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Hyung W. Lim

*Purdue University - Main Campus, bicycle@purdue.edu*

Jeeho Lee

*Purdue University - Main Campus*

Peter Hillsamer

*Sagamore Surg Ctr, Lafayette, IN 47909*

Chang H. Kim

*Purdue University - Main Campus*

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# Human Th17 Cells Share Major Trafficking Receptors with Both Polarized Effector T Cells and FOXP3<sup>+</sup> Regulatory T Cells<sup>1</sup>

Hyung W. Lim,\* Jeeho Lee,\* Peter Hillsamer,<sup>†</sup> and Chang H. Kim<sup>2\*</sup>

**It is a question of interest whether Th17 cells express trafficking receptors unique to this Th cell lineage and migrate specifically to certain tissue sites. We found several Th17 cell subsets at different developing stages in a human secondary lymphoid organ (tonsils) and adult, but not in neonatal, blood. These Th17 cell subsets include a novel in vivo-stimulated tonsil IL17<sup>+</sup> T cell subset detected without any artificial stimulation in vitro. We investigated in depth the trafficking receptor phenotype of the Th17 cell subsets in tonsils and adult blood. The developing Th17 cells in tonsils highly expressed both Th1- (CCR2, CXCR3, CCR5, and CXCR6) and Th2-associated (CCR4) trafficking receptors. Moreover, Th17 cells share major non-lymphoid tissue trafficking receptors, such as CCR4, CCR5, CCR6, CXCR3, and CXCR6, with FOXP3<sup>+</sup> T regulatory cells. In addition, many Th17 cells express homeostatic chemokine receptors (CD62L, CCR6, CCR7, CXCR4, and CXCR5) implicated in T cell migration to and within lymphoid tissues. Expression of CCR6 and CCR4 by some Th17 cells is not a feature unique to Th17 cells but shared with FOXP3<sup>+</sup> T cells. Interestingly, the IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> Th17 cells have the features of both IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup> Th1 and IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> Th17 cells in expression of trafficking receptors. Taken together, our results revealed that Th17 cells are highly heterogeneous, in terms of trafficking receptors, and programmed to share major trafficking receptors with other T cell lineages. These findings have important implications in their distribution in the human body in relation to other regulatory T cell subsets. *The Journal of Immunology*, 2008, 180: 122–129.**

The IL-17 family of cytokines is composed of IL-17A through F (1–3). Among the cytokines, IL-17A and F are produced by a T cell subset now more frequently called Th17 cells (2). There is an increasing body of evidence showing that Th17 cells constitute a novel Th cell lineage distinct from Th1 and Th2 cells (3, 4). It has been reported that the transcription factor retinoic-acid-related orphan receptor- $\gamma$ t is important for generation of Th17 cells in vitro and in vivo (5). TGF- $\beta$ 1 and IL-6 are required for naive T cell differentiation into the IL-17-producing T cells in mice (6). Additionally, cytokines such as IL-23 and IL-21 promote the generation or proliferation of Th17 cells whereas others, such as IFN- $\gamma$ , IL-4, and IL-27, suppress their generation (7–11). Human Th17 cells appear to be quite different from mouse Th17 cells in that TGF- $\beta$ 1 and IL-6 are not required for generation of Th17 cells (12, 13). So far, the most effective cytokines to enhance the generation or expansion of human Th17 cells are IL-1 $\beta$  and IL-23 (12, 13).

Although the function of Th17 cells in infectious diseases is unclear, their roles in induction of autoimmunity are well established. IL-17 and Th17 cells are required for the induction of autoimmune inflammation in several animal models. Th17 cells or

their cytokine IL-17 are required to maximally induce experimental autoimmune inflammation in animal models of experimental allergic encephalomyelitis (14–16), collagen induced arthritis (17, 18), and colitis (19). Consistently, the numbers of Th17 cells are increased in the inflamed tissue sites of patients with multiple sclerosis (20), rheumatoid arthritis (21, 22), and inflammatory bowel diseases (23). Th17 cells are widely found in non-lymphoid tissues (e.g., intestine) and secondary lymphoid tissues (mesenteric lymph nodes, peripheral lymph nodes, spleen, and Peyer's patches) of mice (5).

Generally, trafficking receptors are expressed by effector T cells in a lineage specific manner. Th1 cells express CCR5, CXCR3, and CXCR6 (24–27), whereas Th2 cells express CCR4 and CRTH2 (27–30). Nonpolarized early memory T cells express both CXCR3 and CCR4 (27, 31). Other receptors such as CCR6, CCR7, CXCR4, and CXCR5 are expressed by T cell subsets that migrate to the different compartments of secondary lymphoid tissues (32–35). For example, CCR6 is implicated in T cell development and localization in Peyer's patches (32), whereas CXCR5 is important for localization of B cell helping effector T cells within B cell follicles (35). A limited amount of information is available about the trafficking receptors of Th17 cells. Recent reports suggest that Th17 cells express CCR6, CCR4, and CCR2, but not CXCR3 and CCR5, in human adult peripheral blood (PB)<sup>3</sup> (36–38). However, it is unclear whether this is the universal feature of all Th17 cells in the body or a feature limited only to certain Th17 cells in the PB. It is necessary to determine the complete profile of trafficking receptors expressed by multiple subsets of Th17 cells to address the issue. We investigated in depth the trafficking receptors expressed

