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ORIGINAL ARTICLE

CD4+ T cells from MHC II-dependent thymocyte–thymocyte interaction provide efficient help for B cells

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Recently, a novel CD4+ T-cell developmental pathway was reported that generates thymocyte–thymocyte (T–T) CD4+ T cells. We established a mouse system (CIITA^{tg}CIITApIV$^{-/-}$) where thymic positive selection occurred only by major histocompatibility complex (MHC) class II+ thymocytes. T–T CD4+ T cells selected via MHC class II-dependent T–T interaction are comprised of PLZF-negative and innate PLZF-positive populations. Until recently, the functional role of the PLZF-negative population was unclear. In this study, we demonstrate that naïve T–T CD4+ T cells provide B-cell help to a level comparable with that of naïve conventional CD4+ T cells. Considering the absence of PLZF expression in naïve T–T CD4+ T cells, these results suggest that PLZF-negative naïve T–T CD4+ T cells are functionally equivalent to conventional naïve CD4+ T cells in terms of B-cell help.
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Unlike conventional T cells, a distinct lineage of T cells, termed innate T cells, can arise in the thymus by positive selection on hematopoietic cells.[1] During their developmental progress from CD4+CD8+ thymocytes, they acquire effector functions as a result of maturation processes, rather than as a consequence of activation following an antigenic encounter in the periphery, and thus have innate-like characteristics in terms of rapid cytokine secretion in response to antigenic or other stimuli.[2] To date, invariant natural killer T cells, γδ T cells, mucosa-associated invariant T cells, H2-M3-restricted CD8+ T cells and CD8αα+ intraepithelial lymphocytes have been described as innate T cells.[2–8] In mice, all of these innate-type T cells are mostly restricted to major histocompatibility complex (MHC) class Ib molecules. However, some T cells in humans, called thymocyte–thymocyte (T–T) CD4+ T cells, are selected by recognition of MHC class II self-peptide complexes on thymocytes. The T–T hypothesis was first raised by *in vitro* reaggregate culture systems of human thymocytes, on the basis of their expression of MHC class II molecules,[9,10] and then sequentially evidenced in class II MHC transactivator (CIITA)-transgenic mice[11,12] and human fetuses.[13] They share some characteristics with invariant natural killer T cells, such as SLAM-SAP-dependent development,[14] simultaneous production of interferon-γ (IFN-γ) and interleukin-4 (IL-4),[15] and promyelocytic leukemia zinc-finger protein, PLZF (also known as zbtb16) expression.[13,16] Specifically, PLZF directs the acquisition of innate phenotypes in both invariant natural killer T cells and T–T CD4+ T cells.[13,17–19] However, T–T CD4+ T cells are unique in that they have a diverse T-cell receptor (TCR) repertoire and consist of a PLZF-negative population as well as a PLZF-positive population. Given their innate properties and preferential generation during the prenatal stage in humans, PLZF-positive T–T CD4+ T cells have been implicated in neonatal antiviral immunity.[13,16] In contrast, PLZF-negative T–T CD4+ T cells are more similar to conventional T cells with respect to the absence of activation/memory markers on their surface during the intrathymic maturation process. However, their function in immune response has not yet been fully determined.

The B-cell response to protein antigens requires cognate interactions between antigen-specific B cells and activated antigen-specific CD4+ helper T cells.[20] This cognate help for B cells is a specialized spectrum of effector T-helper cell functions. Alternatively, T-cell help for B cells can be indirect or non-cognate, in which the T cell is not specific for peptide-MHC molecules presented by B cells. In this case, activated T cells support B-cell immune responses by secreting large quantities of cytokines.[21] This type of B-cell help is more likely to be performed by innate T cells, such as natural killer T cells.[22] On the basis of these findings, we investigated whether T–T CD4+ T cells were able to help B-cell responses upon antigen challenge and examined whether B-cell help was performed by PLZF-positive or PLZF-negative T–T CD4+ T cells.

