Loss of IL-7 receptor alpha on CD4+ T cells defines terminally differentiated B cell-helping effector T cell in a B cell-rich lymphoid tissue

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Loss of IL-7 Receptor α on CD4⁺ T Cells Defines Terminally Differentiated B Cell-Helping Effector T Cells in a B Cell-Rich Lymphoid Tissue

Hyung W. Lim and Chang H. Kim

IL-7 plays important roles in development and homeostatic proliferation of lymphocytes. IL-7 uses a receptor composed of IL-7Rα (CD127) and the common γ-chain (CD132) to transmit its signal. It has been unknown how CD127 is regulated during Th cell differentiation to the B cell-helping T cell lineage. In this study, we report that loss of CD127 defines terminally differentiated B cell-helping effector T cells in human tonsils. Although naive CD4⁺ T cells uniformly express CD127, the memory/effector (non-FOXP3⁺) CD4⁺ T cells are divided into CD127⁺ and CD127⁻ cells. The CD127⁻ T cells are exclusively localized within the germinal centers where B cells become plasma and memory B cells, whereas CD127⁺ T cells are found in T cell areas and the area surrounding B cell follicles. Consistently, the CD127⁻ T cells highly express the B cell zone homing receptor CXCR5 with concomitant loss of CCR7. Compared with CD127⁺ memory T cells, CD127⁻ T cells have considerably shorter telomeres, do not proliferate in response to IL-7, and are prone to cell death. The CD127⁻ T cells produce a large amount of the B cell follicle-forming chemokine CXCL13 upon stimulation with B cells and Ags. Most importantly, they are highly efficient in helping B cells produce Igs of all isotypes in a manner dependent on CD40L and ICOS and inducing activation-induced cytidine deaminase and Ig class switch recombination. The selective loss of CD127 on the B cell-helping effector T cells would have implications in regulation and termination of Ig responses. The Journal of Immunology, 2007, 179: 7448–7456.

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3 Abbreviations used in this paper: BCL, B cell leukemia/lymphoma; GC, germinal center; PB, peripheral blood; AID, activation-induced cytidine deaminase; SEB, staphylococcal enterotoxin B; 7-AAD, 7-aminoactinomycin D; MCL, myeloid cell leukemia.

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Materials and Methods

Cell isolation

Tonsil mononuclear cells were prepared by density gradient centrifugation on Histopaque 1077 (Sigma-Aldrich). Tonsil specimens were obtained from young patients (<15 years) undergoing tonsillectomy to relieve obstruction of respiratory passages and improve drainage of the middle ear. T cells were enriched from the mononuclear cells by a sheep RBC rosetting method (25). CD4+ T cells were isolated by the CD4+ T cell isolation kit (Miltenyi Biotec). CD25+Foxp3+ T cells were removed with anti-CD25/magnetic beads to obtain CD4+CD25- cells. Naive and memory CD4+ T cells were isolated by depleting CD45RO+ and CD45RA+ cells, respectively. CD127+ T cells were isolated from CD4+CD25-CD45RA- cells by depleting CD27+ T cells with MACS. CD25+CD127+ memory T cells were isolated by positive selection of CD127+ cells from the CD4+CD25-CD45RA- cells with MACS. Small numbers of contaminating non-CD4+ cells were further removed by positively selecting CD4+ T cells to isolate highly pure CD4+CD25+CD127+ T cells (purities ∼95%) with minimal contamination with Foxp3+ T cells (<2%). Total CD19+ B cells were isolated by depleting T cells with a sheep RBC rosetting method (25). Naive B cells were further isolated from the total B cells by positively selecting IgD+ cells (purities >99%), and CD19+CD38-IgD- GC B cells (purities ∼94%) were isolated by depleting IgD+ cells and positively selecting CD38+ cells as described previously (25). The use of human tonsils for this study has been approved by the Institutional Review Board at Purdue University.

Expression of trafficking receptors and other Ags by T cells

Detection of chemokine receptors and other surface Ags was performed as described previously (26). CD4+CD25+ T cells were stained with Abs to CCR2 (48607.121), CCR4 (clone 205410), CCR5 (45531.111), CCR6 (53103.111), CCR7 (150503), CXCR3 (49801.111), CXCR4 (44717.111), CXCR5 (51505.111), CXCR6 (56811.111), or mouse control IgG2b (CalTAG Laboratories). The Abs to the chemokine receptors were purchased from R&D Systems or BD Biosciences. Cells were further stained with a biotinylated horse anti-mouse IgG (H+L); Vector Laboratories) and then with biotin-conjugated horse-anti-mouse IgG (H+L). Specific percent migration after subtraction of the background migration was calculated.