\*Laboratory of Immunology and Hematopoiesis, Department of Comparative Pathobiology, Purdue Cancer Center, Bindley Bioscience Center, and Birck Nanotechnology Center, Purdue University, West Lafayette, IN 47907; and <sup>†</sup>Sagamore Surgical Center, Lafayette, IN 47909

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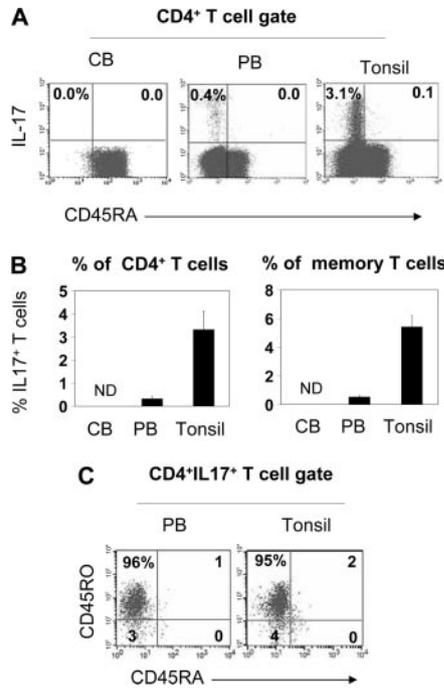
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<sup>2</sup> Address correspondence and reprint requests to Dr. Chang Kim, Department of Comparative Pathobiology, 725 Harrison Street, Purdue University, West Lafayette, IN 47907. E-mail address: chkim@purdue.edu

<sup>3</sup> Abbreviations used in this paper: PB, peripheral blood; Treg, regulatory T cell; <sup>2</sup> LT, secondary lymphoid tissues; CB, cord blood.

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**FIGURE 1.** Pan-Th17 cell subsets in neonatal CB, adult PB, and human tonsils. *A*, FACS dot plots of Th17 cells in CB, PB, and tonsils. *B*, The frequencies of Th17 cells in CB, PB, and tonsils among the total CD4<sup>+</sup> T cells and memory CD4<sup>+</sup> T cells. *C*, The CD45RA and CD45RO phenotype of Th17 cells in PB and tonsils. The cells were activated with PMA and ionomycin for detection of Th17 cells. Representative (*A* and *C*) and combined data (*B*) of five independent experiments are shown.

by several different Th17 cell subsets of tonsils and blood. Our results revealed that Th17 cells express not only the homeostatic trafficking receptors but also Th1/Th2/regulatory T cell (Treg)-associated trafficking receptors implicated in T cell migration to non-lymphoid tissues. We also comparatively investigated the trafficking receptors expressed by the Th17 cell subsets defined by expression of IFN- $\gamma$  (IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> cells and IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells). Our results suggest that Th17 cells have a more diverse and flexible expression program of trafficking receptors than previously reported. The implications of these findings in Th17 cell distribution and regulation of inflammation are discussed.

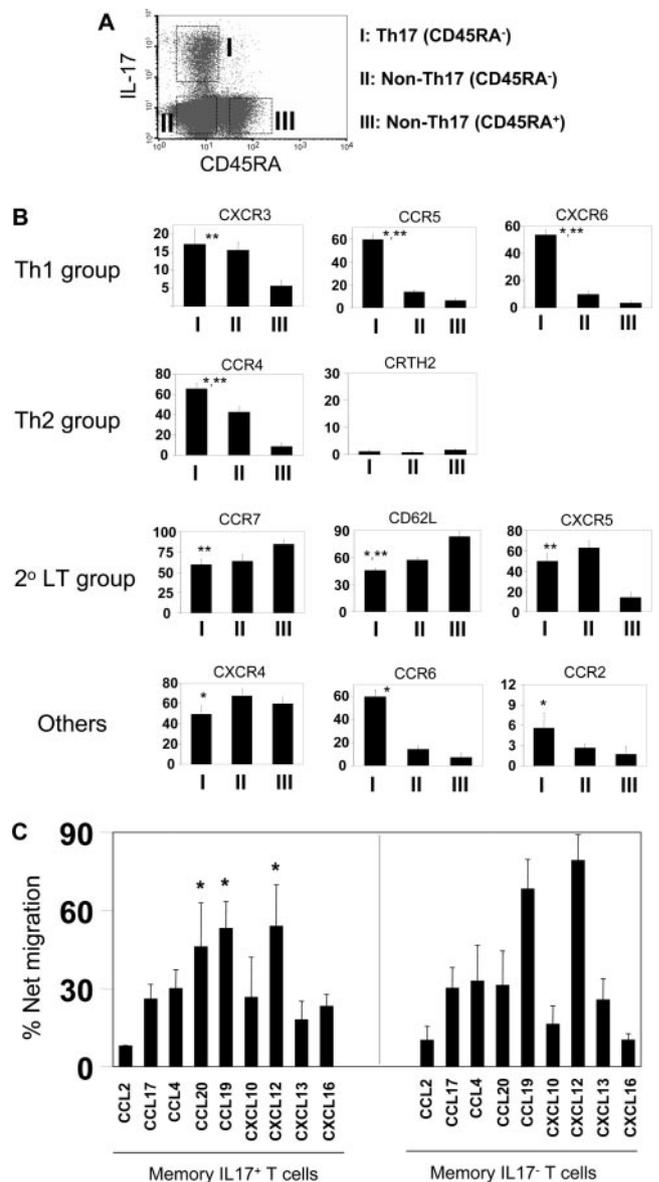
**Materials and Methods**

*Cell preparation from cord blood (CB), adult PB, and tonsils*

Mononuclear cells of CB, tonsils, and adult PB were prepared by density gradient centrifuge on histopaque 1077 (Sigma-Aldrich). T cells were enriched from the mononuclear cells by a sheep-RBC rosetting method (39). In most experiments, these rosetted T cells were used for determination of the chemokine receptor profile of Th17 cells and other T cells. For chemotaxis assay, CD4<sup>+</sup> T cells were isolated by the CD4<sup>+</sup> T cell isolation kit (purity >95%; Miltenyi Biotec). Tonsil specimens were obtained from young patients (<15 years) undergoing tonsillectomy to relieve obstruction of respiratory passages and improve drainage of the middle ear. The use of human CB, PB, and tonsils for this study has been approved by the institutional review board at Purdue.

