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Selective programming of CCR10+ innate lymphoid cells in skin-draining lymph nodes for cutaneous homeostatic regulation

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Abstract

Innate lymphoid cells (ILCs) are preferentially localized into barrier tissues where they function in tissue protection but can also contribute to inflammatory diseases. The mechanisms regulating the establishment of ILCs in barrier tissues are poorly understood. Here we show that under steady-state conditions ILCs in skin-draining lymph nodes (sLNs) were continuously activated to acquire regulatory properties and high expression of the chemokine receptor CCR10 for localization into the skin. $CCR10^+$ ILCs promoted the homeostasis of skin-resident T cells and reciprocally, their establishment in the skin required T cell-regulated homeostatic environments. Foxn1-expressing $CD207^+$ dendritic cells were required for the proper generation of $CCR10^+$ ILCs. These observations reveal mechanisms underlying the specific programming and priming of skin-homing $CCR10^+$ ILCs in the sLNs.

Innate lymphoid cells (ILCs) are innate lymphocytes preferentially enriched in barrier tissues such as the intestine, lung and skin where they play important roles in establishing the local tissue homeostasis[1–4]. Although ILCs do not express lineage-specific cell-surface markers, they express the hematopoietic lineage marker CD45 and surface molecules commonly associated with lymphocytes, such as CD90 and CD127[1]. Based on their

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Contributions

J.Y., S.H. and L.Z. performed experiments. D.K. and G.H.P. provided materials. N.X. and J.Y. designed the study. N.X., J.Y., L.Z. and S.H. analyzed the data and wrote the manuscript. All read and commented on the manuscript.

Competing financial interests

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functional potentials and developmental requirements, and analog to T helper cell (T_H) subsets, ILCs are commonly divided into three groups (ILC1-3)[1]. The ILC1 group comprises natural killer cells and other ILCs that predominantly produce T_H1-type cytokines such as interferon γ (IFN-γ) when activated; ILC2s produce T_H2-type cytokines such as interleukin 5 (IL-5) and IL-13; and ILC3s produce T_H17-type cytokines such as IL-17 and IL-22. Through the production of unique cytokines and direct cell-cell interaction, distinct ILC subsets interact with various other immune cells, such as T cells, mast cells, eosinophils and dendritic cells (DCs) to maintain homeostasis in local tissues[4–7].

Dysregulated activated ILCs are also involved in tissue inflammatory diseases. IL-23- and IL-1β-responsive ILC3-like cells could contribute to intestinal inflammatory diseases[8–10]. IL-13-producing ILC2s were suggested to have a role in various types of lung inflammation[11–14]. ILC2s mediate skin inflammation in mouse models of atopic dermatitis[4, 15–17] while IL-17-producing ILC3s were reported to contribute to Aldara cream-induced psoriatic disease development in mice[18]. It was suggested that pathogenic ILCs might be different from ILCs involved in the tissue homeostatic regulation in term of their surface receptor expression and regulation of activation. For example, among IL-17-producing ILCs, the subset expressing the IL-23 receptor (IL-23R) is pathogenic, while the IL-23R$^-$ subset is not[9]. However, how they are differentially generated is not clear.

Despite the diverse roles of ILCs in both homeostasis and inflammation in different barrier tissues, the mechanisms regulating their tissue-specific localization and functions are poorly understood. It was suggested that ILCs acquire specific homing properties for their preferential localization into barrier tissues during their development in the bone marrow (BM), while their activation happens *in situ* in the periphery[19, 20]. Most circulating ILC2s were found in an inactivated state and expressed a common epithelial tissue homing-molecule CCR6, which might direct their preferential migration into various epithelial tissues[19, 20]. It was also reported that most developing ILC2 precursors in BM and ILC2s in the intestine express CCR9, a homing molecule important for their proper localization in the intestinal lamina propria[21]. However, ILCs, including ILC2s, are found abundantly in other barrier tissues, such as the skin. ILCs isolated from the skin of healthy individuals express CCR10[16], a homing molecule that was previously involved in the localization of T cells into the homeostatic skin through interaction with CCL27, a skin-specific CCR10 ligand expressed by keratinocytes[22, 23]. Considering the complexity of requirements for migration of lymphocytes in different barrier tissues under homeostatic and inflammatory conditions, additional layers of regulation are likely required for specific localization and functions of ILCs.

Here we report that ILCs are programmed in skin-draining lymph nodes (sLNs) to acquire skin-homing properties for the homeostatic establishment of skin ILC pool. In addition, under homeostatic or inflammatory conditions, sLNs programmed the generation of activated ILCs with distinct properties to help regulate the local homeostasis and inflammation.

Results

Skin-specific CCR10$^+$ ILCs are generated in sLNs

The majority of CD45$^+$CD3$^-$Lin$^-$ cells in the skin and sLNs of *Ccr10*$^{+/EGFP}$ reporter mice[23, 24] were CD127$^+$CD90$^+$ CCR10(EGFP)$^+$ ILCs, while CD45$^+$CD3$^-$Lin$^-$ cells in the intestine and lung mucosa, intestine-draining mesenteric lymph nodes (mLNs), the BM or spleens were CCR10$^-$ (Fig. 1a,b). Nearly all CCR10$^+$ skin ILCs and most CCR10$^+$ sLN ILCs expressed other common epithelial tissue-homing and adhesion molecules, such as CCR6, CD103 and CD69 (Fig. 1c). CCR10$^+$ ILCs of the skin and sLNs expressed the lymphocyte activation marker CD44 and significantly higher percentages of them were Ki-67$^+$ than corresponding CCR10$^-$ ILCs (Fig. 1d), suggesting that CCR10$^+$ ILCs were more activated. Higher percentages of CCR10$^+$ skin and sLN ILCs were IL-17$^+$ (ILC3) and IL-5$^+$ (ILC2) than corresponding CCR10$^-$ ILCs but few CCR10$^+$ skin and sLN ILCs produced IFN-γ or IL-10 (Fig. 1e). Most CCR10$^+$ skin and sLN ILCs expressed Gata-3 but no T-bet (Supplementary Fig. 1a), consistent with their expression of IL-5 but no IFN-γ. CCR10$^+$ skin and sLN ILCs did not express Rorγt (Supplementary Fig. 1b)[25] even though a significant portion of them expressed IL-17.