Immunohistochemistry

Frozen sections of tonsils were cold acetone-fixed and stained with monoclonal anti-human CD127 (BioRDR5; eBioscience) and then with biotin-conjugated horse-anti-mouse IgG (H+L; Vector Laboratories). The sections were further stained with PE-conjugated streptavidin (eBioscience). After blocking with 10% mouse serum, the sections were stained with Abs to CD57 (T01), ICOS (DX29), and/or CD4 (RPA-T4). The slides were further stained with Hoechst 33342. Slides were examined with a Nikon E400 microscope equipped with epifluorescence.

Chemotaxis

Chemotaxis was performed as described previously (25). Human CCL19 and CXCL13 proteins were purchased from R&D Systems. A total of 5 × 10⁵ CD4+CD25+ T cells in 10 μl of chemotaxis medium (RPMI 1640 with 0.5% BSA) was placed in each transwell insert (5 μm pore, 24-well format; Corning Costar), and the transwell inserts were placed in 24-well plates containing 600 μl of chemotaxis medium (RPMI 1640 with 0.5% BSA) with optimal concentrations of CCL19 (1000 ng/ml) or CXCL13 (1500 ng/ml). Cells were allowed to migrate for 3 h in a 5% CO2 incubator at 37°C. After chemotaxis, the cells that migrated to the lower chambers were harvested and stained with Abs to CD4 (RPA-T4), CD127 (hIL-7R-M21), and CD45RO (UCHL1). Stained cells were acquired on a FACS Calibur, and specific percent migration after subtraction of the background migration was calculated.

Proliferation, cell survival, and CXCL13 production

For proliferation, sorted T cells were cocultured in U-bottom 96-well plates for 5 days at indicated ratios in the presence of PHA (5 μg/ml), anti-CD3 (5 μg/ml, immobilized) and anti-CD28 (2 μg/ml, soluble), or equal numbers of syngeneic CD19+ B cells with or without streptolysococal enterotoxin B (SEB; 1 μg/ml; Sigma-Aldrich). Cells were further incubated with 1 μCi/well [3H]thymidine for 8 h, and [3H]thymidine incorporation was measured by a β-scintillation counter (Top Count Microplate Scintillation Counter; Packard Instrument). For the cell survival assay, isolated T cell subsets were cultured for 5 days in RPMI 1640/10% FBS and then stained with 7-aminocoumarin D (7-ADD; final 0.5 μg/ml) immediately before flow cytometric detection of dead (7-ADD+ or forward scatter low) cells. The 5-day-old culture medium was examined for CXCL13 production with an anti-hCXCL13 ELISA kit (R&D Systems).

FIGURE 1. The presence of non-FOXP3+ CD4+ CD127- T cells in tonsils. A. Many CD4+CD25- (FOXP3-) T cells are CD127low-. B. FACs dot plots for the tonsil CD4+CD25- T cell subsets defined by CD45RO and CD127. Expression of CXCR4, CXCR5, CCR7, and CD62L by the three CD4+ T cell subsets was examined. Mean fluorescent intensity is shown to indicate the expression levels. C. Chemotaxis of the three cell subsets to indicated chemokines (error bars are SEM of four independent experiments). All experiments were performed at least three times. Representative (A and B) or combined data (C) are shown. *, Significant differences from naïve (●) or CD127+ cells (●●).
SEM of three independent experiments are shown. Differences from naive (differences of duplicated ELISA).

Frozen tonsil sections were stained with Hoechst 33432 and Abs to CD127, CD57, CD4, and/or ICOS.

Table I. Expression of trafficking receptors by tonsil CD4 \(^{+}\)CD25\(^{(-)}\)CD127\(^{(-)}\) T cells