*Surface Ag expression by Th17 cells without ex vivo activation*

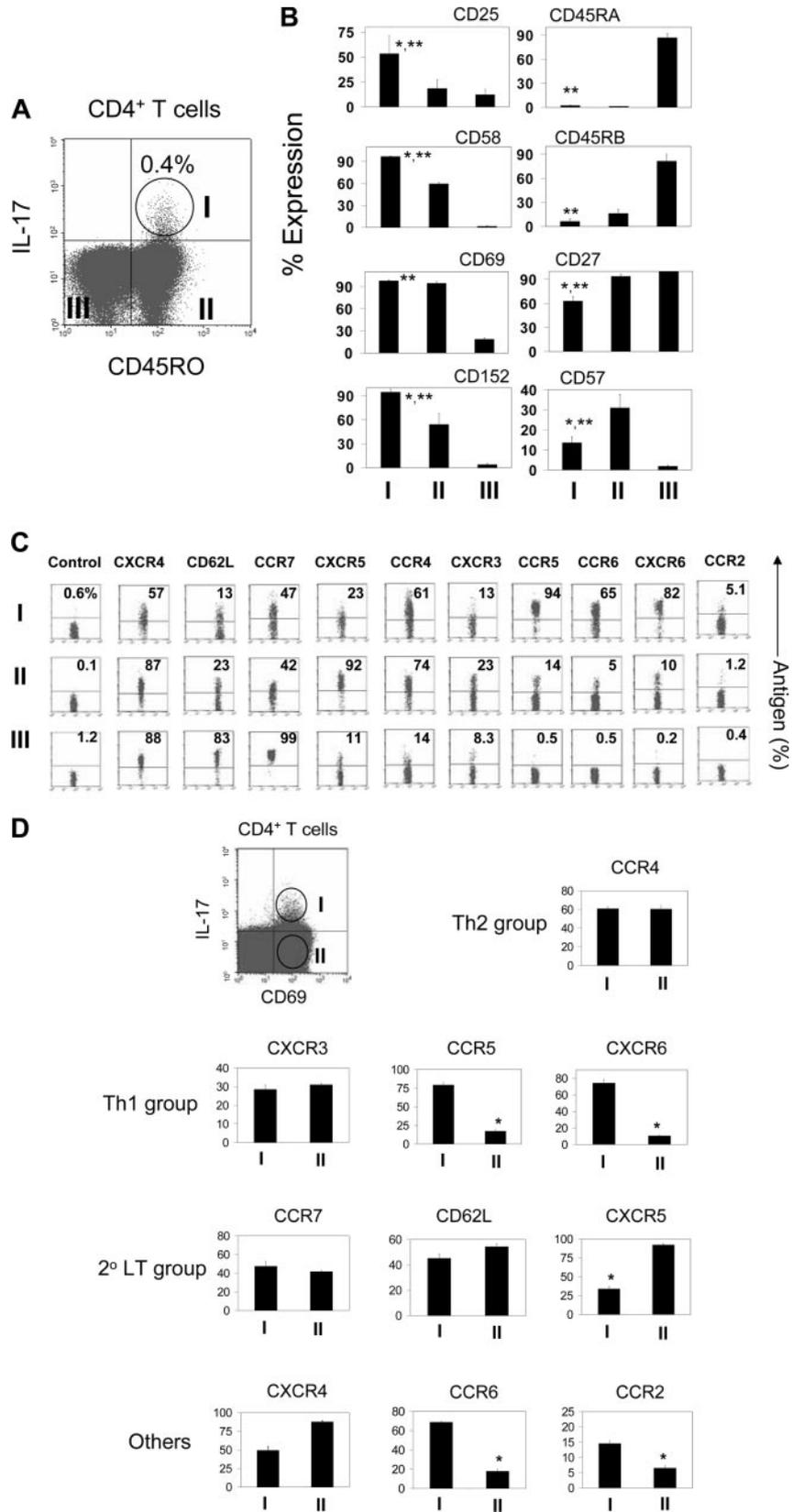
Rosetted T cells were first stained with Abs to CD4 (clone RPA-T4) and CD45RO (clone UCHL1) along with one of the following Abs to surface Ags: CD25 (clone BC96), CD27 (clone M-T271), CD45RA (clone H100), CD45RB (clone MT4), CD57 (TB01), CD58 (LFA-3, clone 1C3), and CD69 (clone FN50). An Ab to CD152 (BNI3) was used for intracellular staining of the Ag. These Abs were purchased from eBioscience, Bio-Legend, and BD Biosciences. The cells were further stained with anti-hIL-17 (eBioscience; clone eBio64CAP17) and acquired on a FACSCalibur.



