thymocytes, because the architecture of the thymus in the FTOC condition is severely damaged and devoid of clear cortex-medulla structure (17). Müller and Kyewski (18–20) have shown that in vivo treatment with a hybrid Ab consisting of anti-Vβ8 Ab and anticortical epithelial cell Ab rescues the generation of Vβ8⁺CD4⁺CD8⁻ thymocytes in class II MHC-deficient mice. However, these reports have not addressed whether the Ab treatment in class II MHC-deficient mice may affect the architecture of thymic medulla and/or the emigration of CD4⁺CD8⁻ thymocytes to the peripheral circulation.

The present study has addressed whether the treatment of class II MHC-deficient mice with anti-TCR Ab may restore the generation of peripheral CD4⁺CD8⁻ T cells and the formation of thymic architecture. Our results show that a single i.p. injection of anti-TCR Ab in class II MHC knockout (KO) mice indeed restored the generation of CD4⁺CD8⁻ T cells in peripheral circulation as well as in the thymus. Interestingly, in vivo anti-TCR treatment also restored the volume of the medullary region in the thymus of class II MHC-deficient mice, suggesting that the architecture of the medulla in the thymus is independently regulated for CD4⁺CD8⁻ thymocytes and CD4⁺CD8⁺ thymocytes.

Materials and Methods

Mice

C57BL/6 (B6) (SLC, Shizuoka, Japan), MHC class I-deficient B6-background (β2-microglobulin gene targeted; The Jackson Laboratory, Bar Harbor, ME), MHC class II-deficient B6-background (Aβ gene targeted; provided by Dr. D. Mathis, Harvard Medical School, Boston, MA) (9), and TCRα-deficient B6-background (The Jackson Laboratory) mice were used in this study. MHC class I/II double-deficient mice were bred in our

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3 Abbreviations used in this paper: DP, double positive; SP, single positive; FTOC, fetal thymus organ culture; KO, knockout; CCL, CC chemokine ligand; BLS, bare lymphocyte syndrome.
animal facility by crossing MHC class I-deficient and MHC class II-deficient mice.

**Anti-TCR Ab**

Hybridoma cells producing the hamster anti-mouse TCRβ Ab H57-597 (21) were cultured in RPMI 1640 medium supplemented with 10% FBS, 50 μM 2-ME, 2 mM l-glutamine, 1% nonessential amino acids, 10 mM HEPES, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Gaithersburg, MD). IgG Ab was purified from the culture supernatant over protein A-coupled Sepharose beads.

**In vivo administration of Ab**

Newborn babies of either MHC class II-deficient mice or TCRα-deficient mice were i.p. injected with 100 μg of the Ab in 50 μl of PBS. Unless otherwise indicated, mice were analyzed on day 5 after the injection.

**FTOC**

FTOC was conducted as previously described (22). Thymus lobes obtained from MHC class II-deficient mice at day 16 or 17 of the gestation were cultured for 5 days in the absence or presence of anti-TCRβ Ab (10 μg).

**Immunofluorescence staining and flow cytometry analysis**

Immunofluorescence staining was performed as described (23). Labeled mAbs were obtained from BD PharMingen (San Diego, CA). Multicolor flow cytometry analysis was performed using two-laser FACScalibur (BD Biosciences, San Jose, CA). CellQuest software was used to obtain data on viable cells that were determined by forward-light scatter intensity and propidium iodide exclusion.

**T cell stimulation**

Thymocytes from untreated or Ab-treated mice were stimulated with either Con A (2.5 μg/ml) or plate-bound anti-CD3 Ab (1 μg/ml) and anti-CD28 Ab (5 μg/ml) in a 12-well plate for 72 h.

**Chemotaxis assay**

Chemotaxis assay was performed as described (24, 25). Briefly, thymocytes (5 × 10^5 cells in 0.1 ml) from Ab-treated or untreated mice were placed on a 5-μm-pore, 6.5-mm-diameter polycarbonate membrane (Costar, Cambridge, MA) in a chamber with 0.6 ml of medium containing 100 nM chemokine in the bottom well. After 90 min at 37°C, cells were counted and stained for flow cytometry analysis.