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Naive T</th>
<th>Memory CD127(^{+})T</th>
<th>Memory CD127(^{-})T</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2</td>
<td>0.7 ± 0.2</td>
<td>3.2 ± 1.0</td>
<td>4.0 ± 1.0*</td>
</tr>
<tr>
<td>CCR4</td>
<td>5.1 ± 1.2</td>
<td>55.1 ± 9.6</td>
<td>62.1 ± 8.0**</td>
</tr>
<tr>
<td>CCR5</td>
<td>0.8 ± 0.3</td>
<td>5.7 ± 1.4</td>
<td>13.1 ± 4.1**</td>
</tr>
<tr>
<td>CCR6</td>
<td>1.5 ± 0.2</td>
<td>25.4 ± 3.4</td>
<td>9.5 ± 1.9**</td>
</tr>
<tr>
<td>CCR7</td>
<td>94.5 ± 1.2</td>
<td>57.2 ± 2.5</td>
<td>26.9 ± 4.2**</td>
</tr>
<tr>
<td>CCR9</td>
<td>7.8 ± 1.7</td>
<td>2.0 ± 0.5</td>
<td>3.3 ± 0.7*</td>
</tr>
<tr>
<td>CXCR3</td>
<td>5.5 ± 1.0</td>
<td>17.6 ± 1.6</td>
<td>23.2 ± 3.4**</td>
</tr>
<tr>
<td>CXCR4</td>
<td>79.1 ± 5.1</td>
<td>85.5 ± 3.3</td>
<td>83.5 ± 0.7</td>
</tr>
<tr>
<td>CXCR5</td>
<td>11.7 ± 3.6</td>
<td>83.9 ± 1.9</td>
<td>95.1 ± 1.2**</td>
</tr>
<tr>
<td>CXCR6</td>
<td>1.2 ± 0.6</td>
<td>5.2 ± 1.9</td>
<td>10.6 ± 5.1</td>
</tr>
</tbody>
</table>

* The percent of cells expressing each chemokine receptor is shown. Significant differences from naive (+) or memory CD127\(^{-}\) T cells (++) are shown. Averages ± SEM of three independent experiments are shown.

Determination of telomere length

The telomere length of sorted naive, memory CD127\(^{+}\), and CD127\(^{-}\) T cells was measured using the Telomere PNA/FITC kit for flow cytometry (DakoCytomation). Briefly, T cells were washed in PBS and resuspended in 300 μl of hybridization solution containing 70% formamide with FITC-conjugated telomere peptide nucleic acid probe. The cells were heated for 10 min at 82°C for denaturation of DNA. Hybridization was performed overnight at room temperature. Cells were heated for 10 min at 40°C and washed with DakoCytomation washing solution twice. Samples were analyzed by flow cytometry.

Assessment of B cell-helping capacity and class switch recombination

Sorted T cells and B cells (10\(^{5}\) each) were cultured in each well of 48-well plates in RPMI 1640 medium supplemented with 10% FBS, gentamicin, streptomycin, and penicillin. When indicated, NaN\(_{3}\)-free mAbs to ICOS (ANC6C6-A3) and/or CD40L (24–31; both obtained from Ancell) or control Abs (mouse IgG1, 11711.11; R&D Systems) were added at 10 μg/ml to neutralize the molecules. For class switch recombination, SEB (1 μg/ml) was added to provide a cognate T-B cell activation signal. Cells were incubated in a 5% CO\(_{2}\) incubator at 37°C for 5 days. The Igs produced from the B cells were detected by ELISA. Activation-induced cytidine deaminase (AID), Ig transcripts, and β-actin were amplified by PCR as previously described (27). Digestion circularization-PCR was performed as previously described using PCR primers for γ\(_{3}\) and α1/2 (28).

RT-PCR analysis of costimulatory molecules and BCL-2-related molecules

Total RNA was extracted with TRizol reagent (Invitrogen Life Technologies) from the sorted T cells and was reverse transcribed into cDNAs with the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies) according to the manufacturer’s protocol. The primers used were 5‘-tg cgc cac tca taa gtc-3’ (forward) and 5‘-gcc aac gga cgt gaa gcc a-3’ (reverse) for CD40L; 5‘-agt aag tca ggc ctc tgg taa ttt-3’ and 5‘-cag aac ttc ggc ctc tgg tgt ttt-3’ for ICOS; 5‘-gca ttc agt gac ctg aca tcc-3’ for BCL-2-related molecules.

**FIGURE 2.** The CD4\(^{+}\)CD127\(^{-}\) T cells are present specifically in GCs and produce the follicle-homing chemokine CXCL13. A. Immunohistochemistry data. Frozen tonsil sections were stained with Hoechst 33432 and Abs to CD127, CD75, CD4, and/or ICOS. B. Isolation of the three CD4\(^{+}\) T cell subsets for functional characterization. C. The three CD4\(^{+}\) T cell subsets differ in their CXCL13 production capacity. The T cell subsets were cultured with PHA for 5 days and the culture medium was examined for CXCL13 by ELISA. D. The CD4\(^{+}\)CD127\(^{-}\) T cells were cultured alone or stimulated with PHA or syngeneic B cells. E. Engagement of the CD4\(^{+}\)CD127\(^{-}\) T cells and B cells with SEB (a superantigen) greatly enhances the CXCL13 production by the CD127\(^{-}\) cells. All experiments were performed at least three times. Representative (A–C) or combined (D and E) data are shown (error bars are the differences of duplicated ELISA). *, Barely detectable.
Kodak imaging system (EDAS 290).