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RESULTS

Normal B-cell development in the presence of T–T CD4+ T cells

The mouse system in which T–T CD4+ T cells develop was previously described.[13,16] In CIITA^{tg}pIV$^{-/-}$ mice, immature CD4+ T cells are positively selected only by MHC class II-expressing cortical thymocytes (Supplementary Figure 1) and subsequent negative selection is normally executed by medullary thymic epithelial cells and dendritic cells.[23] Before addressing a B-cell helper function of T–T CD4+ T cells, we investigated whether B-cell development was compromised in CIITA^{tg}pIV$^{-/-}$ mice. As previously reported, a substantial fraction of T–T CD4+ T cells are PLZF-positive innate cells that can rapidly secret large amounts of IL-4 and IFN-γ. These cells influence CD8+ T cell development.[11,12] In wild-type mice, therefore, it was important to ask whether the presence of T–T CD4+ T cells disturbs B-cell development. In the overall proportion of B cells in bone marrow, spleen and lymph nodes, no significant difference was found between CIITA^{tg} pIV$^{-/-}$ and wild-type B6 mice (Figure 1a). Moreover, dissection of the B-cell population in spleen into mature B cells (IgM+IgD+), follicular B cells (CD19+CD21+CD23+), marginal zone B cells (CD19+CD21+CD23^{lo}), germinal center B cells (GL7+CD19+) and plasma cells (CD138+CD19+) showed a normal distribution of B-cell sub-populations in CIITA^{tg}pIV$^{-/-}$ mice (Figures 1b and c). Thus, T–T CD4+ T cells do not seem to have any influence on B-cell development in terms of proportion of respective B-cell subcompartments.

T–T CD4+ T cells are able to help B-cell responses to T-dependent antigen

To evaluate the B-cell help activity of T–T CD4+ T cells, we immunized CIITA^{tg}pIV$^{-/-}$ and wild-type B6 mice with the T-cell-dependent antigen, 4-hydroxy-3-nitrophenylacetyl (NP)-keyhole limpet hemocyanin (KLH), in alum and measured serum titers of NP-specific antibodies at various time points. Antigen-challenged pIV$^{-/-}$ mice, in which positive selection of CD4+ T cells is almost abolished, served as a negative control. Serum levels of IgG1 and IgG3 antibodies were quantified by ELISA. Compared with wild-type mice, serum levels of NP-specific IgG1 and IgG3 were somewhat lower in CIITA^{tg}pIV$^{-/-}$ mice (Figure 2a). However, CIITA^{tg}pIV$^{-/-}$ mice produced significantly more NP-specific IgG1 and IgG3 than pIV$^{-/-}$ mice, indicating that T–T CD4+ T cells were able to provide help to B lymphocytes for the production of antigen-specific antibodies. NP-specific antibody response was further investigated in mice that were re-challenged with NP-KLH at 60 days after primary immunization. NP-specific antibody titer in serum of CIITA^{tg} pIV$^{-/-}$ mice was still lower than that of wild-type mice (Figure 2b).

In an earlier mouse model of T–T interaction (CIITA^{tg}CIITA$^{-/-}$), the splenic CD4+ T-cell number was slightly higher than that of wild-type littermates.[11] However, negative selection by thymic epithelial and dendritic cells is defective in this system. Thus, we compared the proportion of CD4+ T cells in the thymus and secondary lymphoid tissues of CIITA^{tg}pIV$^{-/-}$ and wild-type mice to determine the effect

