Programmed Downregulation of CCR6 Is Important for Establishment of Epidermal $\gamma$ $\delta$T Cells by Regulating Their Thymic Egress and Epidermal Location

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Unfornate conventional αβ T cells, various subsets of γδ T cells display the properties of innate immune cells. Many of these innate-like γδ T cells preferentially reside in epithelial tissues covering the external and internal surface of the body, such as the skin, reproductive tracts, and lungs, where they function as the first line of defense (1). However, the mechanisms that direct the preferential localization of the innate-like γδ T cells in the epithelial tissues are not well understood. γδ T cells in the skin of mice include some of the most representative epithelial-tissue–resident innate-like γδ T cells. In the epidermis, nearly all of the resident γδ T cells (referred to as skin intraepithelial γδ T lymphocytes [sIEL] or dendritic epithelial T cells) express canonical Vγ3/Vδ1+ γδ TCRs (2). The Vγ3+ sIEL could recognize and be activated by (unidentified) skin-specific Ag(s) induced by the local pathophysiological changes (3). The activated Vγ3+ sIEL have the cytolytic capacity to kill skin tumor cells and are able to express an array of cytokines and factors such as IFN-γ, insulin-like factor 2, and keratinocyte growth factor (KGF) that mediate functions of the sIEL in immune surveillance against cutaneous tumors (4–7), regulation of local inflammatory responses (8–10), and wound healing (3, 11, 12). In the dermis, the γδ T cell repertoire is more diverse than that of epidermal γδ T cells based on their γδ TCR usage. A significant fraction of the dermal γδ T cells, many of which express the Vγ2+ TCR, are the dominant source of IL-17 (therefore referred to as γδT17 cells) during the early innate phase of immune response against the bacterial infection but also involved in the skin inflammatory diseases such as psoriasis (13–16). Functionally similar subsets of γδ T cells are also found in the epidermis and dermis of humans (although they use different γδ TCRs than the murine γδ T cells) (13, 17–20), suggesting evolutionarily conserved development of the skin-resident γδT innate-like lymphocytes of unique functional capacities to protect the local tissue integrity.

We previously reported that the fetal thymic Vγ3+ γδ T cells, which are exclusive precursors for the Vγ3+ epidermal sIEL, are programmed in the thymus for their specific distribution into the epidermis (21, 22). The fetal thymic Vγ3+ sIEL precursors underwent a positive selection-dependent process that results in a coordinate switch in the expression of homing molecules and cytokine receptors that are known or potentially important for their migration and maintenance in (to) the skin (21–23). Particularly, upregulation of chemokine receptor CCR10, integrin CD103, and adhesion molecules E-selectin ligand and P-selectin ligand on the positively selected fetal thymic Vγ3+ sIEL precursors is important for their localization into epidermis (23–25), whereas the upregulation of sphingosine 1-phosphate receptor 1 (SIPR1) is likely important for the thymic egress of the mature Vγ3+ sIEL precursor cells (21, 26). In addition, the upregulation of cytokine receptor CD122 (IL-2R β-chain) on the positively selected fetal thymic Vγ3+ sIEL precursors is critical for their survival/proliferation in the skin, and mice deficient of CD122 or its ligand IL-15 do not have the epidermal γδ T cells (15, 27–29). The dermal γδT17 cells are recently found also predominantly originating from the fetal thymic γδ T cells (14, 30). Although mechanisms regulating localization of the fetal thymic precursors of the γδT17 cells in the dermis are not well studied, the dermal γδT17 cells express different (although overlapping) sets of
chemokine receptors and adhesion molecules than the epidermal γδT cells, and it is likely that localization and maintenance of the different subsets of γδT cells into the different regions of the skin are differentially coordinated during their thymic development stage (14, 15).