PCR products were separated on 1.5% agarose gels and documented with a 64°C, and 72°C progressing toward 60°C with equal decrements over the five and MCL-1 were amplified by a touch-down PCR method (5 cycles of 94°C, were almost equally divided into CD127 cells (hereafter called “CD4 T cells) (Fig. 1). It has been reported that CD25 T cells are different from each other in expression of CXCR5 Tonsil non-FOXP3 T cells (29). The CD4 T cells. CD4 T cells, express CXCR5 at lower levels but CCR7 at higher levels. As expected, CD127 CD45RO naive T cells highly express CCR7 but do not express CXCR5. Consistently, CD127 CD45RO T cells were highly responsive to CCL13 (the CXCR5 ligand), while CD45RO naive T cells were highly responsive to CCL19 (a CCR7 ligand) (Fig. 1C). In comparison, CD127 CD45RO T cells were moderately responsive to both CCL19 and CXCL13. Otherwise, the expression of other chemokine receptors by the CD4 CD127 and CD127 T cell subsets was similar overall (Table I).

CD4 CD127 and CD127 T cells are differentially localized in tonsil microenvironments

We performed immunohistochemistry experiments to determine the microenvironmental localization of CD4 CD127 and CD4 CD127 T cells. The CD127 T cells were almost exclusively localized in the T cell zones. In contrast, the CD127 T cells were localized within the GC areas (marked by the CD57 expression, Fig. 2A). In contrast, the costimulation receptor ICOS was expressed by the T cells in not only GCs but also in the T cell areas, and, thus, its expression is not a good indicator for GC-T cells. One of the key indicators of GC-T cells is the ability to produce the B cell zone chemokine CXCL13 (30). We isolated the three CD4 T cell subsets at high purities to examine the CXCL13 production capacity of the T cells (Fig. 2B). It was the CD4 CD127 T cells that almost exclusively produced CXCL13 among the three T cell subsets (Fig. 2C). Spontaneous production of CXCL13 by the CD4 CD127 T cells in the absence of APCs or activators was hardly detectable by ELISA (Fig. 2D). Activation with T cell activators or coculture with syngeneic B cells greatly enhanced the production rates of CXCL13 from the CD4 CD127 T cells (Fig. 2D). CD4 CD127 T cells were comparable to the previously characterized CD57 GC-T cells (30) in the CXCL13 production capacity (Fig.

Statistical analyses

The Student paired two-tailed t test was used for statistical analysis when indicated. Values of p ≤ 0.05 were considered significant.

Results

Tonsil non-FOX3 CD4 CD127 T cells and CD4 CD127 T cells are different from each other in expression of CXCR5 and CCR7

It has been reported that CD25 FOX3 T cells in peripheral blood (PB) are CD127 (17–19). We found that this is true also for most CD4 CD25 (FOX3) T cells in tonsils and many CD4 CD25 T cells (Fig. 1A). CD4 CD25 CD45RO T cells were almost equally divided into CD127 and CD127 T cells (Fig. 1B). Reciprocal expression of the two chemokine receptors CCR7 and CXCR5 determines the localization of T cells within secondary lymphoid tissues (29). The CD4 CD25 CD127 T cells (hereafter called “CD4 CD127 T cells”) highly express CXCR5 but express CCR7 at reduced levels (Fig. 1B). Expression of the lymph node-homing selectin CD62L was also decreased on the CD4 CD127 T cells. The CD127 CD45RO T cells, when compared with CD127 CD45RO T cells, express CXCR5 at lower levels but CCR7 at higher levels. As expected, CD127 CD45RO naive T cells highly express CCR7 but do not express CXCR5. Consistently, CD127 CD45RO T cells were highly responsive to CCL13 (the CXCR5 ligand), while CD45RO naive T cells were highly responsive to CCL19 (a CCR7 ligand) (Fig. 1C). In comparison, CD127 CD45RO T cells were moderately responsive to both CCL19 and CXCL13. Otherwise, the expression of other chemokine receptors by the CD4 CD127 and CD127 T cell subsets was similar overall (Table I).