A fraction of intestinal ILC3s were reported to express high amounts of MHCII and to have regulatory antigen-presenting cell (APC) capacities[5]. Interestingly, most CCR10$^+$ skin and sLN ILCs expressed high amounts of MHCII, while CCR10$^-$ ILCs expressed no or low amounts of MHCII (Fig. 1f and Supplementary Fig. 1c), regardless of their expression of IL-5 and IL-17 (Supplementary Fig. 1d). In addition, most CCR10$^+$ skin and sLN ILCs expressed the co-inhibitory molecule PD-L1 while they had no or low expression of other co-stimulatory or co-inhibitory molecules CD80, CD86, PD-L2, B7x, B7h, B7H3, OX40L and CD40 (Supplementary Fig. 1e,f). Together, these results suggest that CCR10$^+$ ILCs are skin-specific ILCs with regulatory APC potentials that are generated in sLNs under homeostatic conditions.

Predominant migration of CCR10$^+$MHCII$^+$ ILCs of sLN into the skin

To determine whether CCR10$^+$ sLN ILCs migrate into the skin, we intravenously injected total sLN cells from *Ccr10*$^{+/EGFP}$ mice (CD45.2$^+$) into wild-type mice (CD45.1$^+$CD45.2$^+$). Two days after transfer, about 80% of donor ILCs in the skin of recipients were CCR10$^+$MHCII$^+$ (Fig. 2a), suggesting that injected CCR10$^+$MHCII$^+$ sLN ILCs rapidly migrate into the skin. Confirming this, sorter-purified CCR10$^+$ but not CCR10$^-$ sLN ILCs were found in the skin of wild-type recipients two days after transfer (Fig. 2b), and they were maintained two weeks after transfer (Fig. 2c). In addition, most CCR10$^+$MHCII$^+$ donor ILCs in the skin of recipients became CCR10$^+$MHCII$^-$ two weeks after transfer (Fig. 2c), implying the downregulation of MHCII expression. No CCR10$^+$ donor ILCs could be found in sLNs 2 days or 2 weeks after transfer (Fig. 2b,c), suggesting that CCR10$^+$ ILCs do not recirculate back to sLNs once they have localized into the skin. Thus, adoptively transferred CCR10$^+$MHCII$^+$ sLN ILCs can migrate to the skin where they could also become CCR10$^+$MHCII$^-$ ILCs.

To determine whether a continuous input of CCR10$^+$MHCII$^+$ ILCs from sLNs was required for homeostatic establishment of ILCs in the skin, we fed mice with the drug FTY720, which sequesters lymphocytes in LNs[26]. Compared to un-treated mice, FTY720-fed mice had a significant increase of CCR10$^+$ ILCs in sLNs and a significant reduction of CCR10$^+$, especially CCR10$^+$MHCII$^+$, ILCs in the skin (Fig. 2d and Supplementary Fig. 2a). FTY720 treatment also caused accumulation of CCR10$^+$ T cells in sLNs and their reduction in the skin (Supplementary Fig. 2b). These results demonstrate that the homeostatic presence of ILCs in the skin requires continuous input of CCR10$^+$MHCII$^+$ ILCs from sLNs.

sLNs are essential for the homeostasis of skin CCR10$^+$ ILCs

Previous studies found that ILCs were abundant in the skin of LN-deficient *Rorc*$^{-/-}$ mice[16,27]. However, there were essentially no CCR10$^+$MHCII$^+$ skin ILCs in *Rorc*$^{-/-}$ mice (Fig. 2e and Supplementary Fig. 2c), suggesting that sLNs are critical for the homeostatic establishment of CCR10$^+$ ILCs in the skin. There were also reduced percentages of CCR10$^+$ T cells in the skin of *Rorc*$^{-/-}$ mice compared to wild-type mice (Supplementary Fig. 2d), consistent with the notion that CCR10$^+$ T cells are programmed in sLNs[28]. The percentages of IL-17$^+$ skin ILCs were also reduced in *Rorc*$^{-/-}$ mice compared to wild-type controls (Supplementary Fig. 2e). Similar to Rorγt, aryl hydrocarbon receptor (Ahr) is important for the development of IL-17$^+$ ILC3s in intestines[29], but *Ahr*$^{-/-}$ mice have normal LN development, and they had apparently normal generation and establishment of sLN and skin ILCs and CCR10$^+$ skin T cells (Fig. 2e and Supplementary Fig. 2f–i).

We reconstituted irradiated wild-type mice with total *Rorc*$^{-/-}$ BM cells and *vice versa*. Donor *Rorc*$^{-/-}$ BM cells gave rise to abundant MHCII$^+$CCR10$^+$ skin ILCs in the wild-type recipients while donor wild-type BM cells could not efficiently give rise to CCR10$^+$, particularly MHCII$^+$CCR10$^+$, skin ILCs in *Rorc*$^{-/-}$ recipients (Fig. 2f), indicating that altered host environments, most likely lack of sLNs, were responsible for the impaired establishment of CCR10$^+$ ILCs in the skin of *Rorc*$^{-/-}$ mice.

We then treated wild-type mice *in utero* with lymphotoxin β receptor-immunoglobulin fusion proteins (LTβR-Ig) to selectively block their LN development[30]. When analyzed 1.5–2 months after birth, the treated mice had significantly reduced percentages of CCR10$^+$, particularly MHCII$^+$CCR10$^+$, skin ILCs compared to untreated controls (Fig. 2g and Supplementary Fig. 2j). Together, these results demonstrate that sLNs are critically required to program CCR10$^+$ ILCs for homeostatic establishment of the skin ILC pool.