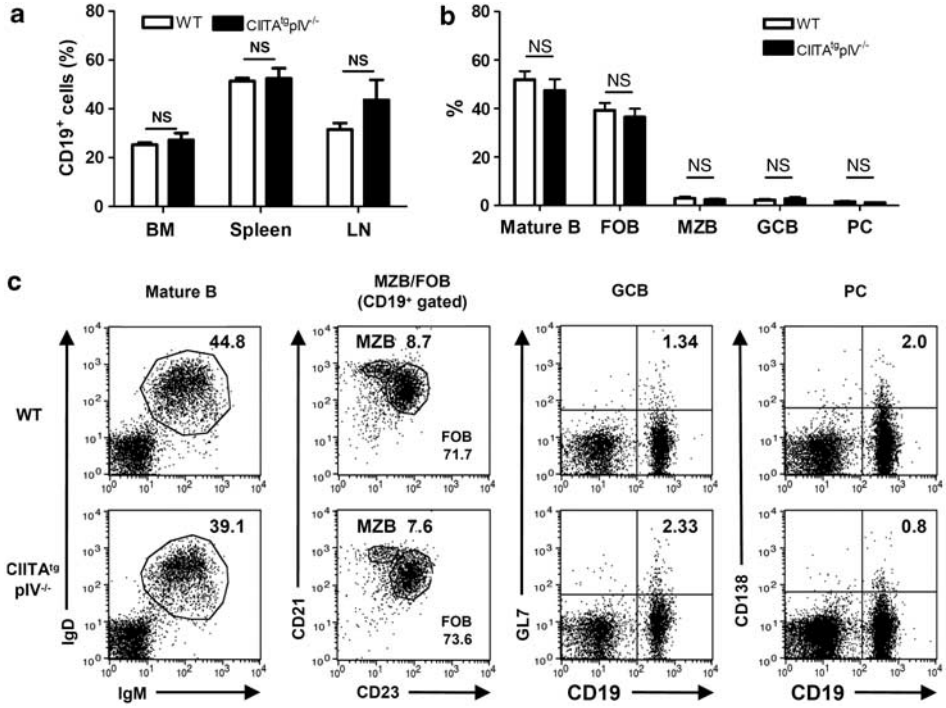

Figure 1 Normal B-cell development in CIITA^{tg}pIV$^{-/-}$ mice. (**a**) Comparison of B-cell percentage in bone marrow (BM), spleen and lymph nodes (LN) between wild-type (WT) and CIITA^{tg}pIV$^{-/-}$ mice. To identify B-cell population, total nucleated cells obtained from BM, spleen and LN of each mouse were stained with CD19 antibody and analyzed via flow cytometry. The percentage of CD19+ cells is shown in each compartment. The data are mean values ± s.e.m. from three animals in each group. (**b**, **c**), Comparison of splenic B-cell subset percentage in spleen between WT and CIITA^{tg}pIV$^{-/-}$ mice. To identify mature B cells, splenocytes from each mouse were stained with anti-IgD and anti-IgM. To reveal marginal zone B cells (MZB) and follicular B cells (FOB), CD19+-gated B cells were analyzed for expression of CD21 and CD23. Germinal center B cells (GCB) were identified with anti-GL7 and anti-CD19 and plasma cells (PC) with anti-CD138 and anti-CD19. In bar graph, the percentage of each subset among total splenocytes of CIITA^{tg}pIV$^{-/-}$ or WT mice is summarized, and the data are mean values ± s.e.m. from three animals in each group (**b**). A representative flow-cytometric profiles of each B-cell subset are also shown, and the numbers are the percentage of given subsets of B cells among total splenocytes (Mature B, GCB and PC) or the percentage of marginal zone B cells and follicular B cells among all CD19+ B cells (**c**). NS, not significant.

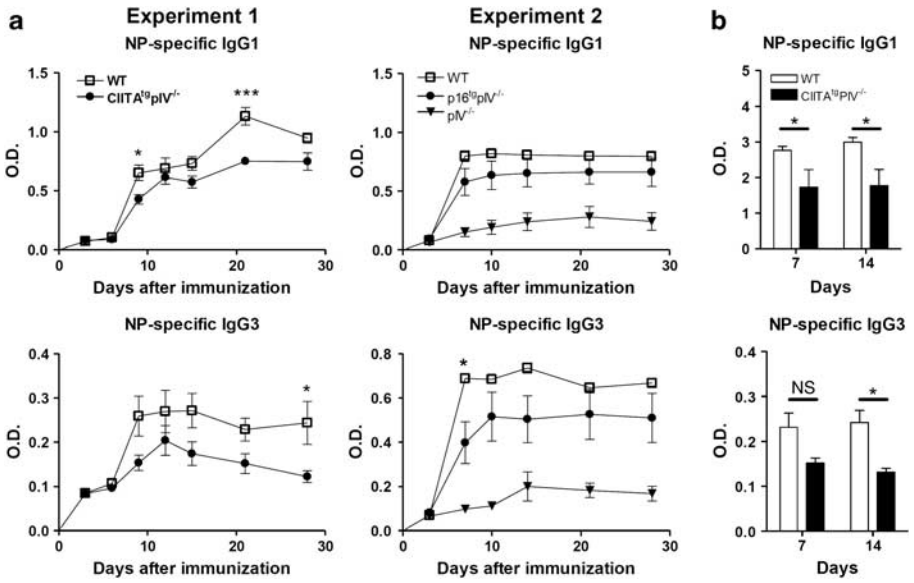

Figure 2 CIITA^{tg}pIV$^{-/-}$ mice are able to mount B-cell responses to a T-dependent antigen, NP-KLH. (**a**) Serum IgG1 and IgG3 antibody responses to NP after primary immunization with NP-KLH. Wild-type (WT), CIITA^{tg}pIV$^{-/-}$, or pIV$^{-/-}$ mice were injected i.p. with 50 μg of NP-KLH on day 0 and bled on days 0, 3, 7, 10, 14, 21 and 28. Serum anti-NP responses were measured, and expressed as arbitrary OD units. Two separately performed experiments (experiment 1 and experiment 2) are shown. The data are mean values ± s.e.m. from five animals in each group. (**b**) Secondary antibody responses to NP. Wild-type and CIITA^{tg}pIV$^{-/-}$ mice were re-challenged with NP-KLH at 60 day after primary immunization, and the anti-NP antibody titers in serum were compared on day 7 and 14. The data are mean values ± s.e.m. from four (CIITA^{tg} pIV$^{-/-}$) or six (WT) animals in each group. NS, not significant; *$P<0.05$; ***$P<0.001$.