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Coengagement of CD4<sup>+</sup>CD127<sup>−</sup>T cells and B cells by the superantigen SEB greatly increased the production capacity of CD4<sup>+</sup>CD127<sup>+</sup>T cells (Fig. 2E).

The cell surface and cytokine phenotype of tonsil CD4<sup>+</sup>CD127<sup>−</sup>T cells

We examined the expression of a number of naive or memory T cell-associated Ags by the CD4<sup>+</sup>CD127<sup>−</sup>T cells to more closely characterize these T cells. CD45RA (a naive T cell marker) and CD31 (platelet endothelial cell adhesion molecule-1, a naive T cell-specific receptor) (31) are not expressed by the CD4<sup>+</sup>CD127<sup>−</sup>T cells (Fig. 3A). In contrast, CD57 (an Ag expressed by a GC T cell subset), CD58 (LFA-3, an adhesion molecule and the ligand of CD2), and CD69 (an early activation Ag and suppressor of sphingosine 1-phosphate receptor) (32) were expressed by many CD4<sup>+</sup>CD127<sup>−</sup>T cells. Thus, the CD4<sup>+</sup>CD127<sup>−</sup>T cells express the cell surface molecules associated with activated memory/effector T cells trapped deep within tissues. Only a few CD4<sup>+</sup>CD127<sup>−</sup>T cells were able to produce IL-4, IL-10, and IFN-γ (Fig. 3B). CD4<sup>+</sup>CD127<sup>−</sup>T cells had somewhat lower capacities in production of IL-2, IFN-γ, and TNF-α, compared with the CD127<sup>+</sup>CD45RO<sup>−</sup>T cells. The CD4<sup>+</sup>CD127<sup>−</sup>T cells were not able to produce TNF-β/lymphotoxin-α (a cytokine specifically produced by naive T cells in humans) in a manner similar to the CD4<sup>+</sup>CD127<sup>−</sup>T cells. Thus, these data suggest that CD4<sup>+</sup>CD127<sup>−</sup>T cells are activated and largely nonpolarized tissue-residing T cells.

Tonsil CD4<sup>+</sup>CD127<sup>−</sup>T cells have short telomeres and are programmed to die

Every chromosome has several thousand repeats of hexameric sequences at both ends, which are called telomeres (33). Telomeres...
become shorter by 50–100 bp with each round of replication, and, therefore, can indicate the replication history of a T cell population (34). As expected, naive CD4⁺CD45RA⁺ T cells have longer telomeres than the memory/activated CD4⁺CD127⁺CD45RO⁺ T cells (Fig. 3C). CD4⁺CD127⁺ T cells have the shortest telomeres among the three CD4⁺ T cell subsets. Generally, high cell death rates are associated with effector T cells. Most CD4⁺CD127⁻ T cells spontaneously died in culture at rates higher than CD4⁺CD127⁺CD45RO⁺ or naive CD4⁺ T cells (Fig. 3D). In this regard, CD127⁻ T cells expressed BCL-2 (a cell survival-associated molecule) at levels lower than the CD127⁺ T cells (Fig. 3E). In contrast, the expression of Bcl-xL and MCL-1 (activation and GC-associated BCL-2-related molecules, respectively (35, 36)) was increased in the CD4⁺CD127⁻ T cells compared with the CD127⁺ T cells. Thus, the low expression of BCL-2 is in line with its proapoptotic nature, and the high expression of Bcl-xL and MCL-1 correlates with their activation status and tissue tropism, respectively.

**Tonsil CD4⁺CD127⁻ T cells do not respond to IL-7 and poorly proliferate upon cell activation**

IL-7 plays positive roles in survival, proliferation, and differentiation of T cells (37). We examined whether the CD4⁺CD127⁻ T cells identified with the Abs really lost their responsiveness to IL-7. We examined the cell survival and proliferative responses driven by IL-7. Although CD4⁺CD127⁻ T cells (naive and CD45RO⁺ cells) vigorously responded to IL-7 in survival and proliferation, the CD4⁺CD127⁺ T cells died and poorly responded to IL-7 in both the presence and absence of T cell activation (Fig. 4, A and B). We also examined CXCL13 production in response to IL-7 (Fig. 4C). IL-7 enhanced the CXCL13 production of naive T cells and CD127⁺CD45RO⁺ cells but not that of CD4⁺CD127⁻ T cells which produced CXCL13 at high levels regardless of the stimulation with IL-7. Therefore, these data demonstrate that the CD4⁺CD127⁻ T cells do not respond to IL-7 and rather die. Before they die, however, CD4⁺CD127⁻ T cells produce a large amount of CXCL13.