**Histological analysis**

Frozen thymuses embedded in OCT compound (Sakura Finetek, Torrance, CA) were sectioned at 5 μm and stained with H&E. The area of the cortex and medulla was analyzed by using NIH Image software, version 1.62. For immunohistological analysis, frozen sections were fixed with acetone, blocked with 2.4G2 anti-Flk1 Ab, and stained with Alexa 488-conjugated anti-mouse CD4 (GK1.5), biotin-conjugated anti-mouse CD8α (53-6.7), and Alexa 546-conjugated UEA-1 (Vector Laboratories, Burlingame, CA). Biotin signals were visualized with Alexa 633-conjugated streptavidin ( Molecular Probes, Eugene, OR). The confocal images were acquired using a Leica (Mannheim, Germany) TCS SP2 confocal laser-scanning unit equipped with Ar and He-Ne lasers (excitation lines, 488, 546, and 633 nm). Images were obtained using Leica confocal software, version 2.0.

**Results**

**Administration of anti-TCR Ab generates CD4^+ CD8^− thymocytes in class II MHC-deficient mice in vivo**

Mice that are deficient for class II MHC molecules are devoid of mature CD4^+ CD8^− SP T cells (9, 10). We have previously shown that the treatment with anti-TCRβ Ab of FTOC from class II MHC KO mice induces the generation of a distinct population of CD4^+ CD8^− thymocytes (Ref. 14; also shown in Fig. 1A). The present study has examined whether the treatment of class II MHC KO mice with anti-TCRβ Ab might also cause the generation of CD4^+ CD8^− thymocytes in vivo. As shown in Fig. 1B, 5 days after the injection of anti-TCR Ab into the peritoneal cavity of newborn class II MHC KO mice, thymocytes from class II MHC KO mice contained a significantly higher number of CD4^+ CD8^− cells than thymocytes from untreated class II MHC KO mice. The increase of CD4^+ CD8^− cells was specific for anti-TCRβ Ab, because the injection of other hamster mAbs with irrelevant specificity did not increase CD4^+ CD8^− cells (data not shown). The treatment with anti-TCRβ Ab did not elevate the number of CD4^+ CD8^− cells in TCRα KO mice (Fig. 1C), suggesting that an increase of CD4^+ CD8^− cells is indeed caused by the engagement of TCRαβ Ag receptor.

It should be noted that the in vitro treatment in FTOC (Fig. 1A) gave more severe reduction of CD4^-CD8^- cells than the in vivo treatment (B) at the Ab concentrations used for these experiments. However, it was also found that a higher concentration of anti-TCRβ Ab caused a more severe reduction of CD4^-CD8^- cells in the in vivo condition, and that a lower concentration of anti-TCRβ Ab caused a less severe reduction of CD4^-CD8^- cells in the in vitro FTOC condition (data not shown). Thus, we think it is likely

**FIGURE 1.** Anti-TCR Ab restores the generation of CD4^+ CD8^− thymocytes in class II MHC-deficient mice. A, Day 16 fetal thymus lobes from class II MHC KO mice were placed in organ culture for 5 days with or without 10 μg/ml anti-TCRβ Ab. Recovered cells were analyzed for expression of CD4 and CD8 by flow cytometry. The numbers beside each box indicate the frequency of cells in that box. Bar graphs indicate means and SEs of absolute cell numbers in CD4^-CD8^−, CD4^+ CD8^-; and CD4^-CD8^− gates per lobe from the cultures in the absence (□) or presence (■) of anti-TCRβ Ab. Asterisks indicate statistical significance (p < 0.05) by Student’s t test. B, Newborn class II MHC KO mice were injected with 100 μg of anti-TCRβ Ab on the day of birth. Thymocytes were analyzed at 5 days of age. C, Newborn TCRα KO mice were injected with 100 μg of anti-TCRβ Ab on the day of birth, and thymocytes were analyzed at 5 days of age. Representative data from three independent experiments are shown.
that a higher local concentration of anti-TCR Ab within the thymus architecture in the condition shown in Fig. 1A than in Fig. 1B caused a higher frequency of apoptosis in CD4\(^{+}\)CD8\(^{-}\) thymocytes.