CCR10 and CCR6 co-regulate the localization of ILCs in the skin

To determine the role of CCR10 in the migration and establishment of CCR10$^+$ ILCs into the skin we compared the presence of EGFP$^+$ ILCs in the sLNs, skin and spleens of *Ccr10*$^{EGFP/EGFP}$ mice, which did not express CCR10 proteins[24], and *Ccr10*$^{+/EGFP}$ littermates. *Ccr10*$^{EGFP/EGFP}$ mice had similar, if not higher, percentages and numbers of EGFP$^+$MHCII$^+$ ILCs in sLNs compared to *Ccr10*$^{+/EGFP}$ littermates (Fig. 3a and Supplementary Fig. 3a). In contrast, there were decreased percentages of EGFP$^+$MHCII$^+$ ILCs in the skin of *Ccr10*$^{EGFP/EGFP}$ mice, while EGFP$^+$MHCII$^-$ or EGFP$^-$MHCII$^-$ ILCs increased or did not change significantly compared to corresponding *Ccr10*$^{+/EGFP}$ controls

(Fig. 3a and Supplementary Fig. 3a,b). Considering that EGFP$^+$MHCII$^+$ ILCs in the skin are "newcomers" emigrating from sLNs (Fig. 2a–c), these results suggest that CCR10 is required for their localization into the skin but increased maintenance of other subsets of ILCs might compensate in absence of CCR10 expression on EGFP$^+$MHCII$^+$ ILCs. There were significantly higher percentages of EGFP$^+$ ILCs in the spleens of *Ccr10*$^{EGFP/EGFP}$ mice than *Ccr10*$^{+/EGFP}$ mice (Fig. 3a), suggesting that CCR10-deficient EGFP$^+$ ILCs abnormally accumulate in lymphoid organs that do not express CCR10 ligands. CCR10 deficiency did not affect expression of other homing or activation molecules or cytokine production by skin or sLNs EGFP$^+$ ILCs (Supplementary Fig. 3c).

To assess the intrinsic requirement for CCR10 expression for ILC establishment in the skin, we co-transferred similar numbers of *Ccr10*$^{+/EGFP}$ and *Ccr10*$^{EGFP/EGFP}$ total BM cells into irradiated wild-type mice. Two months after transfer, *Ccr10*$^{+/EGFP}$ donors contributed significantly more to EGFP$^+$ and EGFP$^-$ skin ILCs than *Ccr10*$^{EGFP/EGFP}$ donors (Fig. 3b,c), indicating that impaired localization of *Ccr10*$^{EGFP/EGFP}$ EGFP$^+$ sLN ILCs into the skin affected both EGFP$^+$ and EGFP$^-$ skin ILCs. Donor *Ccr10*$^{EGFP/EGFP}$ EGFP$^+$ ILCs were also reduced in sLNs (Fig. 3b,c), suggesting that CCR10 deficiency impaired the proper maintenance of CCR10$^+$ ILCs in sLNs, an effect that might be homeostatically compensated at steady state in *Ccr10*$^{EGFP/EGFP}$ mice. *Ccr10*$^{EGFP/EGFP}$ donor cells contributed more than *Ccr10*$^{+/EGFP}$ donor cells to the EGFP$^+$ ILC population in the spleen of hosts (Fig. 3b,c), consistent with abnormal accumulation of EGFP$^+$ ILCs in spleens of *Ccr10*$^{EGFP/EGFP}$ mice (Fig. 3a). In addition, *Ccr10*$^{EGFP/EGFP}$ EGFP$^+$ sLN ILCs migrated much less efficiently into the skin than *Ccr10*$^{+/EGFP}$ EGFP$^+$ sLN ILCs when co-transferred into the same wild-type mice (Fig. 3d and Supplementary Fig. 3d). These results demonstrate that CCR10 is important for the migration of CCR10$^+$MHCII$^+$ sLN ILCs into the skin for homeostatic establishment of skin ILCs.

CCR6 was expressed on most sLN and skin CCR10$^+$ and CCR10$^-$ ILCs (Fig. 1c). Compared to CCR6-sufficient littermates, *Ccr6*$^{-/-}$ mice had similar percentages of CCR10$^+$MHCII$^+$ ILCs (Fig. 3a), but reduced numbers of total CCR10$^+$ ILCs in the skin, largely due to reduced percentages of total ILCs in the CD45$^+$ population (Supplementary Fig. 3a,b). However, *Ccr6*$^{-/-}$ mice did not show increased accumulation of CCR10$^+$ ILCs in spleens (Fig. 3a). Compared to *Ccr10*$^{+/EGFP}$ mice, *Ccr10*$^{EGFP/EGFP}$*Ccr6*$^{-/-}$ mice had even fewer EGFP$^+$MHCII$^+$ ILCs in the skin and more EGFP$^+$ ILCs in spleens than *Ccr10*$^{EGFP/EGFP}$ mice (Fig. 3a and Supplementary Fig. 3a). Thus, CCR6 and CCR10 coordinately regulate the localization and maintenance of CCR10$^+$ and CCR10$^-$ ILCs in the skin.