**Tonsil CD4⁺CD127⁻ T cells are highly efficient helpers for B cells**

The microenvironmental tissue tropism and other phenotype of CD4⁺CD127⁻ T cells suggest that they are terminally differentiated effector Th cells in B cell follicles. We examined the B cell-helping activity of the CD4⁺CD127⁻ T cells and other tonsil CD4⁺ T cells (Fig. 5A). When cocultured with syngeneic B cells, the CD4⁺CD127⁻ T cells were most efficient in inducing the production of IgM, IgG, IgA, and IgE. In contrast, naive T cells and CD4⁺CD45RO⁺CD127⁺ T cells were able to only weakly stimulate the production of the Abs by B cells.

ICOS and CD40L are costimulatory molecules and have been implicated in GC formation and regulation of humoral immune responses (38–41). ICOS and CD40L were more highly expressed at protein and mRNA levels by the CD127⁺ T cells than by naive or CD127⁻ T cells (Fig. 5B). To determine the costimulation molecule requirement for the CD127⁺ T cell-dependent B cell production of Abs, we used neutralizing Abs to CD40L and ICOS (Fig. 5C). A neutralizing Ab to CD40L suppressed the CD4⁺CD127⁺ T cell-mediated B cell Ig production by ~80%. Similarly, a neutralizing Ab to ICOS effectively suppressed the CD4⁺CD127⁺ T cell-mediated B cell Ig production. These data suggest that CD40L and ICOS are required for the B cell-helping function of the CD4⁺CD127⁺ T cells.

We further investigated whether the increased Ab production by the B cells stimulated by CD4⁺CD127⁻ T cells accompanies increased expression of AID and Ig class switch recombination (Fig. 6). AID is a key enzyme involved in the DNA cleavage for somatic hypermutation and class-switch recombination (42). When B cells and Th cells are stimulated with the superantigen SEB, expression of AID, switch DNA circles, and productive Ig RNA transcripts were induced (Fig. 6, A–C). We found that the CD4⁺CD127⁺ T cells were most efficient in induction of AID and class switch recombination (indicated by switch DNA circles and productive Ig RNA transcripts) among the three CD4⁺ T cell subsets (Fig. 6, B and C). Please note that the freshly isolated naive B cells and GC B cells (included as negative and positive controls) showed low background and high levels of class switch recombination, respectively. The CD4⁺CD127⁻ T cells displayed a B cell-helping activity comparable to the previously characterized CD57⁺ GC-Th cells (21) in induction of B cell Ig production (Fig. 6D). Overall, these results suggest that the tonsil CD4⁺CD127⁻ T cells are highly efficient in induction of the processes required for Ig production in B cells.

**The non-FOXP3⁺CD4⁺CD127⁻ T cells in PB are composed of heterogeneous memory/effector T cell subsets**

In PB, only ~8% of CD4⁺CD25⁻ T cells were CD127⁺ T cells, which is considerably lower than in tonsils (Fig. 7A). To find similarities and differences between tonsil and PB non-FOXP3⁺CD4⁺CD127⁻ T cells, we examined the surface Ag and cytokine expression phenotype of the PB CD4⁺CD25⁻CD127⁺ T cells. PB CD4⁺CD25⁺CD127⁻ T cells expressed CD45RO and CD58
(memory/effector cell Ags) but do not express CD45RA, CD31 (naive T cell Ags), and CD69 (an activated cell Ag) (Fig. 7, A and B). PB CD4⁺ CD127⁻ T cells highly expressed CCR5, CXCR3 (a Th1-associated receptor), CCR6 (memory), and CCR7 (circulation and T cell area homing), compared with the tonsil CD4⁺ CD127⁻ T cells (Fig. 7C). In contrast, expression of CXCR4 (a homeostatic homing receptor) and CXCR5 (B cell area homing) was reduced on the PB CD4⁺ CD127⁻ T cells. The PB CD127⁻ T cells contained Th1 (IFN-γ⁺) cells, Th2 (IL-13⁺), and Th17 cells (IL-17⁺ cells) (Fig. 7D). These results suggest that, unlike the tonsil CD4⁺ CD127⁻ T cells, the PB CD4⁺ CD127⁻ T cells are composed of heterogeneous memory/effector T cell subsets.