of negative selection on the population of mature T–T CD4$^+$ T cells. As expected, MHC class II expression in thymic medullary epithelial cells and dendritic cells in CIITA^{tg}pIV$^{-/-}$ mice led to a significant reduction in the percentage of CD4$^+$ T cells in the thymus, spleen and lymph nodes compared with that of wild-type mice (Figure 3a). On the contrary, we were not able to find any significant difference in the BrdU-labeled fraction of the peripheral CD4$^+$ T cells from CIITA^{tg}pIV$^{-/-}$ and wild-type mice after the daily injection of BrdU during 9 days (Figure 3b), suggesting that CD4$^+$ T-cell turnover was not overtly affected in CIITA^{tg}pIV$^{-/-}$ mice. Therefore, these findings raised the possibility that the lower level of NP-specific antibody production in CIITA^{tg} pIV$^{-/-}$ mice might result from the decrease in CD4$^+$ T-cell number.

To investigate this possibility, CD4$^+$ T cells were isolated from the spleens of CIITA^{tg}pIV$^{-/-}$ mice and wild-type littermates at day 14 after primary or secondary immunization, and 3×10^5 CD4$^+$ T cells were re-stimulated with KLH in the presence of irradiated antigen-presenting cells. When the *ex vivo* T-cell responses were compared via thymidine incorporation, the CD4$^+$ T cells of CIITA^{tg}pIV$^{-/-}$ mice showed a lower proliferative response in both primary and secondary response, compared with that of wild-type mice (Figure 3c). These data suggested that the generation of antigen-specific CD4$^+$ T cells *per se* was somewhat attenuated in CIITA^{tg}pIV$^{-/-}$ mice.

Next, to compare B-cell help activity of the same number of CD4$^+$ T cells in *in vivo*, 5×10^5 splenic CD4$^+$ T cells were isolated from CIITA^{tg}pIV$^{-/-}$ and wild-type mice and adoptively transferred into pIV$^{-/-}$ mice. Thereafter, these mice were immunized with NP-KLH in alum, and the serum titers of NP-specific antibodies were compared with those in pIV$^{-/-}$ mice where CD4$^+$ T cells were not adoptively transferred (Figure 3d). Similar to those in CIITA^{tg}pIV$^{-/-}$ mice, adoptively transferred T–T CD4$^+$ T cells were able to help the antibody response by host B cells. However, both IgG1 and IgG3 antibody responses in recipients of T–T CD4$^+$ T cells were still somewhat lower than in mice provided with wild-type CD4$^+$ cells. These data suggest that T–T CD4$^+$ T cells have a clear ability to provide B-cell help, but seemingly to a slightly lower level than that of wild-type CD4$^+$ T cells.

B-cell helper activity of the naïve population of T–T CD4$^+$ T cells was comparable to that of conventional naïve CD4$^+$ T cells

Unlike conventional CD4$^+$ T cells, a substantial fraction of T–T CD4$^+$ T cells express PLZF and have innate properties.[13] To investigate the possibility that the PLZF-positive innate T cells, which comprise a substantial fraction of total T–T CD4$^+$ T cells, might affect their total B-helper activity, T–T CD4$^+$ T cells were fractionated into naïve and activation/memory cells, based on the expression of CD62L and CD44, and then transferred to pIV$^{-/-}$ mice. Thereafter, we investigated the B-cell helper activity of each population of T–T CD4$^+$ cells separately. As reported previously,[13] T–T CD4$^+$ T cells had an even lower fraction of CD62L^{high}CD44^{low} naïve cells, and PLZF was not expressed in this population (Figure 4a). To see the helper response for antibody production, the recipient mice were immunized with NP-KLH and serum anti-NP antibodies were measured at indicated time points. Naïve T–T CD4$^+$ T cells were able to induce fairly good NP-specific antibody responses in recipient mice that were comparable to those of conventional naïve CD4$^+$ T cells (Figure 4b). In contrast, recipients of T–T and conventional activation/memory cells showed attenuated responses (Figure 4c). Thus, it was evident that PLZF-negative naïve T–T CD4$^+$ cells were responsible for sufficient B-cell help, indeed, comparable to that of conventional naïve CD4$^+$ T cells. These data indicate that the lower antibody response in mice provided with unfractionated T–T CD4$^+$ T cells was due to the increased frequency of activation/memory CD4$^+$ T cells and their decreased B-cell helper activity.