T cells and immune homeostasis help program CCR10$^+$ ILCs

Because CCR10 also controls the T cell localization in the skin[23], we investigated the presence of CCR10$^+$ skin and sLN ILCs in the absence of T cells in *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice. Few skin ILCs in *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice expressed CCR10 or MHCII, while they still had high expression of CCR6, CD103 and CD44 (Fig. 4a and Supplementary Fig. 4a,b). IL-17 and IL-5 expression was also similar in CCR10$^-$ *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ skin ILCs and CCR10$^+$ *Ccr10*$^{+/EGFP}$ skin ILCs (Supplementary Fig. 4c). There were still high percentages

of CCR10$^+$MHCII$^+$ ILCs in sLNs of *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ (Fig. 4b). But CCR10$^-$ sLN ILCs in *Rag1*$^{-/-}$ *Ccr10*$^{+/EGFP}$ mice were also activated, as they had increased expression of CD44, CCR6 and CD103 and more proliferative Ki-67$^+$ cells compared to CCR10$^-$ sLN ILCs in *Ccr10*$^{+/EGFP}$ mice (Fig. 4c,d and Supplementary Fig. 4b). Two days after the intravenous injection of total *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ sLN ILCs into *Rag1*$^{-/-}$ recipients, both CCR10$^+$ and CCR10$^-$ donor ILCs were found in the skin (Supplementary Fig. 4d), suggesting that both CCR10$^+$ and CCR10$^-$ sLN ILCs could migrate into the recipient skin. Most donor ILCs in the skin of *Rag1*$^{-/-}$ recipients were MHCII$^-$ (Supplementary Fig. 4d), suggesting its downregulation. Supporting this notion, purified *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ or *Ccr10*$^{+/EGFP}$ CCR10$^+$MHCII$^+$ sLN ILCs all became MHCII$^-$CCR10$^-$ four weeks after transferred into *Rag1*$^{-/-}$ recipients (Supplementary Fig. 4d). These results suggest that T and B cells are important for proper programming of ILCs in sLNs and the homeostatic presence of CCR10$^+$ ILCs in the skin.

We then injected intravenously wild-type splenic CD4$^+$ T cells into *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ recipients. The T cell reconstitution increased the percentages of CCR10$^+$ and CCR10$^+$MHCII$^+$ ILCs in the skin 2 and 4 folds compared to untreated *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice (Fig. 4e), but had no or small effect on the activation of CCR10$^-$ and CCR10$^+$ ILCs in the sLNs of recipients (Supplementary Fig. 4e), suggesting that CD4$^+$ T cells help the maintenance of CCR10$^+$ ILCs in the skin. We also injected T_{reg}-depleted wild-type splenic CD4$^+$ T cells or T_{reg} cells separately into *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ recipients. Neither of the transfer increased the percentage of CCR10$^+$ skin ILC in the *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ recipients (Fig. 4e). Therefore, both effector T and T_{reg} cells are required for the homeostatic presence of CCR10$^+$ ILCs in the skin.

We additionally analyzed sLN and skin ILCs in *Foxp3*$^{-/-}$ mice, which lack all T_{reg} cells and develop severe organ inflammation. Compared to wild-type mice, *Foxp3*$^{-/-}$ mice had severely reduced percentages and numbers of CCR10$^+$ ILCs in the skin as well as sLNs (Fig. 4f,g and Supplementary Fig. 4f), suggesting that inflammation might affect the generation of CCR10$^+$ ILCs in sLNs and their presence in the skin. Consistent with this, topical treatment with calcipotriol, which induces atopic dermatitis-like skin inflammation[15–17], resulted in 2- and 1.5-fold reduction of CCR10$^+$ ILCs in sLNs and the skin compared to untreated controls (Fig. 4h and Supplementary Fig. 4g). Therefore, the generation and presence of CCR10$^+$ ILCs in sLNs and the skin depend on local immune homeostasis.

Skin ILCs promote the resident T cell homeostasis in the skin

We next transferred splenic CD4$^+$ T cells from *Ccr10*$^{+/EGFP}$ mice into *Ccr10*$^{EGFP/EGFP}$*Rag1*$^{-/-}$ recipients to test whether skin CCR10$^+$ ILCs are required to help the skin T cell homeostasis. Compared to *Rag1*$^{-/-}$ recipients, *Ccr10*$^{EGFP/EGFP}$*Rag1*$^{-/-}$ recipients had significantly reduced percentages of T_{reg} cells of total donor cells in the skin (Fig. 5a), The frequencies of donor CD4$^+$ T cells that produced IL-17, IFN-γ and IL-5 were similar in *Ccr10*$^{EGFP/EGFP}$*Rag1*$^{-/-}$ vs. *Rag1*$^{-/-}$ recipients (Supplementary Fig. 5a). Therefore, CCR10$^+$ ILCs are particularly important to support T_{reg} cell presence in the skin.

We also transferred CD4$^+$ splenic T cells into *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice, which lack all ILCs[3, 31, 32]. The percentage of T_{reg} cells of total donor cells in the skin of *Il2rg*$^{-/-}$*Rag2*$^{-/-}$

recipients was reduced 2–3 folds compared to that of *Rag1*$^{-/-}$ recipients (Fig. 5b). In addition, nearly all donor CD4$^+$ T cells in the skin of *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ recipients were CCR10$^-$ (Fig. 5b) and expressed significantly higher IL-17A and IL-5, but much lower IFN-γ, than T cells in *Rag1*$^{-/-}$ recipients (Fig. 5c, d). These results suggest that skin ILCs regulate the skin T cell homeostasis.

Foxn1 and CD207$^+$ DCs are required for programming CCR10$^+$ ILCs

We next investigated the mechanisms underlying the specific programming of CCR10$^+$ ILCs in sLNs. Foxn1 is a transcription factor selectively expressed in thymic and skin epithelial cells, such as keratinocytes[33, 34]. Like *Rag1*$^{-/-}$ mice, *Foxn1*$^{-/-}$ mice, which have impaired T cell and skin development, had severely reduced percentages of CCR10$^+$, particularly CCR10$^+$MHCII$^+$ skin ILCs (Fig. 6a and Supplementary Fig. 6a). Additionally, *Foxn1*$^{-/-}$ mice had significantly reduced CCR10$^+$ ILCs in sLNs compared to *Rag1*$^{-/-}$ mice (Fig. 6a), suggesting that Foxn1 is involved in regulating molecules or cells important for the generation of sLN CCR10$^+$ ILCs.