Most CD4\(^{+}\)CD8\(^{-}\) cells generated in anti-TCR-treated class II MHC KO mice expressed a high level of CD5, an undetectable level of CD25, and a very low level of CD69 (Fig. 2A), resembling the phenotype of newly generated mature T cells rather than extensively proliferating T cells derived from a few CD4\(^{+}\)CD8\(^{-}\) thyromocytes that might have been present before anti-TCR stimulation. To assess the functional maturity of CD4\(^{+}\)CD8\(^{-}\) thyromocytes generated upon the anti-TCR injection, thyromocytes obtained from anti-TCR-treated or untreated class II MHC KO mice were stimulated with either Con A or anti-CD3 plus anti-CD28 Abs. Thus, the newly generated CD4\(^{+}\)CD8\(^{-}\) thyromocytes contained the cells that became blastoid and CD25 positive upon stimulation with either Con A or anti-CD3 plus anti-CD28 Abs. These results indicate that the administration of anti-TCR Ab generates CD4\(^{+}\)CD8\(^{-}\) mature thyromocytes in class II MHC-deficient mice in vivo.

**Peripheral supply of CD4\(^{+}\)CD8\(^{-}\) T cells in anti-TCR-treated class II MHC-deficient mice**

To investigate whether anti-TCR-induced CD4\(^{+}\)CD8\(^{-}\) thyromocytes are capable of emigrating from the thymus, thyromocytes from anti-TCR-injected class II MHC KO mice were examined for their chemotactic responsiveness to CC chemokine ligand (CCL)19 and CCL21. It has been shown that these CCR7-ligand chemokines are involved in the emigration of mature T cells from the thymus to the circulation in newborn mice (23). As shown in Fig. 3, either CCL19 or CCL21 efficiently attracted CD4\(^{+}\)CD8\(^{-}\) thyromocytes generated in anti-TCR-treated class II MHC KO newborn mice.

We then examined whether CD4\(^{+}\)CD8\(^{-}\) thyromocytes in anti-TCR-treated class II MHC KO mice indeed emigrated to the peripheral circulation. Five days after the injection of anti-TCR Ab, CD4\(^{+}\)CD8\(^{-}\) cells were found in the peripheral blood and in the spleen of class II MHC KO newborn mice (Fig. 4A). Thus, a single injection of anti-TCR Ab is sufficient to restore circulating CD4\(^{+}\)CD8\(^{-}\) T cells as well as CD4\(^{+}\)CD8\(^{-}\) thyromocytes in class II MHC-deficient mice.

It is interesting to note that, within 5 wk after the injection of anti-TCR Ab, CD4\(^{+}\)CD8\(^{-}\) spleen cells in anti-TCR-treated class II MHC KO mice were not higher in numbers than CD4\(^{+}\)CD8\(^{-}\) spleen cells in untreated class II MHC KO mice (Fig. 4B), whereas the number of CD4\(^{+}\)CD8\(^{-}\) thyromocytes was still higher in anti-TCR-treated mice even after 5 wk of the injection (Fig. 4B). These results are consistent with a previously described notion that long-term survival of naive CD4 T cells in the periphery requires TCR signals through class II MHC molecules (26). The results also suggest that the maintenance of CD4\(^{+}\)CD8\(^{-}\) thyromocytes is regulated differently from the maintenance of peripheral CD4\(^{+}\)CD8\(^{-}\) T cells and occurs even in the absence of class II MHC molecules or peripheral CD4\(^{+}\)CD8\(^{-}\) T cells. It was also found that the number of CD4\(^{+}\)CD8\(^{-}\) T cells in the spleen of H57-597-treated mice was significantly decreased after 5 wk of the treatment (Fig. 4B), which is possibly due to the apoptosis of CD8 T cells mediated by TCR stimulation.