**FIGURE 2.** The trafficking receptor profiles of Th17 cells and non-Th17 memory CD4<sup>+</sup> T cells in tonsils. *A*, Definition of Th17 cells and non-Th17 memory CD4<sup>+</sup> T cells based on the expression pattern of IL-17 and CD45RA. *B*, Trafficking receptor profiles of Th17 cells (I), non-Th17 memory T cells (II), and CD45RA<sup>+</sup> T cells (III) were determined by flow cytometry. The data shown are percent cells expressing a chemokine receptor within each cell population. Combined data from six independent experiments. \*,\*\*, Significant differences from subset II (\*) or III (\*\*). *C*, A chemotaxis assay using Transwells was performed with various chemokine ligands at their optimal concentrations, determined by titration experiments. The cells were activated with PMA and ionomycin for detection of Th17 cells after chemotaxis and staining for surface Ags. Net chemotaxis rates after subtraction of the background migration rates are shown. Combined data from three independent experiments is shown. \*, Significant differences between IL17<sup>+</sup> and IL17<sup>-</sup> T cell subsets.

*Expression of trafficking receptors and other Ags by Th17 cells and Th1 cells*

The rosetted T cells were stained with Abs to CCR2 (clone 48607.121), CCR4 (clone 205410), CCR5 (clone 45531.111), CCR6 (clone 53103.111), CCR7 (clone 150503), CXCR3 (clone 49801.111), CXCR4 (clone 44717.111), CXCR5 (clone 51505.111), CXCR6 (clone 56811.111), CD62L (clone Dreg 56), CRTH2 (clone BM16), or mouse control IgG2b. The Abs were purchased from R&D Systems, BD Biosciences, and Invitrogen Life Technologies. Cells were further stained with a biotinylated