Total *Ccr10*$^{+/EGFP}$ BM cells were transferred into irradiated *Foxn1*$^{-/-}$ mice. Significantly lower percentages of *Ccr10*$^{+/EGFP}$ donor CCR10$^+$ and CCR10$^+$MHCII$^+$ ILCs were found in the sLNs (Fig. 6b) and skin (Supplementary Fig. 6b) of *Foxn1*$^{-/-}$ recipients compared to controls of wild-type recipients, suggesting the Foxn1 expressed in “radio-resistant” cells, such as keratinocytes and epidermal DCs, was important for the generation and establishment of CCR10$^+$MHCII$^+$ ILCs in sLNs and the skin.

To identify the Foxn1$^+$ cells important for the generation and maintenance of CCR10$^+$ ILCs in sLNs and skin, we first used the *Foxn1*$^{+/Cre}$*Gt(ROSA)26Sor*$^{+/RFP}$ reporter mice to assess the current or previous Foxn1-expressing cells. In these mice, Foxn1 was selectively and highly expressed in a large fraction of CD207 (langerin)$^+$ CD45$^+$CD3$^-$CD11c$^+$ DCs of the skin and sLNs while DCs from the intestine and mLNs did not express Foxn1 (Fig. 6c). Both CD103$^-$ epidermis-origin and CD103$^+$ dermis-origin CD207$^+$ DCs expressed Foxn1 (Supplementary Fig. 6c).

We temporarily depleted CD207$^+$ DCs by injecting diphtheria toxin (DT) into MuLanDTR mice that express transgenic receptors for DT (DTR) in all CD207$^+$ DCs[35]. Compared to untreated MuLanDTR mice, MuLanDTR mice treated with DT for 8 days had reduced percentages of CCR10$^+$MHCII$^+$ ILCs in sLNs but not in the skin (Fig. 6d and Supplementary Fig. 6d), The percentage of CCR10$^+$ T cells in sLNs was unchanged (Supplementary Fig. 6e). A longer period (17–21 days) of DT treatment reduced the percentages of CCR10$^+$MHCII$^+$ ILCs in sLNs and skin compared to untreated mice (Fig. 6d) and also slightly reduced the percentages of CCR10$^+$ T cells in sLNs and the skin (Supplementary Fig. 6e). We then specifically depleted epidermal CD207$^+$ DCs by injecting DT into HuLanDTR mice[36]. DT-treated HuLanDTR mice had decreased percentages of CCR10$^+$MHCII$^+$ ILCs in sLNs and the skin compared to untreated HuLanDTR mice (Fig. 6e), although the reduction was smaller than that observed in treated MuLanDTR mice. DT treatment did not significantly reduce the percentages of sLN and skin CCR10$^+$ T cells in HuLanDTR mice (Supplementary Fig. 6f). Thus, both epidermal and dermal CD207$^+$ DCs are involved in generation and maintenance of CCR10$^+$ ILCs in sLNs and the skin.

Discussion

Our studies here reveal that, like T_H cells, ILCs are programmed in sLNs with specific homing and functional capacities for localization and function in the skin. In the skin, $CCR10^+$ ILCs promote T_{reg} and effector T cell homeostasis. Reciprocally, T cells, including T_{reg} cells, are required for homeostatic presence of skin $CCR10^+$ ILCs. These results suggest a functional cross-talk between $CCR10^+$ ILCs and T cells in the skin for their tissue maintenance and function.

ILCs originate in BM. Our findings reveal that after leaving BM, ILCs still could undergo further differentiation. Such ability to keep differentiating might be important in allowing the cells to enter different peripheral tissues in response to different stimulations under homeostatic and inflammatory conditions. Under homeostatic conditions, $CCR10^+$ ILCs generated in sLNs, expressing high amounts of MHCII and the inhibitory mediator PD-L1, are likely involved in the homeostatic regulation of T_H cells, including T_{reg} cells, after migrating into the skin. However, whether ILCs directly regulate the homeostatic regulation of T_H cells is still yet to be determined. ILCs likely also modulate the homeostasis of mast cells or other immune cells in the skin[4]. Reciprocally, maintenance of $CCR10^+$ ILCs in the skin was dependent on other resident immune cells, including $CD4^+$ T cells. How $CCR10^+$ ILCs, $CD4^+$ T cells and other immune cells crosstalk to regulate skin immune homeostasis and response under various stimulatory conditions is an important question for the future study. In one of our preliminary studies, $CD4^+$ T cell-reconstituted *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice had lower innate skin inflammation in response to topical application of the irritant 2,4-dinitro-1-fluorobenzene (DNFB) than same reconstituted *Rag1*$^{-/-}$ or *Ccr10*$^{EGFP/EGFP}$*Rag1*$^{-/-}$ mice (data not shown), suggesting that ILCs might have an important role in driving early immune response to stimulations on the skin. It will be interesting to test whether ILCs are involved in regulation of adaptive T cell response to the DNFB re-challenge.

ILC2s and ILC3s were reported to contribute to the skin inflammation[4, 15–18]. In the various mouse models of skin inflammation and homeostatic dysregulation we tested, we noticed a reduction of $CCR10^+$ ILCs and an increase of $CCR10^-$ ILCs in sLNs and skin. Considering this shift, it is possible that pathogenic ILCs mediating skin inflammation might be different from homeostatic ILCs. However, $CCR10^-$ ILCs in the inflamed skin and sLNs have same capacities to produce IL-17 and IL-5 as $CCR10^+$ ILCs in the homeostatic skin, suggesting that cellular properties other than cytokine production potential might be involved in their function as regulatory versus inflammatory ILCs.