The programming of Vγ3+ sIEL precursors in the fetal thymus is not only associated with upregulation of the homing molecules and cytokine receptors important for their location and survival in the skin but also downregulation of other molecules such as chemokine receptor CCR6 (21, 31). Because the ligand for CCR6, CCL20, is expressed in the thymus (32, 33), the downregulation of CCR6 might be a part of the regulation process that allows for the egress of mature fetal thymic Vγ3+ sIEL precursors from the thymus (21). In addition, considering that the dermal γδT cells express CCR6, the regulated downregulation of CCR6 on the Vγ3+ sIEL precursors might be also involved in their distribution into the epidermal but not dermal regions of the skin. To address these questions, we generated a strain of CCR6-transgenic (CCR6Tg) mice that maintain a constitutive expression of CCR6 on the mature sIEL precursors and sIEL. Using the CCR6Tg mice, we show that the programmed downregulation of CCR6 on the mature fetal thymic Vγ3+ sIEL precursors is involved in regulation of their thymic egress as well as proper distribution in the epidermis of the skin.

Materials and Methods

CCR6Tg mice

To generate CCR6Tg mice, a DNA fragment containing the coding sequence of mouse CCR6 was inserted into a transgenic expression vector under the control of the human CD2 promoter (CD2-P) and enhancer (CD2-E) (Fig. 1A) (34). The CCR6Tg vector was injected into fertilized eggs of C57BL/6 (B6) mice (National Cancer Institute, Bethesda, MD) to generate transgenic founders. The CCR6Tg founder mice were crossed with B6 CD2-E (CD2) (Fig. 1A) (34). The CCR6Tg vector was injected into fertilized eggs of C57BL/6 (B6) mice (National Cancer Institute, Bethesda, MD) to generate transgenic founders. The CCR6Tg founders were crossed with B6 mice, and two lines of germline-transmitted CCR6 transgenic mice were obtained, as identified by PCR using primers specific for the transgenic (but not endogenous) CCR6 sequences (the primers CCR6Tg-1F and CCR6Tg-1R that span the start codon of transgenic CCR6 and primers CCR6Tg-2F and CCR6Tg-2R that span the stop codon of transgenic CCR6) (Fig. 1B). To determine copy numbers of the CCR6 transgenic genomes, the genomic DNA of the transgenic mice were subject to a real-time quantitative PCR (qPCR) analysis with primers CCR6-2F and CCR6-2R that span the stop codon of transgenic CCR6Tg-2F and CCR6Tg-2R that span the stop codon of transgenic CCR6Tg mice with those of wild-type (WT) mice, which have two copies of the endogenous CCR6 gene. Based on the qPCR analysis, we calculated that both lines of the transgenic mice have two copies of CCR6 transgenes (Fig. 1C). The real-time RT-PCR analysis of mRNA from splenic T cells found that transcript levels of CCR6 of both endogenous and transgenic CCR6 genes are higher in the splenic T cells found that transcript levels of CCR6 in total splenic T cells are higher in the CCR6Tg mice than WT controls (Fig. 1D). The transcript level of CCR6 in total mRNA from splenic T cells found that transcript levels of CCR6 in total mRNA from splenic T cells are higher in the CCR6Tg mice than WT controls (Fig. 1D). The transcript level of CCR6 in total mRNA from splenic T cells are higher in the CCR6Tg mice than WT controls (Fig. 1D).

In vitro chemotaxis assay

The experiment was performed as previously described (21). Briefly, 5×10^4 E17 fetal thymocytes of WT or CCR6Tg mice suspended in DMEM/10% FBS were plated into the upper chamber of a Transwell plate containing 5-μm pore filters (Costar) and incubated with CCL20 or medium only in the bottom chamber for 4 h. Cells migrating into the bottom chamber were collected and analyzed by flow cytometry for Vγ3+ T cells along with other markers.