**Discussion**

In an effort to understand the IL-7Rα/CD127 expression by human Th cells specialized in helping B cells, we investigated the phenotype and function of the CD4⁺ T cell subsets defined by CD127 and CD45RO within the non-FOXP3⁺ T cell population. We found that the tonsil CD4⁺ CD127⁻ T cells have all of the features of the effector T cells within the B cell-helping T cell lineage and demonstrated the usefulness of CD127 as a marker for these Th cells. We found that, in combination with other surface Ags such as CXCR5 and CD45RO, CD127 allows specific identification of B cell-helping effector CD4⁺ T cells in tonsils. Moreover, the regulation of CD127 by naive to fully differentiated B cell-helping effector T cells provides insights into how Th cells regulate their responsiveness to IL-7 and effector function during their differentiation.

The surface and chemokine receptor phenotype of tonsil CD4⁺ CD127⁻ T cells is interesting in that they highly express CXCR5 (a B cell zone-homing receptor) but express CCR7 at greatly reduced levels (a T cell zone-homing receptor). Coordinated expression of these two receptors regulates the positioning of lymphocytes in secondary lymphoid tissues because of the specific expression of CXCL13 (for CXCR5) in the B cell zones and of CCL19/CCL21 (for CCR7) in T cell zones (29). The selectin molecule CD62L, required for lymphocyte recirculation into lymph nodes, is also down-regulated on the CD4⁺ CD127⁻ T cells. Therefore, the phenotype suggests that these T cells do not circulate the blood-lymphoid system but they would rather position themselves in GCs deep into the B cell areas. Indeed, the immunohistochemistry results demonstrate that almost all of the CD4⁺ T cells in GCs are CD127⁻. In contrast, ICOS and CXCR5 are expressed by the majority of memory/activated T cells regardless of their localization in tonsils (Figs. 1 and 2). All of these CD127⁻ T cells are CD69, and they can produce CXCL13, which has the lymphoid tissue and GC-organizing activity (43). CD69 is expressed by activated T cells and down-regulates the expression of sphingosine 1-phosphate receptor (32) for prevention of T cell exit from lymphoid tissues (44). A genome-wide gene expression study determined that CXCL13 is expressed specifically by a GC-Th cell subset (30). Tonsil CD4⁺ CD127⁻ T cells are the T cells that produce CXCL13 potentially for maintaining GCs in a manner similar to follicular dendritic cells (45). Their overall phenotype suggests that the tonsil CD4⁺ CD127⁻ T cells are sessile and engaged in cell-cell interaction with other cells in GCs. This interpretation is strongly supported by their expression of CD57 (HNK-1, ~40%), CD58 (LFA-3, ~75%), adhesion molecules that are implicated in cell-cell interaction (46, 47). In contrast, the CD4⁺ CD127⁻ T cells lack the expression of CD31, CD45RA, and lymphoxygen-α, the Ags specifically expressed by naive CD4⁺ T cells in humans (48, 49), demonstrating their nature as memory/effector T cells.

By definition, effector T cells should be highly efficient in performing specialized functions. The tonsil CD4⁺ CD127⁻ T cells are remarkably efficient in helping B cells to produce all subsets of Abs (IgM, IgG, IgA, and IgE) (Fig. 5). This nonselective nature of B cell-helping activity in terms of Ig isotypes is considered an important feature of basic B cell-helping effector T cells. Based mainly on in vitro data, it has been postulated that Th2 cells would be a major effector T cell subset providing B cell help (50, 51). However, Th2 cells do not express the B cell zone-homing receptor CXCR5 (52), and, thus, cannot migrate toward B cells to stimulate them. In addition, Th2 cells do not induce Ab class switch to all subsets of Abs but selectively induce the production of subsets of Abs (IgG4 and IgE) in humans through IL-4. Moreover, IL-4 is not essential for T-dependent Ab responses (53). In contrast, CD127⁻ T cells are nonpolarized T cells, and, thus, can induce the
Ig class switch to all isotypes of Abs without any preference. The Ab production profile induced by the tonsil CD4+ CD127 T cells is characterized by high IgG/IgM, intermediate IgA and low IgE, which is a typical serum pattern in healthy people. As potential effecter/costimulatory molecules, we examined CD40L and ICOS, which are required to maintain humoral immunity in humans and mice (38–41, 54). Blockade of CD40L or ICOS effectively incapacitated the B cell-helping ability of CD127 T cells (Fig. 5), suggesting that CD4+ CD127 T cells need these molecules to induce B cell production of Abs. CD127+ T cells are more efficient than CD127− T cells in inducing AID expression required for Ig gene class switch recombination (Fig. 6). In this study, we stimulated the T cells to drive T cell activation, a situation where even naive T cells can induce class switch recombination at certain levels due to the activation signal. Even in this situation, CD4+ CD127+ T cells were more efficient than other T cells in class switch recombination.