The transcription factor Fonx1, which is selectively expressed in skin epithelial cells and skin and sLN-specific $CD207^+$ DCs, but not in epithelial cells and DCs of other mucosal tissues, was at least partially responsible for the specific programming of $CCR10^+$ ILCs in sLNs. Potentially, Fonx1 could regulate the expression of soluble factors and membrane ligands in both skin epithelial cells and $CD207^+$ DCs for the proper programming of $CCR10^+$ ILCs. Paralleling this notion, skin epithelial cells and skin-derived DCs are both implicated in the programming of skin-homing T cells[28, 37, 38]. However, the molecular events and cellular interactions involved in the specific programming of skin-homing $CCR10^+$ ILCs need further investigation.

Together with our recent finding that CCR10 expressed on skin-resident T cells is important in the homeostatic maintenance and prevention of over-activation of T cell responses to the skin stimulations[23], our observation that CCR10$^+$ ILCs are also involved in regulation of the skin immune homeostasis fully establish CCR10 as a skin immune homeostatic regulator. Previous studies have found that CCL27 is upregulated in the inflamed skin of humans with psoriasis and atopic dermatitis[39, 40], but downregulated in severe skin lesions of psoriasis[41, 42], suggesting that the lack of the CCR10-ligand signals is associated with the severe psoriatic inflammation. In addition, it was reported that the vast majority of ILCs in the healthy skin of humans express CCR10[16]. It will be interesting to determine whether a severe psoriatic skin lesion is associated with decrease of CCR10$^+$ ILCs. Considering the role of CCR10 in the skin immune regulation, it is likely that enhancing, rather than inhibiting, the CCR10-ligand signal should represent a useful therapeutic strategy in restoration of immune homeostatic status in treatment of skin inflammatory diseases such as psoriasis.

Online method section

Mice

Ccr10-knockout/*Egfp*-knockin mice on C57BL/6 background (CD45.2$^+$) were described[24], and crossed to B6 mice bearing CD45.1 alleles (Jackson lab) to obtain *Ccr10*$^{EGFP/EGFP}$ and *Ccr10*$^{+/EGFP}$ mice bearing CD45.1 and/or CD45.2 alleles. *Rag1*$^{-/-}$ mice, *Rorc*$^{-/-}$ mice, *Ccr6*$^{-/-}$ mice, *Foxp3*$^{-/-}$, *Foxn1*$^{-/-}$, *Foxn1*$^{Cre/Cre}$, *Gt(ROSA)26Sor*-loxp-STOP-loxp-RFP (*Gt(ROSA)26Sor*$^{RFP/RFP}$), MuLanDTR and Foxp3-RFP reporter mice were all purchased from Jackson lab. HuLanDTR mice were previously described[36]. *Ahr*$^{-/-}$ mice were kindly provided by Christopher Bradfield (University of Wisconsin). All the mice were on C57BL/6 background (CD45.2$^+$). These mice were also crossed to *Ccr10*-knockout/*Egfp*-knockin mice on proper CD45.2$^+$ and CD45.1$^+$ C57BL/6 background to introduce one or two *Ccr10*-knockout/*Egfp*-knockin alleles for the purpose of reporting the CCR10 expression with EGFP or total *Ccr10*-knockout. *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice were from Taconic. All mouse experiments were performed in specific pathogen-free conditions (SPF) in accordance with protocols approved by The Pennsylvania State University Institutional Animal Care and Use Committee.

Reagents

Monoclonal antibodies purchased from commercial sources are listed in Supplementary Table 1. Anti-mouse PDL-2, anti-mouse B7x, anti-mouse B7h and anti-mouse B7H3 antibodies were kindly provided by Xingxing Zang (Albert Einstein College of Medicine, Bronx, NY). A cocktail of antibodies against the lineage markers [CD5, CD45R (B220), CD11b, Gr-1 (Ly-6G/C), 7–4, and Ter-119 antibodies] was purchased from Miltenyi Biotec (San Diego, CA). Streptavidin-PE-Texas Red was from BD Biosciences (San Jose, CA). Foxp3/Transcription Factor Staining Buffer Set was from eBioscience (San Diego, CA). LTβR-Ig fusion protein was kindly provided by Biogen Idec MA Inc. (Cambridge, MA). Calcipotriol ointment was purchased from a local pharmacy.

Cell isolation

Isolation of lymphocytes from the skin was performed similarly as previous described[23]. Isolation of lymphocytes from intestines and lungs was also performed similarly as reported[43]. BM cells were isolated by flushing tibias and femurs. Splenic and lymph node cells were prepared by pressing the tissues through cell strainers using the end of a sterile plunger of a 10-mL syringe.

Cell staining, flow cytometric analysis and cell sorting

Antibody staining for cell surface markers and flow cytometric analysis of the staining and EGFP signals were previously described[24]. Intracellular Foxp3 transcription factor staining was performed as described[23]. For the intracellular cytokine staining, cells were stimulated in culture with PMA/inomycin for 2–4 hours in presence of brefeldin A first and then stained similarly as for the intracellular transcription factor staining. Cells stained with a single antibody were used to calibrate signal output and compensation. Staining with isotype control antibodies was used to set gating for corresponding antigen-specific antibody staining. Flow cytometric analyses were performed on FC500 (Beckman Coulter) or BD Fortessa LSRII (BD Biosciences, San Jose, CA). Data were analyzed with FlowJo software (TreeStar, Ashland, OR). Cell gating is indicated in figures. Cell sorting was performed using BD Cytopeia's Influx (BD Biosciences).

Bone marrow reconstitution

The experiment was performed as previously described[43].

In vitro migration assay

EGFP^{+} sLN ILCs of *Ccr10*$^{EGFP/EGFP}$ and *Ccr10*$^{+/EGFP}$ mice were labeled with CellTrace^{TM} Violet dye (Invitrogen), injected into WT mice and analyzed two days later, performed similarly as previously described[43].