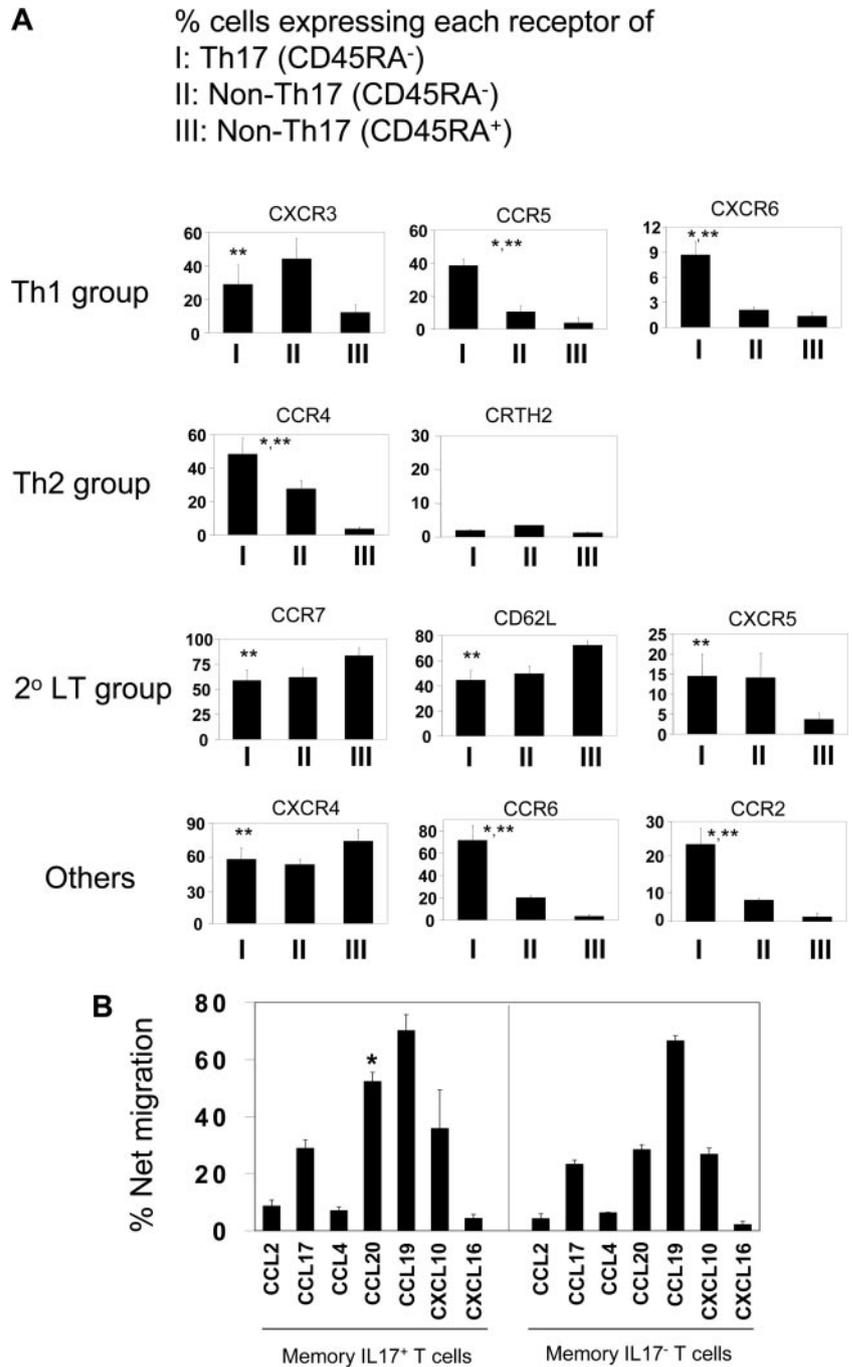


**FIGURE 3.** The surface Ag and trafficking receptor phenotype of a Th17 cell subset producing IL-17 in response to antigenic stimulation in vivo. *A*, Tonsil CD4<sup>+</sup> T cells were stained with an Ab to IL-17 without any artificial stimulation in vitro. *B*, The surface Ag phenotype of the in vivo-stimulated Th17 cells. \*\*\*, Significant differences from subset II (\*) or III (\*\*). *C*, The trafficking receptor profile of the in vivo-stimulated Th17 cells. *D*, Comparison of the chemokine receptors expressed by comparably activated CD69<sup>+</sup>IL-17<sup>+</sup> and CD69<sup>+</sup>IL17<sup>-</sup>CD4<sup>+</sup> T cells. \*, Significant differences between the two subsets. Representative (*A* and *C*) or combined (*B* and *D*) data from three independent experiments.

horse anti-mouse IgG (H+L) Ab or goat anti-rat IgG (H+L) (Vector Laboratories) for 20 min followed by staining with PerCP-streptavidin (BD Biosciences) and anti-CD4 Ab. The stained cells were activated for 4 h in RPMI 1640 (10% FBS) with PMA (50 ng/ml) and ionomycin (1 μM) in the presence of monensin (2 mM) to prevent the loss of surface Ag expression. The cells were fixed, permeabilized, and stained with PE or allophycocyanin-conjugated Abs to IL-17 and/or IFN-γ (40). The cells were acquired on a FACSCalibur.

*Chemotaxis*

Human CCL2 (MCP-1), CCL17 (TARC), CCL4 (MIP-1β), CCL20 (LARC), CXCL10 (IP-10), CXCL12 (SDF-1), CCL19 (ELC), CXCL13 (BLC), and CXCL16 proteins were purchased from R&D Systems or Pepro-Tech. A total of 5 × 10<sup>5</sup> T cells in 100 μl of chemotaxis medium (RPMI 1640 with 0.5% BSA) were placed in each Transwell insert (5 μm pore,



**FIGURE 4.** The trafficking receptor profile of Th17 cells in adult PB. *A*, The trafficking receptor profiles of Th17 cells (I), non-Th17 memory T cells (II), and CD45RA<sup>+</sup> T cells (III) were determined by flow cytometry. Combined data from three independent experiments are shown. \*,\*\*, Significant differences from subset II (\*) or III (\*\*). *B*, A chemotaxis assay using Transwells was performed. The concentrations of chemokines were identical to those used in Fig. 2. The cells were activated with PMA and ionomycin for detection of Th17 cells. Net migration after subtraction of the background migration (5–10%) is shown. Combined data from three independent experiments are shown. \*, Significant differences between IL17<sup>+</sup> and IL17<sup>-</sup> T cell subsets.

24-well format; Corning Costar), and the Transwell inserts were placed in 24-well plates containing 600  $\mu$ l of the chemotaxis medium with optimal concentrations of chemokines. The concentrations used in this study were: CCL2 (500 ng/ml), CCL17 (1000 ng/ml), CCL4 (500 ng/ml), CCL20 (2000 ng/ml), CCL19 (1500 ng/ml), CXCL10 (1000 ng/ml), CXCL12 (100 ng/ml), CXCL13 (1500 ng/ml), or CXCL16 (2000 ng/ml). Cells were allowed to migrate for 3 h in a 5% CO<sub>2</sub> incubator at 37°C. After chemotaxis, the cells that migrated to the lower chambers were harvested and stained with Abs to CD4 and CD45RO. Surface Ag-stained cells were activated and stained for IL-17 as described above. Stained cells were acquired on a FACSCalibur, and percent migration of the cells in the inputs was calculated.

*Statistical analyses*

All experiments were repeated at least three times. Student’s paired 2-tailed *t* test was used to determine the significance of the differences between two groups of samples. A value of *p*  $\leq$  0.05 was considered significant. All error bars shown in this paper are SEM.

**Results**

*Identification of Th17 cell subsets in human tonsils, adult PB, and neonatal blood*

We examined the frequencies of pan-Th17 cells (total IL-17<sup>+</sup>CD4<sup>+</sup> T cells) in human neonatal CB, adult PB, and tonsils. CB does not contain significant numbers of Th17 cells but PB contains detectable numbers of Th17 cells (Fig. 1). Approximately 0.4% of CD4<sup>+</sup> T cells were IL17<sup>+</sup>CD4<sup>+</sup> T cells. The frequency of pan-Th17 cells is considerably higher in tonsils. In tonsils, ~3.5% of CD4<sup>+</sup> cells and ~5.5% of memory CD4<sup>+</sup> T cells were Th17 cells (Fig. 1B). Most Th17 cells in PB and tonsils were CD45RA<sup>-</sup>CD45RO<sup>+</sup> memory T cells (Fig. 1C). These data suggest that the frequencies of Th17 cells increase with age and only small numbers of Th17 cells are in the blood circulation.