Statistical analyses

All data are expressed as means ± SDs. Statistical significance was determined by two-tailed Student t tests. A p value <0.05 is considered significant.
Results

Reduced numbers of Vγ3+ γδT cells in epidermis of CCR6Tg mice

To determine whether the temporally regulated downregulation of CCR6 on positively selected mature fetal thymic Vγ3+ sIEL precursors is important for the sIEL development, we compared numbers of sIEL by immunofluorescent microscopy in epidermal sheets of littermates of WT and CCR6Tg mice in which expression of the CCR6 transgene is under control of human CD2 regulatory elements that were previously reported to promote the expression of its regulated gene in mature T cells that express CD2 (Fig. 1A–D) (34). Comparing to their WT littermate controls, both lines of CCR10-transgenic mice have significantly reduced numbers of Vγ3+ sIEL in the epidermis (Fig. 2A, 2B). In addition, the higher extent of the percentage reduction of sIEL in the #3 line of transgenic mice than in the #1 line is consistent with the higher transgenic expression level in the former (Fig. 1D).

CCR6 transgene predominantly compensates for downregulation of the endogenous CCR6 gene expression in mature Vγ3+ fetal thymic sIEL precursors and alters their response to CCL20

Considering the downregulation of CCR6 is associated with the positively selected mature fetal thymic Vγ3+ sIEL precursors (21), the impaired sIEL development in the CCR6Tg mice suggests that the constitutive expression of CCR6 transgene likely affected the migration and seeding of the mature Vγ3+ sIEL precursors into the skin early in the fetal/neonatal stages. Consistent with this, the impaired development of sIEL in CCR6Tg mice was also observed early in the neonatal stage of days 1 and 7 mice (Fig. 2C, 2D). To dissect this further, we then determined how the transgenic CCR6 affects expression of CCR6 in the mature CD122+ and immature CD122−Vγ3+ fetal thymic γδT cell populations and their response to CCL20, the ligand for CCR6.

Based on the real-time RT-PCR analysis for CCR6 transcripts, expression of CCR6 was downregulated in the mature CD122+ Vγ3+ sIEL precursors compared with the immature CD122− Vγ3+ fetal thymocytes in WT mice (Fig. 3A) (23). The CCR6 expression in CD122+Vγ3+ fetal thymic sIEL precursors of CCR6Tg mice was comparable to that in the immature CD122− Vγ3+ fetal thymic γδT cells of WT littermates (Fig. 3A), suggesting that the CCR6 transgene compensated for downregulation of the endogenous CCR6 expression to result in a constitutive expression of CCR6 in the mature fetal thymic Vγ3+ sIEL precursors as well as in the immature Vγ3+ cells. The expression of CCR6 in the immature CD122− Vγ3+ fetal thymic γδT cells was also increased in the CCR6Tg mice compared with that of the WT littermates (Fig. 3A). Therefore, the upregulated expression of CCR6 in both immature and mature fetal thymic Vγ3+ γδT cells could potentially contribute to the impaired sIEL development.

Flow cytometric analysis of the surface expression of CCR6 on CD122− and CD122+ fetal thymic Vγ3+ γδT cells of WT and CCR6Tg mice confirmed the findings of the RT-PCR analysis. In WT mice, CD122− fetal thymic Vγ3+ γδT cells expressed low levels of CCR6, whereas CD122+ fetal thymic Vγ3+ γδT cells did not express CCR6 (Fig. 3B). CCR6 transgene resulted in enhanced expression of CCR6 on both CD122− and CD122+ fetal thymic Vγ3+ γδT cells compared with their respective WT counterparts (Fig. 3B). The cell-surface level of CCR6 on CD122+ fetal thymic Vγ3+ γδT cells of CCR6Tg mice was lower than that on their CD122+ counterpart of CCR6Tg mice but comparable with that on CD122− fetal thymic Vγ3+ γδT cells of WT mice (Fig. 3B).