Cell adoptive transfer

Adoptive transfer of total lymph node cells and isolated T cells or innate lymphoid cells into *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice was performed similarly as previously described[23].

FTY720 treatment

Mice were fed with drinking water containing 10 μg/ml of FTY720 for 14 days.

Topical treatment with calcipotriol

Mice were topically applied with calcipotriol on the back similarly as reported[44].

LTβR-Ig treatment

Pregnant mice were injected intravenously with 100μg LTβR-Ig fusion protein at E11, E14 and E17, as previously described[30]. Impairment of skin-draining inguinal LN development was visually confirmed in all the treated mice at the time when they were euthanized at the age of 1.5–2 months.

Diphtheria toxin treatment

Mice were injected intraperitoneally with 1μg diphtheria toxin (List Biological Labs, Inc, Campbell, CA) per week and analyzed 8–21 days after injection.

Statistical analyses

Data are expressed as means ± standard errors (SEM). Paired or unpaired two-tailed student T tests or ANOVA test were used to determine statistical significance. $P < 0.05$ is considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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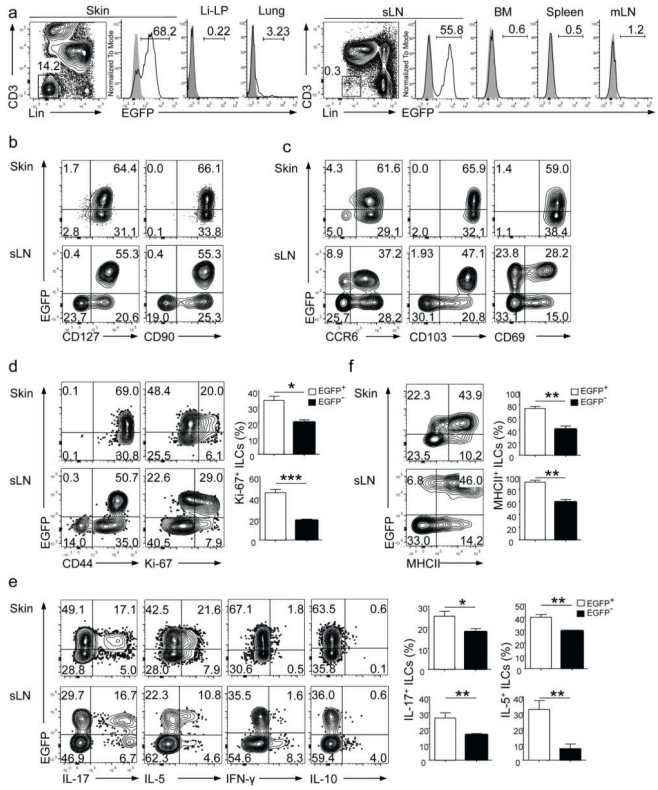

Figure 1.

Selective programming of skin-specific CCR10^{+} ILCs in sLNs. (**a**) Flow cytometric analysis of CCR10 (EGFP) expression in CD45^{+}CD3^{-}Lin^{-} ILCs in the skin, sLNs, BM, spleen and mLNs, or CD45^{+}CD3^{-}Lin^{-}IgA^{-}IgM^{-}CD138^{-}CD19^{-} ILCs in the lamina propria of large intestine (Li-LP) and lungs of *Ccr10*$^{+/EGFP}$ mice. Left: gating strategy for skin and sLN ILCs, gated on CD45^{+} cells. Right: expression of CCR10 (EGFP) in ILCs of indicated tissues. Gray areas: EGFP signals in wild-type (WT) ILCs used as negative controls. Representative of >50 mice for skin, 7 for lungs, 4 for intestines, >30 for sLNs, >10 for spleen, 2 for BM and mLNs. (**b**, **c**) Flow cytometric analysis of expression of CD127 and CD90 (b), and CCR6, CD103 and CD69 (c) on EGFP^{+} and EGFP^{-} CD45^{+}CD3^{-}Lin^{-} ILCs of the skin and sLNs of *Ccr10*$^{+/EGFP}$ mice. N=3 each. (**d–f**) Flow cytometric analysis of expression of CD44 and Ki-67 (d), IL-17, IL-5, IFN-γ and IL-10 (e), and MHCII (f) on EGFP^{+} and EGFP^{-} CD45^{+}CD3^{-}Lin^{-} ILCs of the skin and sLNs of *Ccr10*$^{+/EGFP}$ mice (left). Right: The bar graphs compare average percentages of Ki-67^{+} cells (d), IL-17^{+} and IL-5^{+} cells (e), and MHCII^{+} cells (f) in EGFP^{+} and EGFP^{-} ILCs of the skin and sLN. N=3 for the skin and 5 for sLNs in (d), 6–7 for IL-17, 4 for IL-5, 2 each for IFN-γ and IL-10 in (**e**), 5 for the skin and 4 for sLNs in (f). $^{*}P$<0.05. $^{**}P$<0.01, ***P<0.001.

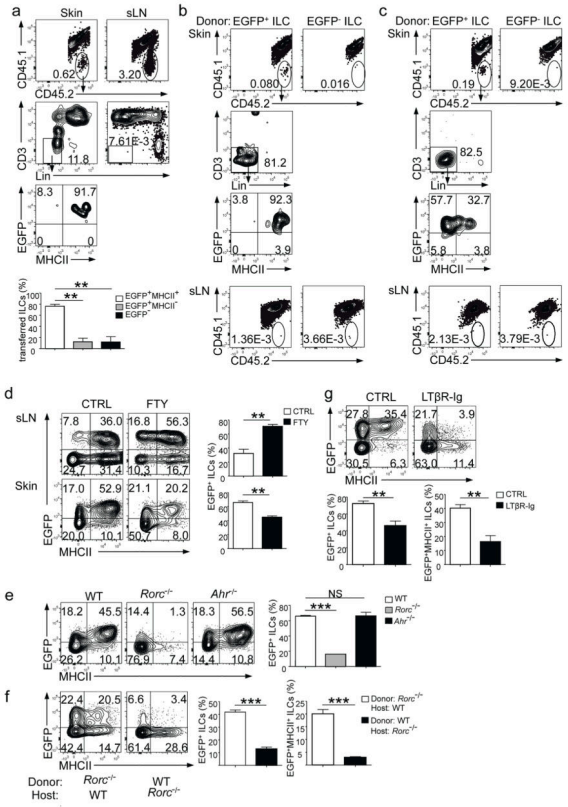

Figure 2.