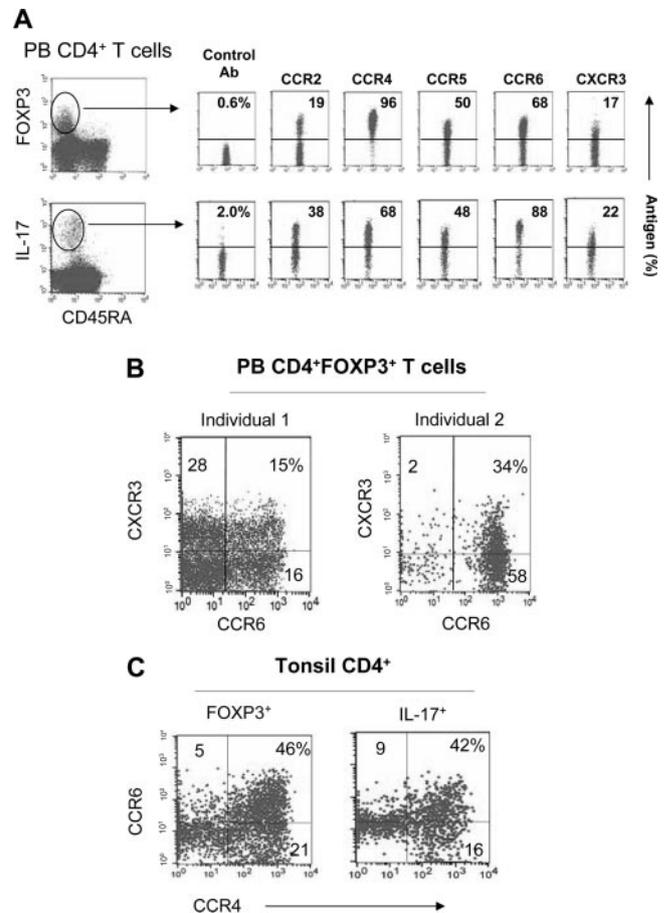
altered by the artificial activation with anti-CD3 or PMA and ionomycin, commonly used to detect Th17 cells. The *in vivo* stimulated Th17 cells were CD45RO<sup>+</sup>, CD25<sup>+</sup>, CD58<sup>+</sup>, CD69<sup>+</sup>, and CD152<sup>+</sup>, suggesting that they are activated (based on the expression of CD69, CD25, CD152/CTLA-4, and CD58/LFA3) and memory (based on the expression of CD45RO) T cells. In contrast, they don't express the markers of naive T cells such as CD45RA and CD45RB. CD27 expression is considerably decreased, and ~10% of the *in vivo* stimulated Th17 cells expressed the germinal center T cell marker CD57 (41). For comparison of similarly activated IL17<sup>+</sup> and IL17<sup>-</sup>CD4<sup>+</sup> cells, we examined the chemokine receptor expression phenotype of CD69<sup>+</sup>IL17<sup>+</sup>CD4<sup>+</sup> T cells and CD69<sup>+</sup>IL17<sup>-</sup>CD4<sup>+</sup> T cells (Fig. 3D). Although the two T cell subsets were similar in expression of CCR4, CXCR3, and CCR7, some receptors such as CCR5, CXCR6, CCR6, and CCR2 were more preferentially expressed by IL17<sup>+</sup> T cells than the similarly activated IL17<sup>-</sup> T cells. In contrast, CXCR4 and CXCR5 were expressed by many more IL17<sup>-</sup> T cells. Thus, the *in vivo* stimulated Th17 cells (Fig. 3) and the total IL17<sup>+</sup> T cells (pan-Th17 cells) detected with *in vitro* stimulation (Fig. 2) are similar to each other in expression of chemokine receptors.

#### The chemokine receptor profile of circulating pan-Th17 cells in adult blood

The T cells in the blood circulation are the mixture of emigrating cells from thymus, 2° LT, and non-LT. Since Th17 cells are generated in the periphery, the Th17 cells in adult PB are probably from 2° LT and non-LT. The PB Th17 cells expressed CCR5, CCR4, CXCR6, CCR6, and CCR2 at levels higher than non-Th17 memory CD4<sup>+</sup> T cells. The PB Th17 cells responded well to CCL17, CCL4, CCL20, CCL19, and CXCL10 in chemotaxis, suggesting that the receptors of these chemokines are fully functional on Th17 cells (Fig. 4B). In a manner similar to tonsil Th17 cells, the migration of PB Th17 cells to CXCL16 or CCL2 was not efficient, reflecting the fact that their receptors, CCR2 and CXCR6, are expressed by only a small subset of Th17 cells and suggesting that not all of the receptors are functional.

#### Chemokine receptors of Th17 subsets (IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> and IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells) vs IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup> Th1 cells in tonsils and PB

The expression of Th1-associated chemokine receptors by the Th17 cells of tonsils and PB is interesting. To directly compare the chemokine receptors expressed by Th17 and Th1 cell subsets, we costained the T cells with Abs to IFN- $\gamma$  and IL-17 to identify Th1 and Th17 cell subsets within the same samples (Fig. 5A). We found that the human Th17 cells were composed of IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> and IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells in tonsils and PB. It is important to examine the trafficking receptors of the IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double positive cells because this subset has features of both Th17 and Th1 cells in cytokine production. The average frequencies of the IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double positive cells among total IL-17<sup>+</sup> cells were not insignificant with ~20% (tonsils) and ~18% (PB) (Fig. 5A). IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> Th17 cells were different from IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup> Th1 cells in tonsils in expression of CCR4, CXCR6, and CCR6 (at higher frequencies by Th17) and CXCR3 (at lower frequencies, Fig. 5B). However, the two subsets were similar in expression of other receptors. The IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double positive cells were similar to IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> Th17 cells in expression of CCR6 in tonsils and PB. The IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double positive cells expressed



**FIGURE 6.** Trafficking receptors of Th17 cells vs Tregs. *A*, PB pan-Th17 cells and FOXP3<sup>+</sup>CD4<sup>+</sup> T cells were compared for expression of trafficking receptors. The PB T cells were activated by PMA and ionomycin for detection of Th17 cells. Tregs were detected without the *in vitro* stimulation with an anti-hFOXP3 Ab. *B*, The presence of CCR6<sup>+</sup>/CXCR3<sup>+</sup> Tregs in PB. The T cells were costained with Abs to CCR6 and CXCR3. *C*, Shared coexpression of CCR6 and CCR4 by Th17 cells and FOXP3<sup>+</sup> T cells in tonsils. The *in vivo* (naturally) stimulated tonsil IL17<sup>+</sup>CD4<sup>+</sup> cells, shown in Fig. 3, and FOXP3<sup>+</sup> T cells were examined. Representative data from at least three independent experiments are shown.