DISCUSSION

In this study, our primary question was whether T–T CD4$^+$ cells had the ability to recognize peptides that were processed in B cells and dendritic cells. Thymocytes are less likely to be equipped with a full

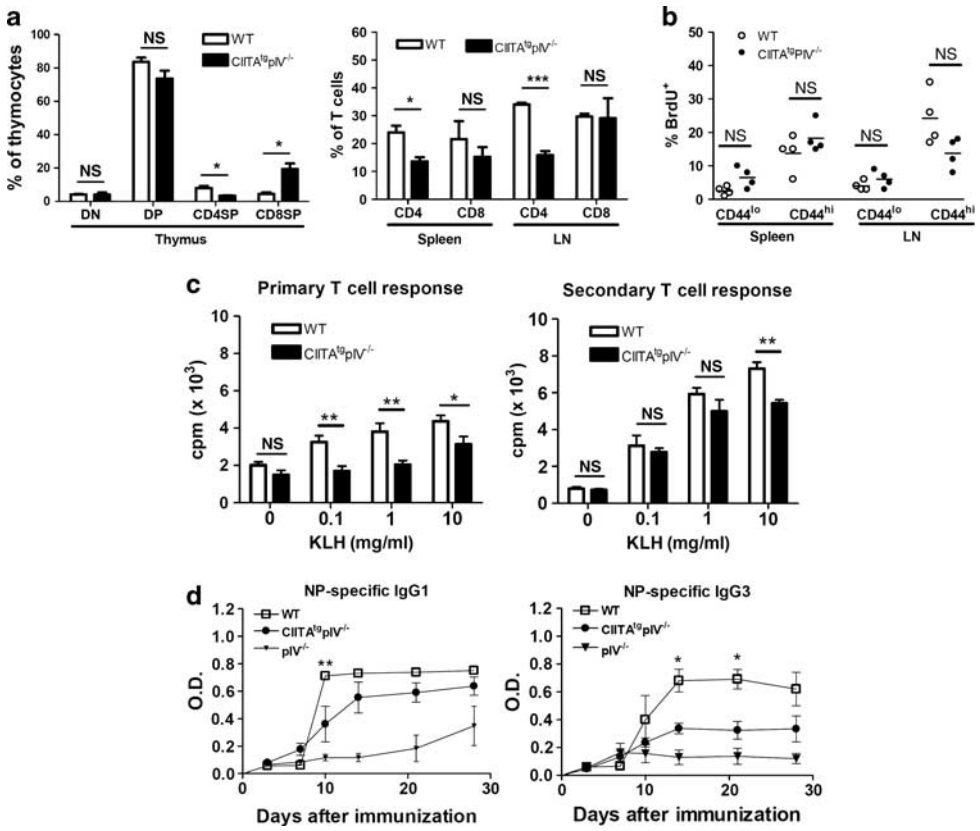

Figure 3 T–T CD4+ T cells help B-cell responses to a T-dependent antigen, NP-KLH, to a lower level than WT CD4+ T cells. (**a**) Comparison of the percentage of thymic or splenic CD4+ cells between WT and CIITA^{tg} pIV$^{-/-}$ mice. (**b**) Assessment of T-cell turnover rate via *in vivo* BrdU labeling of CD4+ T cells in spleen and lymph nodes of WT and CIITA^{tg}pIV$^{-/-}$ mice given BrdU for 9 days. Each symbol represents an individual mouse, and the bars mark the mean percentage of BrdU-positive population in each subset. (**c**) Comparison of T-cell responses after first (left) and second (right) immunization with NP-KLH in WT and CIITA^{tg}pIV$^{-/-}$ mice. Mice were immunized with NP-KLH on day 0 and 60, and CD4+ T cells isolated from spleen at 14 days after each immunization were re-stimulated with KLH in the presence of irradiated antigen-presenting cells. Proliferative response of T cells was measured as [^{3}H]thymidine uptake. The data are mean values ± s.e.m. of quadruplicate reactions. (**d**) Serum antibody response to NP in pIV$^{-/-}$ mice, which received un-fractionated CD4+ T cells from WT or CIITA^{tg}pIV$^{-/-}$ mice. The pIV$^{-/-}$ mice without CD4+ T-cell transfer were used as a negative control. The pIV$^{-/-}$ mice with or without CD4+ T-cell adoptive transfer were injected i.p. with 50 μg of NP-KLH on day 0, and serum anti-NP responses were measured and expressed as arbitrary OD units. The data are mean values ± s.e.m. from three animals in each group. NS, not significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