**Defective formation and anti-TCR-mediated restoration of thymic medulla in class II MHC-deficient mice**

It has been shown that the development of the thymic medulla is dependent on the generation of SP thyromocytes (27). However, it has been unclear how CD4 SP and CD8 SP thyromocytes individually regulate the medulla formation. In particular, it is not known...
whether the lack of either class I MHC molecules or class II MHC molecules may affect medulla formation. We have measured the three-dimensional volume of the cortical and medullary regions in the thymus, by summing software-calculated volumes of 5-μm slices throughout the thymus. The medullary and cortical regions were distinguished by H&E staining (Fig. 5) as well as by three-color immunohistological staining for CD4, CD8, and medullary epithelial cell-specific reagents such as UEA-1 (Fig. 6). We find that the volume of thymic medulla is not optimal in either class I MHC KO mice or class II MHC KO mice (Figs. 5, A and B, and 6, A–D). Thus, it should be important to point out that CD4⁺CD8⁻ thymocytes do not compensate for the loss of CD4⁺CD8⁺ thymocytes to form optimal medulla size in class I MHC KO mice (Figs. 5C and 6B). These results suggest that the formation of medullary areas for class I MHC-dependent CD4⁺CD8⁻ thymocytes and class II MHC-dependent CD4⁺CD8⁻ thymocytes are mutually independent.

Interestingly, the treatment of class II MHC KO mice with anti-TCR Ab restored the reduced volume of the thymic medulla, whereas the volume of their cortex was not significantly affected (Figs. 5, A and B, and 6, C, E, and F), indicating that the injection of anti-TCR Ab restores not only the generation of CD4⁺CD8⁻ thymocytes but also optimal formation of the medullary microenvironment in the thymus. Individual medulla in the anti-TCR-treated class II MHC KO mice always contained newly generated CD4⁺CD8⁻ thymocytes and residual CD4⁺CD8⁻ thymocytes together (Fig. 6, E).

**FIGURE 4.** Peripheral supply of CD4⁺CD8⁻ T cells in anti-TCR-treated class II MHC-deficient mice. Newborn class II MHC KO mice were injected with 100 μg of anti-TCR Ab on the day of birth. A, Thymocytes, peripheral blood leukocytes (PBL), and splenocytes were analyzed at 5 days of age. Bar graph for PBL shows the number of cells per microliter of blood. B, Thymocytes and splenocytes were analyzed at 5 wk of age. Asterisks indicate statistical significance (*, p < 0.05; **, p < 0.005; ***, p < 0.0005) by Student’s t test. Representative data from three independent experiments are shown.

**FIGURE 5.** Defective formation and anti-TCR-mediated restoration of the thymic medulla in class II MHC-deficient mice. A. Histological analysis by H&E of the thymus from indicated mice (3 wk of age). B, Three-dimensional analysis for the volume (cubic millimeters) of medullary and cortical regions, defined by H&E staining, in indicated mice. C, Absolute cell numbers of CD4⁺CD8⁻ and CD4⁺CD8⁺ cells in the thymus and spleen of indicated mice (5 wk of age). Asterisks indicate statistical significance (*, p < 0.05; **, p < 0.005; ***, p < 0.0005) by Student’s t test. Representative data from three independent experiments are shown.
and F), indicating that medullary areas formed for CD4⁺CD8⁻ and CD4⁺CD8⁺ thymocytes are tightly localized rather than remotely generated.