The downregulation of endogenous CCR6 expression in the mature CD122+ fetal thymic Vγ3+ sIEL precursors is associated with the upregulation of CCR10 that is important for the migration of the mature Vγ3+ sIEL precursors to the skin, and the CD122− CCR10+ fetal thymic Vγ3+ sIEL precursors ready to exit the thymus (22, 23). To determine the stage of the fetal thymic Vγ3+ sIEL development on which the CCR6 transgene might affect, we crossed CCR6Tg mice with CCR10GFP reporter mice in which expression of CCR10 is reported by the EGFP signal (23). Then we performed an in vitro migration assay to assess how the CCR6 transgene affected responses of the fully mature CD122+CCR10+ and the less mature CCR10− fetal thymic Vγ3+ sIEL precursors toward CCL20. In contrast to the mature CCR10+ fetal thymic Vγ3+ γδ T cells of WT mice that do not migrate toward CCL20, CCR10+ fetal thymic Vγ3+ γδ T cells of CCR6Tg mice migrate toward CCL20 efficiently (Fig. 3C). In contrast, the CCR10− Vγ3+ fetal thymic γδT cells of CCR6 transgenic mice only had a marginal increase in migration toward the CCL20 attraction when compared with the CCR10− fetal thymic Vγ3+ γδ T cells of WT mice (Fig. 3D). Considering that the thymus express CCL20, the predominant effect of CCR6 transgene on the response of the CCR10+ fetal thymic Vγ3+ sIEL precursors toward CCL20 most likely altered their migration out of the thymus that could contribute to the impaired development of sIEL in CCR6Tg mice. The migration of fetal thymic CD3+ Vγ3− T cells of CCR6Tg mice to CCL20 was also increased compared with that of WT controls (Fig. 3E), suggesting that the CCR6 transgene increased the functional expression of CCR6 on at least some of other mature T cells and could affect their migration from the thymus as well.

Abnormal accumulation of mature Vγ3+ sIEL precursors correlates with their failed downregulation of CCR6 expression in CCR6Tg mice

To determine directly whether the CCR6 transgene specifically affected thymic egress of the mature fetal thymic Vγ3+ sIEL
precursors in vivo, we compared numbers of Vγ3+ fetal thymic gd T cells of the different maturation stages in the CCR6Tg and WT littermates. Compared to the WT controls, there were increased percentages and numbers of Vγ3+ γδT cells in the fetal thymus of CCR6Tg mice, suggesting their abnormal accumulation in the fetal thymus and consistent with the impaired thymic egress of the mature Vγ3+ sIEL precursors (Fig. 4A, 4B). When the total Vγ3+ fetal thymic γδT cells were analyzed further for their maturation status based on the expression of CCR10 and CD122, the relative percentage of the fully mature CD122+CCR10+ population within the Vγ3+ γδT cells was higher in CCR6Tg mice than in WT mice (Fig. 4A). Based on total numbers of fetal thymocytes and percentages of Vγ3+ γδT cells of the different maturation stages within them, we calculated numbers of Vγ3+ fetal thymic γδT cells of the different maturation stages (Fig. 4B). Notably, compared with their respective WT controls, the numbers of the fully mature CD122+CCR10+ fetal thymic Vγ3+ cells increased most, whereas the partially mature CD122−CCR10− increased less significantly (Fig. 4B). In contrast, numbers of the immature CD122−CCR10− Vγ3+ fetal thymic γδT cells were not significantly different in CCR6Tg and WT mice (Fig. 4B). Together, these results demonstrate that the failure of downregulation of CCR6 in the mature Vγ3+ fetal thymic sIEL precursors results in their impaired egress from the thymus in the CCR6Tg mice, which

FIGURE 2. Impaired sIEL development in CCR6Tg mice. (A) Representative immunofluorescent microscopy of epidermal sheets of adult CCR6Tg and WT mice stained for identification of Vγ3+ sIEL. Epidermal sheets were costained with anti-Vγ3 Ab (green), anti-CD3 Ab (red), and counterstained with DAPI (blue). The pictures were taken at original magnification ×20. (B) Quantitative comparison of numbers of Vγ3+ sIEL in adult CCR6Tg and WT mice (at ages of 5–7 wk). The number of Vγ3+ sIEL is per field (original magnification ×20) and calculated from enumeration of Vγ3+ sIEL of at least five pictured fields of the immunofluorescent microscopy of one ear epidermal sheet of one mouse. One dot represents the number of Vγ3+ sIEL per field from one mouse. The short flat lines in the middle of dots indicate average numbers of sIEL per field in mice of the different genotypes. (C) Representative immunofluorescent microscopy of epidermal sheets of 7-d-old CCR6Tg and WT mice stained for identification of Vγ3+ sIEL as in (A). (D) Comparison of numbers of Vγ3+ sIEL in 7- and 1-d-old CCR6Tg and WT littermates. The number of Vγ3+ sIEL was per field as presented in (B). *p < 0.05, ***p < 0.001.