spectrum of antigen-presenting machinery for the positive selection of other thymocytes, compared with cortical thymic epithelial cells. For example, thymus-specific serine protease, that is expressed in cortical thymic epithelial cells and crucial for the positive selection of some CD4+ T cells, is not expressed in thymocytes.[24,25] Moreover, TCR-transgenic CD4+ T cells, such as AND or DO11.10 TCR transgenic T cells, are hardly selected by T–T interaction.[12] Thus, it is possible that T–T CD4+ T cells might be biased to recognize extracellularly generated peptides loaded onto MHC class II, regardless of the action of H2-DM, and thus are selected by peptides in a type-B presentation.[26–28] To evaluate this possibility, we initially generated a mixed bone-marrow chimera, in which OT-II TCR transgenic thymocytes were induced to be selected by MHC class II-expressing thymocytes in pIV$^{-/-}$ host. However, T–T interaction in this chimera did not allow the generation of sufficient OT-II T cells (Supplementary Figure 2). Thus, as an alternative, CIITA^{tg}pIV$^{-/-}$ mice were immunized with NP-KLH, and we found that T–T CD4+ T cells could respond against KLH antigen presented by antigen-presenting cells and then provide B-cell help.

It is well known that, in conventional CD4+ T cells, the TCR repertoire of the naïve cell population is more diverse than that of memory subsets acquired through previous antigenic selection.[27,29] Memory CD4+ T cells are less likely to offer appropriate help for newly encountered antigens, because they were selected by previously exposed antigens and their repertoire is more restricted than that of naïve CD4+ T cells. In our experiments, this suggestion was supported by the adoptive transfer study. The pIV$^{-/-}$ hosts that were repopulated with memory CD4+ T cells from un-sensitized CIITA^{tg}pIV$^{-/-}$ or wild-type mice showed a lower antibody response against NP-KLH immunization, compared with pIV$^{-/-}$ mice which received naïve CD4+ T cells. On the basis of this, as well as the abundance of already existing memory CD4+ T cells in CIITA^{tg}pIV$^{-/-}$ mice compared with those in wild-type mice before antigen challenge, our interpretation was that the lower B-cell helper activity of overall T–T CD4+ T cells was due to the decreased number of naïve CD4+ T cells in the peripheral lymphoid organs of CIITA^{tg}pIV$^{-/-}$ mice. In the adoptive transfer experiments of sorted cells, naïve T–T CD4+ T cells were as good as naïve conventional CD4+ T cells in their B-cell help capability. Regarding the TCR diversity of T–T CD4+ T cells, we observed that T–T CD4+ T cells had a diverse TCR-Vβ usage, similar to that of conventional CD4+ T cells,[11] which further supports our interpretation.