Telomeromas become shorter as eukaryotic cells divide unless the telomerase is expressed to prolong the shortened telomeres (33). Evidence that the CD4+ CD127+ tonsil T cells have the shortest telomeres among the tonsil CD4+ T cell subsets, reflecting their extensive cell division history and the trafficking receptors to circulate the T cell areas of secondary lymphoid tissue, and support immunoglobulin production. They also express polarized memory/effector cell-associated chemokine receptors such as CCR4, CCR5, CCR6, and CXCR3. Unlike the tonsil CD127− T cells, PB CD4+ CD127− T cells are not activated based on the CD69 expression. Taken together, the available information suggests that, unlike the more homogeneous and sessile tonsil CD4+ CD127− T cells, the PB CD4+ CD127− T cells are composed of many different circulating memory/effector T cell subsets.

To summarize, we found biologically interesting features of the tonsil non-FOXP3+ CD4+ CD127− T cells, which include 1) loss of IL-7Rα and the responsiveness to IL-7; 2) high expression of CXCR5 but reduced expression of CCR7 and CD62L; 3) high expression of effector T cell adhesion molecules; 4) specific localization in GC; 5) high activities in helping B cells and inducing Ig class switch recombination; 6) production of lymphoid tissue-organizing CXCL13; 7) short telomeromas; 8) nonproliferative nature; and 9) being proapoptotic. Taken together, our study provides the evidence that the CD4+ CD127+ T cells in the B cell-dominant lymphoid tissue tonsils are terminally differentiated effector T cells specialized in helping B cells.

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Disclosures
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Telomeromas become shorter as eukaryotic cells divide unless the telomerase is expressed to prolong the shortened telomeres (33). Effector T cells typically have short telomeromas (55), which is the evidence of their proliferation history. Consistently, CD4+ CD127+ T cells have the shortest telomeres among the tonsil CD4+ T cell subsets, reflecting their extensive cell division history before they become the terminally differentiated effector cells. Although effector T cells are highly efficient in their function as discussed above, they usually do not proliferate and are programmed to undergo cell death (56). In this regard, the proapoptotic and nonproliferative nature of CD4+ CD127+ tonsil T cells also supports that they are terminally differentiated effector T cells. Blocking the death of effector T cells by deleting death receptors or overexpressing cell survival molecules can induce lymphoproliferative diseases (57). Therefore, it is possible that the programmed death of CD4+ CD127+ tonsil T cells would help terminate the humoral immune responses in GCs. The loss of an IL-7Rα chain specifically by these effector T cells would program them to die rather than survive and expand in response to IL-7 upon completion of their effector function.

Although both the PB and tonsil (non-FOXP3+) CD4+ CD127− T cells are memory/effector cells, they are different in many aspects. The CD4+ CD127− T cells are rare in PB and appear to be highly heterogeneous containing various memory/effector T cells such as Th1, CXCR5+ T cells, Th2, and Th17 cells. The PB non-FOXP3+ CD4+ CD127− T cells highly express CCR7 and CD62L, the trafficking receptors to circulate the T cell areas of secondary lymphoid tissues and the blood. They also highly express polarized memory/effector cell-associated chemokine receptors such as CCR4, CCR5, CCR6, and CXCR3. Unlike the tonsil CD127− T cells, PB CD4+ CD127− T cells are not activated based on the CD69 expression. Taken together, the available information suggests that, unlike the more homogeneous and sessile tonsil CD4+ CD127− T cells, the PB CD4+ CD127− T cells are composed of many different circulating memory/effector T cell subsets.

To summarize, we found biologically interesting features of the tonsil non-FOXP3+ CD4+ CD127− T cells, which include 1) loss of IL-7Rα and the responsiveness to IL-7; 2) high expression of CXCR5 but reduced expression of CCR7 and CD62L; 3) high expression of effector T cell adhesion molecules; 4) specific localization in GC; 5) high activities in helping B cells and inducing Ig class switch recombination; 6) production of lymphoid tissue-organizing CXCL13; 7) short telomeromas; 8) nonproliferative nature; and 9) being proapoptotic. Taken together, our study provides the evidence that the CD4+ CD127+ T cells in the B cell-dominant lymphoid tissue tonsils are terminally differentiated effector T cells specialized in helping B cells.
IL-7R+NEGATIVE B CELL-HELPING EFFECTOR T CELLS