Discussion

It has been shown previously that in vitro treatment with anti-TCR Ab could restore the generation of CD4⁺CD8⁻ thymocytes in FTOCs of class II MHC-deficient mice, indicating that anti-TCR Ab could mimic TCR signals that induce positive selection of CD4⁺CD8⁻ thymocytes (14–16). It has also been shown that in vivo treatment with a hybrid Ab consisting of anti-Vβ8 Ab and anticortical epithelial cell Ab rescues the generation of Vβ8⁺CD4⁺CD8⁻ thymocytes in class II MHC-deficient mice (18–20). However, it was not known whether CD4⁺CD8⁻ thymocytes generated by the anti-TCR treatment could emigrate the thymus to supply CD4 T cells in the periphery. It was also unknown whether the formation of the medullary region might be impaired in class II MHC-deficient mice and might be affected with the Ab treatment. In the present study, we show that i.p. injection of anti-TCR Ab into class II MHC-deficient newborn mice caused the generation of phenotypically and functionally mature CD4⁺CD8⁻ thymocytes. We find that anti-TCR treatment in class II MHC KO mice restored the generation of CD4 T cells in the peripheral circulation and optimal architecture of the medullary region.

Functionally mature CD4 T cells were restored in class II MHC-deficient mice by a single administration of anti-TCR Ab, indicating that a transient TCR ligation of immature thymocytes is sufficient for generating CD4 T cells in class II MHC KO mice. However, we do not think that a single hit of transient TCR ligation is sufficient for supporting entire process of positive selection, because we have found that, unlike class II MHC KO mice, class I/class II MHC double-KO mice did not generate CD4⁺CD8⁻ thymocytes upon a single injection of 100 μg of anti-TCRβ Ab (data not shown). We have also found that two shots of anti-TCR administration within a 24-h interval restored the generation of CD4⁺CD8⁻ thymocytes in class I/class II MHC double-KO mice (our unpublished results), consistent with a previous report showing that two separable TCR signals are required for positive selection of CD4 T cells in class I/class II MHC double-KO mice in

![Figure 6. Immunofluorescence analysis of the thymus sections from B6 (A), class I MHC KO (B), class II MHC KO (C), class I and class II MHC double-KO (D), and anti-TCR-treated class II MHC KO (E and F) mice. Green, CD4 fluorescence; blue, CD8 fluorescence; red, UEA-1 fluorescence, which indicates medullary area. Cyan color indicates the merge of green and blue fluorescence, indicating CD4⁺CD8⁻. Scale bars, 80 μm. Representative data from three independent experiments are shown.](http://www.jimmunol.org/)

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Peripheral CD4 T cells restored in class II MHC KO mice disappeared within 5 wk after the injection of anti-TCR Ab, consistent with the previous finding that the long-term survival of naive CD4 T cells requires the interaction with class II MHC molecules in the periphery (26). Our results also show that CD4+CD8− thymocytes were maintained long term in the Ab-treated class II MHC KO mice even after 5 wk, at which time peripheral CD4 T cells have disappeared. It is not clear whether CD4+CD8− thymocytes were maintained for 5 wk by a continuous supply of newly generated CD4+CD8− thymocytes or by a long-term persistence of initially generated CD4+CD8− thymocytes. Nevertheless, these results suggest that 1) unlike peripheral CD4+CD8+ T cells, long-term survival of CD4+CD8− T cells in the thymus can be supported in the absence of class II MHC molecules, and 2) the thymic environment that supports the survival of CD4+CD8− T cells does not belong to open circulation. It has been shown that T cell emigration out of the thymus is a process that is actively regulated by chemokine-mediated signals, including CCR7 ligands (23, 30, 31), even though it is known that mature circulating T cells can recirculate into the thymus (32).