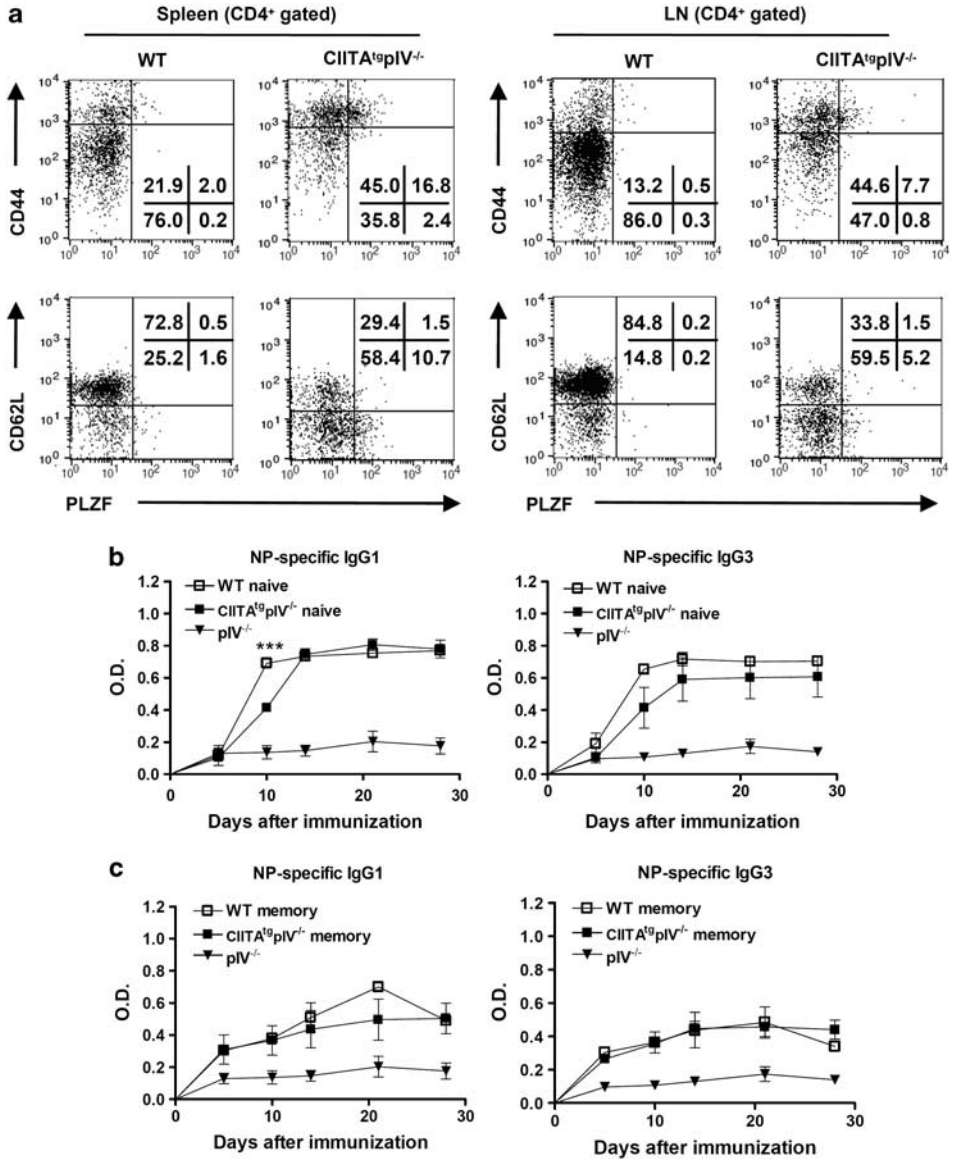

Figure 4 Naïve T–T CD4+ T cells demonstrate B-cell helper activity. (**a**) A representative profile of PLZF expression in naïve and activation/memory populations in gated splenic and lymph node CD4+ T cells from WT and CIITA^{tg} pIV$^{-/-}$ mice. Cells were assessed for the expression of CD44 and CD62L and the intracellular expression of PLZF. The numbers indicate the percentage of cells in each quadrant. (**b**, **c**) Serum antibody responses to NP in pIV$^{-/-}$ mice after adoptive transfer of naïve (**b**) or activation/memory (**c**) CD4+ T cells from WT or CIITA^{tg}pIV$^{-/-}$ mice. The pIV$^{-/-}$ mice without CD4+ T-cell transfer were used as a negative control. The pIV$^{-/-}$ mice with or without CD4+ T-cell adoptive transfer were injected i.p. with 50 μg of NP-KLH on day 0, and serum anti-NP responses were measured and expressed as arbitrary OD units. The data are mean values ± s.e.m. from three animals in each group. ***$P<0.001$.

In summary, our work demonstrated that T–T CD4+ T cells have the ability to support B-cell immune responses and that this B-cell helper activity primarily resides in the naive population. Considering that the most of PLZF-negative T–T CD4+ T cells in thymus are naïve in their phenotype, the development of this population is implicated in the generation of additional capacity of naïve T cells for providing B-cell help because of their addition to conventional CD4+ T cells in terms of T-cell diversity.

METHODS

Mice

C57BL/6 (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). The plck-CIITA^{tg} mice expressing CIITA in T cells were generated previously in our laboratory.[11] Mice carrying a deletion in promoter IV of the *Mhc2ta* gene for CIITA (pIV$^{-/-}$) mice were provided by Hans Acha-Orbea (University of Lausanne, Switzerland).[23] The plck-CIITA^{tg} mice were back-crossed to pIV$^{-/-}$ to generate CIITA^{tg}pIV$^{-/-}$. All the mice were maintained under specific pathogen-free conditions in the animal facility at the Center for Animal Resource Development, Seoul National University College of Medicine. Experiments were performed after receiving approval of the Institutional Animal Care and Use Committee of the Institute of Laboratory Animal Resources, Seoul National University.