CXCR3 (tonsil) and CCR4 (tonsil and PB) at intermediary levels between those of IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> Th17 cells and IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup> Th1 cells. The IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double positive cells expressed CCR5 (tonsil and PB), CXCR6 (tonsil), and CCR2 (PB) at high levels. Thus, the IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> double positive cells have a chemokine receptor phenotype associated with both Th17 and Th1 cells.

#### Chemokine receptors of Th17 vs FOXP3<sup>+</sup> Tregs

A recent report suggests that PB Th17 cells express CCR6 and CCR4, but not CXCR3, and can be isolated by use of the receptors as surrogate markers with minimal contamination with other lineages (37). Because of the shared expression of chemokine receptors among Th17 and non-Th17 cells described in this report, we further compared the trafficking receptors of Th17 cells with those of FOXP3<sup>+</sup> Tregs (Fig. 6A). Many PB Th17 cells and FOXP3<sup>+</sup> T cells shared the expression of CCR2, CCR4, CCR5, CCR6, CCR7, and CXCR3, which raises the concern that the phenotype (CCR6<sup>+</sup>CXCR3<sup>-</sup>) would not be unique to the PB Th17 cells. We further examined the expression of CCR6 and CXCR3 by FOXP3<sup>+</sup> Tregs to determine whether some of these T cells would have the same phenotype (CCR6<sup>+</sup>CXCR3<sup>-</sup>). Indeed, many

Table I. Shared trafficking receptors among Th17 and other major T helper cell lineages in PB<sup>a</sup>

	IL17 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> Th17	IL17 <sup>+</sup> IFN- $\gamma$ <sup>+</sup> Th1	Th1	Th2	Memory Tregs
CCR2	++	+++	++	+	+
CCR4	+++	++	+	+++	+++
CCR5	++	+++	++	+	+++
CCR6	+++	+++	++	+	+++
CCR7	+++	+++	+++	+++	++
CXCR3	++	+++	+++	+	++
CXCR5	+	+	+	+	+
CXCR6 <sup>b</sup>	+	+	+	-	++

<sup>a</sup> High expression (+++):  $\geq 50\%$  (% cells expressing each receptor of Th17, Th1, Th2, or Tregs); medium expression (++) :  $< 50\%$  and  $\geq 25\%$ ; low expression (+) :  $< 25\%$ ; and no or very low expression (-) :  $< 10\%$ . This table is based on the data shown in Figure 5 and previously published papers on Th2 and Tregs (27, 39). The data are based on human PB T cells.

<sup>b</sup> CXCR6 is expressed by a small subset of Th1 cells, but  $\sim 50\%$  of CXCR6<sup>+</sup> cells are Th1 cells, and CXCR6<sup>+</sup> cells contain very few Th2 cells (40). Unlike the PB Th17 cells shown in this table, CXCR6 is highly expressed by tonsil Th17 cells.

FOXP3<sup>+</sup> Tregs were CCR6<sup>+</sup>CXCR3<sup>-</sup>, sharing the same receptor profile as some Th17 cells (Fig. 6B). In some individuals, the majority of FOXP3<sup>+</sup> Tregs had this phenotype, demonstrating that the phenotype is not unique to the Th17 cell lineage. Furthermore, the coexpression of CCR6 and CCR4 by some Th17 cells is not a Th17 cell-specific phenotype because the tonsil FOXP3<sup>+</sup> T cells have the same phenotype (Fig. 6C).

In Table I, we compiled the major chemokine receptors expressed by Th17, Th1, Th2, and Tregs. Again, we found considerable overlap between Th17 cells and other effector or suppressor T cell lineages in expression of the chemokine receptors: CCR2 with Th1, CCR4 with Th2 and Tregs, CCR5 with Th1 and Tregs, CCR6 with Th1 and Tregs, CCR7 with all of the subsets, CXCR3 with Th1 and Tregs, and finally CXCR6 with Th1 and Tregs.

## Discussion

Th17 cells have been receiving a lot of attention because of their ability to induce inflammation at the effector sites. T cell migration is regulated not only by the homeostatic trafficking signals of the thymus and 2<sup>o</sup> LT for development and differentiation but also by the trafficking signals at tissue sites of inflammation. The fact that Th17 cells are efficient inducers of tissue inflammation suggests that these T cells would have the trafficking receptors that can recognize the inflammatory trafficking signals. Despite the previous reports on the trafficking receptors of Th17 cells (36, 37), the comprehensive profile of Th17 cell trafficking receptors has been unknown. We have taken a systematic approach to identify several Th17 cell subsets in the blood circulation and 2<sup>o</sup> LT (tonsils) and determined their comprehensive trafficking receptor profiles. During the study, we identified a novel in vivo-stimulated Th17 cell subset in tonsils, which expresses IL17 without the artificial in vitro stimulation.