The thymic microenvironment is composed of a network of epithelial and reticular cells, which forms the cortex and medulla (27, 33). The development of the thymic microenvironment in vivo is regulated by thymocyte subsets (27). In particular, the formation of the medullary environment is tightly dependent on the generation of mature SP thymocytes as a result of positive selection, so that mice deficient for undergoing positive selection by the lack of TCRα or ζ-associated protein 70 carry a severely reduced and disorganized medulla in the thymus (Fig. 5) (34). Thus, it was reasonable to find severe reduction in the mass of the medullary region in class I and class II MHC double-KO mice in which positive selection is severely affected (Figs. 5 and 6). However, it was unclear whether the development of the thymic medulla might be optimal in mice that are deficient for either class I MHC or class II MHC molecules. Our results indicate that the medulla in the thymus of class I MHC KO mice or class II MHC KO mice are smaller in volume than the medulla of normal mice (Fig. 5B). Consistently, either CD4+CD8− thymocytes in class I MHC KO mice or CD4+CD8− thymocytes in class II MHC KO mice were not significantly different in number than those in normal mice (Fig. 5C). Thus, CD4+CD8− thymocytes do not compensate for the loss of CD4+CD8+ thymocytes in forming the medulla of class I MHC KO mice. Likewise, CD4−CD8+ thymocytes do not compensate for the loss of CD4+CD8− thymocytes in forming the medulla of class II MHC KO mice. These results suggest that, in terms of cell numbers and medulla-occupying volume, class I MHC-dependent CD4+CD8− thymocytes and class II MHC-dependent CD4+CD8− thymocytes are generated and maintained in a mutually independent manner. Additionally, it should be pointed out that the numbers of CD4 T cells and CD8 T cells are also independently regulated in the spleen, because there was no compensation between these two T cell populations (Fig. 5C).

Molecular mechanisms that determine the size of medullary areas for CD4+CD8− and CD4+CD8+ thymocytes must await further analysis.

Our results also show that a single injection of anti-TCR Ab is sufficient to reconstitute the volume of the medulla in class II MHC KO mice. As in normal mice, the individual medulla in the anti-TCR-treated class II MHC KO mice always contained CD4+CD8− thymocytes and CD4+CD8+ thymocytes together (Fig. 6). These results indicate that medullary areas formed for CD4+CD8− and CD4+CD8+ thymocytes are closely localized rather than remotely generated. Thus, it is conceivable that the medullary areas for CD4+CD8− and CD4+CD8+ thymocytes are independently generated but are tightly associated with each other. Epithelial cells that are involved in generating medullary areas for CD4+CD8− and CD4+CD8+ thymocytes may be derived from common progenitor cells (35).

Finally, we would like to suggest that anti-TCR Ab may be useful for the treatment of MHC deficiencies. MHC class II deficiency in humans, also referred to as bare lymphocyte syndrome (BLS), is a congenital immunodeficiency disease characterized by the absence of MHC class II molecules at the cell surface (36). Clinical manifestations typical of BLS include recurrent infections of the gastrointestinal tract, pneumonitis, and bronchitis, essentially caused by the lack of generation and function of CD4 T cells. At present, the only curative treatment of BLS, an otherwise lethal immunodeficiency, consists of bone marrow transplantation (36). However, the success rate of bone marrow transplantation in BLS patients is still low (37, 38). The present results suggest that in vivo anti-TCR treatment could also restore CD4 T cells in BLS patients. CD4 T cells restored by the anti-TCR treatment may be less effective in restoring immune functions than CD4 T cells that are restored by bone marrow transplantation, because the repertoire of Ab-generated CD4 T cells will not be positively selected by self-MHC molecules (15), and the long-term survival of Ab-generated CD4 T cells will not be maintained or activated by the lack of class II MHC-bearing APCs (26). Possible activation and/or apoptosis of CD8 T cells by anti-TCR Ab should also be considered. However, transiently recovered CD4 T cells may at least contribute to immune functions that belong to innate immunity and/or class I MHC-restricted responses. Moreover, certain BLS patients are known to express class II MHC molecules in a cell type-specific manner (36, 39), in which CD4 T cells recovered by anti-TCR Ab may persist and recognize peptide-Ags presented by class II MHC molecules. Furthermore, the administration of anti-TCR Ab would be far easier and widely available than bone marrow transplantation. Thus, our results suggest that the treatment with anti-TCR Ab could help at least certain BLS patients in transiently recovering CD4 T cells.

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