Antibodies and flow-cytometric analysis

The following fluorochrome- or biotin-labeled monoclonal antibodies were purchased from BD Pharmingen (San Diego, CA, USA) eBioscience (San Diego, CA, USA) or Dinona (Seoul, Korea): anti-mouse CD3 (145–2C11), CD4

(RM4.5), CD5 (L17F12), CD8 (53–6.7), CD62L (MEL-14), CD44 (IM7), CD11a (M17/4), CD19 (1D3), CD11b (M1/70), CD19 (1D3), CD21 (7G6), CD23 (B3B4), CD43 (S7), CD138 (281–2), I-A^b (AF6–120.1), IgM (G155-228), IgD (11-26c.2a), GL-7 and BrdU (3D4). After staining with fluorochrome-conjugated antibodies for 30 min at 4 °C, live cells (that is, the propidium iodide (PI; Sigma, St Louis, MO, USA)-negative population) were analyzed using a FACSCalibur (Becton-Dickinson, Mountain View, CA, USA) equipped with CellQuest Pro software (Becton-Dickinson).

Immunization and ELISA for serum immunoglobulin measurement

Mice were injected i.p. or s.c (in the footpad) with 50 μg of alum-precipitated NP-KLH (Biosearch Technologies, Novato, CA, USA). Imject Alum was purchased from Pierce (Rockford, IL, USA). For analysis of serum anti-NP antibodies, plates (Nunc Maxisorp; Thermo Fisher Scientific, Waltham, MA, USA) were coated overnight with 5 μg ml^{-1} NP_{14}-bovine serum albumin (Biosearch Technologies, Novato, CA, USA). Plates were blocked for 1 h at 37 °C with blocking buffer (Sigma-Aldrich, St Louis, MO, USA). Sera were diluted in blocking buffer, added to the NP-bovine serum albumin-coated plates, and incubated for 1 h. Unbound antibodies were removed by washing and bound antibodies were detected using anti-mouse IgG1-horseradish peroxidase or IgG3-horseradish peroxidase (SouthernBiotech, Birmingham, AL, USA). After washing with phosphate-buffered saline with 0.05% Tween-20 three times, 50 μl per well of the horseradish peroxidase substrate tetramethylbenzidine was added for color development. The optical density of samples at an absorbance of 450 nm was measured.

Ex vivo analysis of antigen-specific T cell response

Ex vivo analysis of antigen-specific T cell response was carried out as previously described.[30] In brief, CD4+ T cells were isolated from spleen of mice using a MACS CD4+ T-cell isolation kit (Miltenyi Biotec, Auburn, CA, USA) at day 14 after immunization. After washing, the cells were passed through LS columns within the MACS device. The buffers used throughout the whole procedure were phosphate-buffered saline supplemented with 0.5% fetal calf serum. The cells were washed and then cultured in the presence of irradiated (2000 cGy) T-cell depleted splenocytes from B6 mice. CD4+ T cells were incubated with various concentrations of KLH for 4 days. Cultures were pulsed with 1 μCi per well [^{3}H]thymidine (Amersham Bioscience, Uppsala, Sweden) for the final 18 h, and the mean incorporation of thymidine in DNA was measured in quadruplicate wells by liquid scintillation counting.

Assessment of T cell turnover rate

T cell turnover rate was assessed according to the methods described by Tough and Sprent.[31] In brief, mice daily received intraperitoneal injection of 2 mg BrdU (Sigma) in phosphate-buffered saline for 9 days, and single cell suspension from spleen and lymph nodes was then stained with anti-CD4, anti-CD44 and anti-CD62L antibodies, fixed using a Cytofix/Cytoperm Kit (BD Pharmingen), and stained with anti-BrdU antibody. BrdU-positive fraction was detected by flow cytometry.

Cell sorting and adoptive transfer

MACS-purified CD4+ T cells were incubated with anti-CD62L and anti-CD44 antibodies for 30 min at 4 °C on ice. A FACS Aria system (Becton Dickinson, San Jose, CA, USA) was used to sort CD4+ T cell sub-populations according to CD62L and CD44 expression at purities above 95% (Supplementary Figure 3). Isolated naïve or memory CD4+ T cells (5×10^5) from B6 and $CIITA^{tg}$ $pIV^{-/-}$ mice were adoptively transferred into $pIV^{-/-}$ mice.

Statistical analyses

All data were analyzed using the Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Three to five mice per group were evaluated for all strains. Significance between two animal groups in bar graphs was computed by *t* test, and two groups in time curve were compared statistically using a two-way analysis of variance (ANOVA).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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