FIGURE 3. Transgenic expression of CCR6 specifically affects response of the mature fetal thymic Vγ3+ sIEL precursors to CCL20. (A) Quantification of expression of the CCR6 transcripts in the CD122− and CD122+ E17 fetal thymic Vγ3+ γδT cells of CCR6Tg and WT littermates by the real-time RT-PCR. Three mice of each genotype were analyzed. (B) Flow cytometric analysis of E17 fetal thymic CD122− and CD122+Vγ3+CD3+ T cells of WT and CCR6Tg mice for the cell surface expression of CCR6. The gray area is of isotype-matched control Ab staining. In vitro migration analysis of CCR10+ (C) and CCR10− (D) fetal thymic Vγ3+ cells of CCR6 transgenic and WT mice toward CCL20. The migration index is calculated as a ratio of numbers of Vγ3+ cells migrating into the bottom chamber in presence of CCL20 versus medium only. The experiments were repeated twice for a total of six samples. (E) In vitro migration analysis of fetal thymic Vγ3+ γδT cells of CCR6Tg and WT mice toward CCL20. The experiments were repeated twice in a total of six samples and performed as in (C) and (D). *p < 0.05, **p < 0.01, ***p < 0.001.
FIGURE 4. Abnormal accumulation of mature Vγ3+ sIEL precursors in the fetal thymus of CCR6Tg mice. (A) Flow cytometric analysis of E17 fetal thyocytes for Vγ3+ T cells of CCR6Tg and WT littermates and their expression of CD122 and CCR10 (EGFP). (B) Quantitative comparison of numbers of Vγ3+ γδT cells of different maturation stages (total, CD122+CCR10+, CD122−CCR10+ , and CD122−CCR10−) in E17 fetal thyms of CCR6 transgenic and WT littermates. Numbers of the different Vγ3+ γδT cell subsets were calculated from total numbers of fetal thyocytes and the percentages of Vγ3+ fetal thymic γδT cells of different developmental stages as determined in (A). One dot represents the number of cells from one mouse. (C) Flow cytometric analysis of E17 fetal thymic Vγ3+ γδT cells of WT and CCR6Tg littermates for expression of CD44 and CD24. The cells in the histogram are of the gated Vγ3+CD3+ population. The numbers in each quadrant are average percentage ± SD of cells in the quadrant. At least nine mice of each genotype were analyzed. (D) Quantitative comparison of numbers of E17 fetal thymic Vγ3− T cells in CCR6Tg and WT littermates. The numbers were calculated similarly as in (B). *p < 0.05, **p < 0.01.

would be likely responsible for the impaired development of sIEL. In contrast, the CCR10 expression level was similar on CD122+CCR10+ fetal thymic Vγ3+ γδT cells of WT and CCR6Tg mice (Fig. 4A), suggesting that the failed downregulation of CCR6 on the mature sIEL precursors of CCR6Tg mice probably would not affect the CCR10-mediated skin homing. In addition, the finding that the mature CD122+ fetal thymic Vγ3+ γδT populations were increased, whereas the immature CD122− fetal thymic Vγ3+ γδT cells were not affected in CCR6Tg mice suggests that CCR6 transgene does not affect intrathymic maturation process of the Vγ3+ sIEL precursors. Supporting this notion, fetal thymic Vγ3+ γδT cells of CCR6Tg mice also showed a normal maturation process based on their expression of the maturation marker CD44 and immature marker CD24 (Fig. 4C) (38). The CCR6 transgene resulted in accumulation of other T cells in the fetal thymus (Fig. 4D), consistent with the fact that CCR6 transgene is functionally expressed in T cells other than Vγ3+ fetal thymic γδT cells (Fig. 3E).