The comprehensive trafficking receptor profile of Th17 cells determined in this report shows that Th17 cells express more diverse trafficking receptors than previously reported. It is particularly interesting that Th17 cells share many trafficking receptors with Th1 cells. The receptors that are shared by pan-Th17 cells and IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup> Th1 cells are CCR2, CCR5, CCR7, CXCR3, and CXCR6. As shown in Table I, pan-Th17 cells share CCR4 and CCR7 with Th2 cells. Thus, Th17 cells have the receptors of both Th1 cells and Th2 cells. This trafficking receptor profile is true also for the in vivo-stimulated tonsil Th17 cell subset. Many of these receptors are involved in T cell migration into inflamed tissue sites, and, therefore, the receptor expression profile of Th17 cells is consistent with

their inflammatory activities in non-lymphoid tissues. The shared expression of chemokine receptors by Th17 cells and Th1/Th2 polarized T cells suggests that many Th17 cells probably comigrate to inflamed tissue sites with the polarized Th1/Th2 cells rather than migrate to unique tissue sites where Th1/Th2 won't migrate to. The trafficking receptor expression profile is indeed well supported by our unpublished observation that Th17 cells and polarized T cells are detected in the same tissue sites in mice. Significant numbers ( $\sim 20\%$ ) of Th17 cells were IFN- $\gamma$  producers. Interestingly, the IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells are associated with both IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup>Th17 and IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup>Th1 cells in expression of chemokine receptors. Although the functional relationship between IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> cells and IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> T cells is currently unclear, these data suggest that the IL17<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells would coexist to a certain degree in the body with both IL17<sup>+</sup>IFN- $\gamma$ <sup>-</sup> Th17 and IL17<sup>-</sup>IFN- $\gamma$ <sup>+</sup>Th1 cells.

Th17 cells and FOXP3<sup>+</sup> Tregs are often compared because both lineages need TGF- $\beta$ 1 for their induction in the periphery, at least in mice, and they have the functions opposite to each others (42, 43). When we directly compared the trafficking receptor profiles of Th17 cells and Tregs (Fig. 6 and Table I), we found surprisingly high similarity between FOXP3<sup>+</sup> Tregs and Th17 cells. FOXP3<sup>+</sup> Tregs highly express CCR4, CCR5, CCR6, CXCR3, and CXCR6 (39, 44), which are highly expressed also by Th17 cells. This trafficking receptor profile is consistent with the fact that Th17 cells and Tregs often coexist in many tissue sites. This would have a potentially important role in reigning in the proinflammatory activity of Th17 cells by Tregs. A difference between Th17 cells and Tregs in development is that Tregs can be made in both the thymus as naive cells and 2<sup>o</sup> LT as memory cells, but Th17 cells are made only as memory/effector T cells in 2<sup>o</sup> LT. Naive Tregs express lymphoid tissue homing receptors, such as CD62L, CCR7, and CXCR4, but not the non-lymphoid tissue homing receptors (39, 44), and, thus, Th17 cells have a trafficking receptor profile similar to that of memory, but not naive, Tregs.

A recent report described that Th17 cells in human adult blood are CCR6<sup>+</sup>CCR4<sup>+</sup>CXCR3<sup>-</sup> (37). They used CCR6 and CXCR3 as surrogate markers to enrich Th17 cells in PB T cells. Our results show that this strategy can be used for enrichment of a subset of Th17 cells but is not appropriate for isolation of pure Th17 cells. Some Th17 cells are CXCR3<sup>+</sup>, CCR4<sup>-</sup>, or CCR6<sup>-</sup> and therefore do not fit into the phenotype (CCR6<sup>+</sup>CCR4<sup>+</sup>CXCR3<sup>-</sup>). Another group has reported that Th17 cells in the adult PB are CCR2<sup>+</sup>CCR5<sup>-</sup>. Our results show that CCR2 is preferentially expressed by a small subset ( $< 20\%$ ) of Th17 cells, but the correlation between the CCR2 expression and the IL17 production capacity is not absolute because the majority of IL17<sup>+</sup> T cells do not express CCR2, nor can the majority of CCR2<sup>+</sup> cells express IL-17. Moreover, we found that many Th17 cells in the human PB ( $\sim 30\%$ ) and tonsils ( $\sim 60\%$ ) express CCR5. Utilization of a few trafficking receptors to enrich a subset of Th17 cells may be used, but these Th17 cells would not represent the phenotype and function of the Th17 cell population. The biggest problem is contamination with other T cell lineages, such as Tregs, as shown in Fig. 6. Another problem is the low enrichment factor after isolation of the cells with the strategy due to the paucity of Th17 cells and shared chemokine receptor phenotype with other T cells.

Taken together, Th17 cells express highly diverse trafficking receptors. Th17 cells share virtually all of their trafficking receptors with Th1, Th2, and/or Tregs. This profile of Th17 cells' trafficking receptors is consistent with their wide distribution but concentration in selected non-lymphoid tissues in the body. Our

results suggest also that Th17 cells themselves are not homogeneous in terms of trafficking receptors. They are probably composed of heterogeneous subsets with distinct trafficking receptor profiles induced at various tissue sites. In the future, it is important to identify the potentially distinct functions of these Th17 cell subsets. One of the challenges in the research, however, is to find better methods to isolate live primary Th17 cells at high purities or generate highly enriched human Th17 cells *in vitro*.

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## Disclosures

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