Improper distribution of Vγ3+ γδ T cells in dermis of CCR6Tg mice

In contrast to the absent expression of CCR6 in the mature fetal thymic Vγ3+ sIEL precursors and sIEL, all the Vγ3− dermal γδT cells express CCR6 (14, 15). Considering that the CCR6 ligand CCL20 is also expressed in the skin, the differential expression of CCR6 in Vγ3+ sIEL and Vγ3− dermal γδT cells might be involved in their proper localization within the different regions of skin. Therefore, the impaired sIEL development in CCR6Tg mice could be also associated with an altered distribution of the Vγ3+ γδT cells within the different regions of skin. To test this, we isolated cells from epidermis and dermis of CCR6Tg and WT mice separately and analyzed them for Vγ3+ γδT cells by flow cytometry. Confirming that the sIEL development is impaired in the CCR6Tg mice, there were significantly lower percentages of Vγ3+ T cells in the epidermis of CCR6Tg mice than in WT controls at both adult and neonatal stages (Fig. 5A–C). In contrast, percentages of Vγ3+ γδ T cells in cells isolated from the dermis of CCR6Tg mice were much higher than those of the WT littermates (Fig. 5A–C). As controls, numbers of the total Vγ3+ γδT cells and individual subsets of Vγ3+ and Vγ1.1+ γδT cells in the dermal region of CCR6Tg mice are not different from those of WT mice, suggesting any additional CCR6Tg expression over the endogenous CCR6 expression in the dermal Vγ3+ γδT cells did not have impact on their localization in the skin (Fig. 5D, 5E). In addition, all of the dermal 17D1+ cells, which contain both Vγ3+ and Vγ4+ cells (36), stained positive for Vγ3 in both WT and CCR6Tg mice, suggesting that no Vγ4+ cells were in dermis of either WT or CCR6Tg mice (Fig. 5F). These results reveal the specific effect of CCR6Tg expression on the localization of Vγ3+ γδT cells but not the other γδT cell subsets within the skin. Together, our findings demonstrate that the programmed downregulation of CCR6 in the mature fetal thymic Vγ3+ sIEL precursors is not only important for their thymic egress but also involved in proper distribution of the Vγ3+ cells within epidermis of the skin.

Similar functional capacities of epidermal and dermal Vγ3+ γδ T cells in WT and CCR6Tg mice

The different localization of Vγ3+ γδT cells in epidermis and dermis could potentially affect their functions. We therefore tested whether Vγ3+ γδT cells isolated from epidermis and dermis of WT and CCR6Tg mice have different function capacities in production of KGF and IL-17, two factors uniquely associated with epidermal sIEL and dermal γδT17 cells, respectively (13–15, 39). As reported, the epidermal Vγ3+ γδT cells but not dermal Vγ3+ γδT cells of WT mice produce KGF (Fig. 6A). Similar to the epidermal Vγ3+ γδT cells, dermal Vγ3+ γδT of WT mice also express KGF (Fig. 6A). In addition, the CCR6 transgene had no
effect on production of KGF by the epidermal Vγ3+, dermal Vγ3+, and Vγ3− γδT cell subsets (Fig. 6A).

A significant percentage of dermal Vγ3−, but not Vγ3+, T cells express IL-17 in WT mice (Fig. 6B). Vγ3− γδT cells isolated from dermis of CCR6Tg mice did not produce IL-17 either (Fig. 6B). In addition, similar percentages of the dermal Vγ3 T cells are capable of producing IL-17 in WT and CCR6Tg mice (Fig. 6B). Therefore, although the CCR6 transgene affects localization of Vγ3+ γδT cells within epidermis versus dermis of the skin, it has no effect on their functional potentials.

**Discussion**

Since we first reported that a positive selection is required for the developmental distribution of fetal Vγ3+ sIEL precursors in the skin, it has become clear that the intrathymic γδTCR signaling and the intrinsic property of sIEL precursors are both involved in coordinating expression of an array of chemokine receptors and adhesion molecules on the sIEL precursors for their localization into epidermis (22, 40). In contrast, whether the TCR signaling is required for maintenance of sIEL in the skin is not completely clear. Although some publications suggest that the TCR signaling...
In addition, the expression of CCR10 on the sIEL is downregulated from the level of the fetal thymic sIEL precursors, which is importantly involved in the positioning of the sIEL in the epidermal region of the skin through calibration of the interaction strength of CCR10 of sIEL and its ligand CCL27 expressed by neighboring keratinocytes (23). Therefore, the spatially and temporally regulated up- and downregulation of multiple homing and adhesion molecules are involved in both migration into and maintenance of sIEL in the skin. Our finding that the programmed downregulation of CCR6 on the fetal thymic Vy3+ sIEL precursors is involved in the efficient thymic egress and proper epidermal localization of the Vy3+ sIEL provide a further support to the notion that the intrathymic programming plays an important role in determining peripheral tissue distribution of specific γδ T cell subsets and helps understanding the molecular events mediating the tissue-specific distribution.

Considering that the ligand for CCR6, CCL20, is expressed in the thymus (32, 33), it has been long suggested that the CCR6/CCL20 interaction might be involved in the thymic T cell development. However, up to now, there is no clear evidence that the CCL20/CCR6 pair is involved in the thymic T cell development, and CCR6-knockout mice do not have any obvious defect in the conventional T cell development in the thymus. One explanation for this could be that loss of the low-level expression of CCR6 on the developing thymic T cells could be functionally compensated for by other chemokine receptors. However, despite its low level expression, our study in this paper suggested that the appropriately regulated expression of CCR6 in thymic Vy3+ γδ T cells nevertheless plays an important role in the development of thymic T cells, at least regarding the fetal thymic Vy3+ cells. Considering that the transgene-driven CCR6 expression in the mature Vy3+ sIEL is at a comparable level to that of the immature Vy3+ sIEL (Fig. 3), the transgene-associated phenotypes likely reflect a role of the downregulation of endogenous CCR6 in allowing the efficient thymic egress and proper location of sIEL in the epidermis but are due to significant overexpression of CCR6γδ over the level of endogenous CCR6. In this regard, CCR9, another chemokine receptor involved in mediating the entry of early bone marrow progenitor cells into the thymus (49, 50), is also expressed on the immature thymic T cells but downregulated in mature thymic αβ T cells (51). However, in contrast to the CCR6 downregulation reported in this study, the CCR9 downregulation is not essential for thymocyte export because a transgene-mediated forced expression of CCR9 on mature thymic T cells did not affect numbers of thymic CD4+ or CD8+ T cells (52). In light of our finding, it is possible that the programmed downregulation of CCR9 on thymic mature T cells could be involved in their peripheral localization.

Although the CCR6 downregulation plays a role in the thymic egress of Vy3+ sIEL, it may not be necessarily a universal mechanism controlling the thymic export of all T cell populations. There were reports that specific mature thymic αβ T cell and Vyγδ γδ T cell subsets express high levels of CCR6 in adult mice (48, 53). Whether this specific expression of CCR6 is involved in the intrathymic migration and egress is not clear. Considering that all of the dermal γδ T17 cells express CCR6 and originate from the fetal/perinatal thymic γδ T17 cells (14, 15), it will be interesting to determine whether the fetal thymic precursors of the dermal γδ T17 cells express CCR6 and its role in the localization of γδ T17 cells in the dermis. Furthermore, because significant numbers of Vy3+ sIEL were still found in the epidermis of CCR6 transgenic mice, it is apparent that the downregulation of CCR6 on the Vy3+ sIEL precursors is not absolutely necessary for their thymic export and epidermal localization. This is in contrast with the upregulation of S1PR1, which is critically important for thymic egress.
of both conventional mature αβT cells as well as γδT cells. Likely, the downregulation of CCR6 is one of multiple molecular events involved in the thymic egress and peripheral tissue localizations of the fetal thymic Vγ3δ γδT cells.

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Disclosures

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References