Homeostatic establishment of CCR10^{+} ILCs in the skin depends on continuous input of CCR10^{+}MHCII^{+} ILCs from sLNs. (**a–c**) Flow cytometric analysis of CD45.2^{+}CD3^{-}Lin^{-} donor ILCs and their expression of CCR10 and MHCII in the skin and sLNs of wild-type (CD45.1^{+}CD45.2^{+}) mice that receive transfer of total sLN cells (a) or sorter-purified EFGP^{+} and EGFP^{-} sLN ILCs (b, c) of *Ccr10*$^{+/EGFP}$ mice. Analyzed 2 days (a, b) or 2 weeks (c) after transfer. Bar graphs show average percentages of EGFP^{+}MHCII^{+}, EGFP^{+}MHCII^{-} and EGFP^{-} cells among total donor ILCs in skin of recipients (a). N=3 in (a), 1 in (b) and 3 in (c). (**d**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on gated CD45.2^{+}CD3^{-}Lin^{-} ILCs of sLNs and skin of *Ccr10*$^{+/EGFP}$ mice that were fed with FTY720 (FTY) for two weeks or untreated (CTRL). Bar graphs show percentages of EGFP^{+} cells among ILCs in sLNs and the skin. N=3. (**e**) Flow cytomentric analysis of expression of CCR10 (EGFP) and MHCII on gated CD45.2^{+}CD3^{-}Lin^{-} skin ILCs of *Ccr10*$^{+/EGFP}$ (WT), *Rorc*$^{-/-}$*Ccr10*$^{+/EGFP}$ and *Ahr*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice. Bar graph shows percentages of EGFP^{+} cells of skin ILCs. N=3. (**f**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on gated CD45.2^{+}CD3^{-}Lin^{-} skin ILCs of *Rorc*$^{-/-}$ BM donor cells in wild-type hosts or of wild-type BM donor cells in *Rorc*$^{-/-}$ hosts. Bar graphs show percentages of EGFP^{+} and EGFP^{+}MHCII^{+} cells of donor cell-derived skin ILCs. N=3. (**g**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on gated CD45.2^{+}CD3^{-}Lin^{-} skin ILCs of *Ccr10*$^{+/EGFP}$ mice that were treated with LTβR-Ig *in utero* (LTβR-Ig) or untreated (CTRL). Bar graphs show percentages of EGFP^{+} and EGFP^{+}MHCII^{+} cells of total skin ILCs. N=6 for

CTRL and 10 for LTβR-Ig-treated mice. $^{**}P<0.01$, $^{***}P<0.001$; NS: no significant difference.

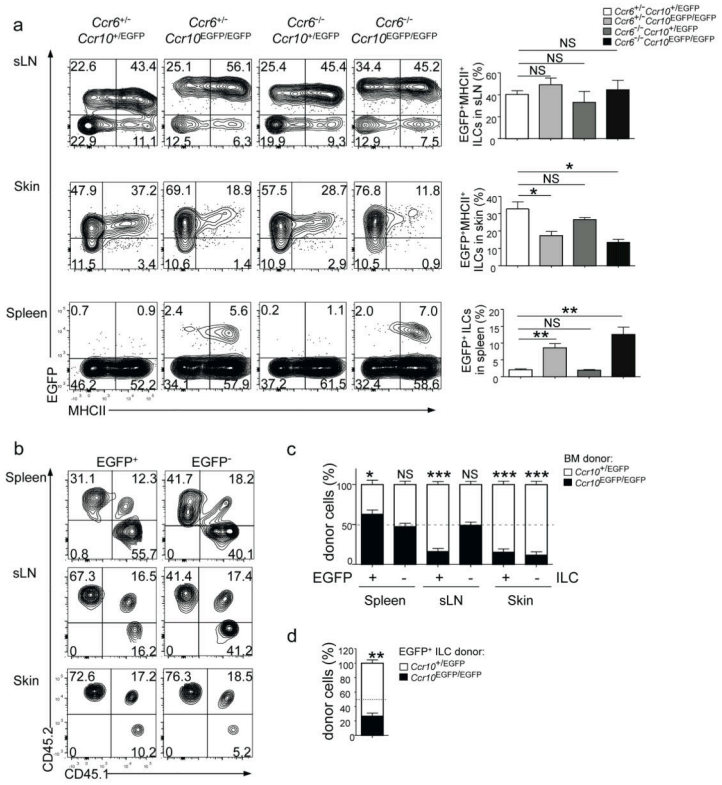

Figure 3.

CCR10 and CCR6 co-regulate specific localization and maintenance of ILCs in the skin. (**a**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on gated CD45.2$^+$CD3$^-$Lin$^-$ ILCs of sLNs (top), skin (middle) and spleen (bottom) of *Ccr6*$^{+/-}$*Ccr10*$^{+/EGFP}$, *Ccr6*$^{+/-}$*Ccr10*$^{EGFP/EGFP}$, *Ccr6*$^{-/-}$*Ccr10*$^{+/EGFP}$, and *Ccr6*$^{-/-}$*Ccr10*$^{EGFP/EGFP}$ mice. Bar graphs show percentages of EGFP$^+$MHCII$^+$ cells among ILCs in sLNs (N=3), skin (N=3), and EGFP$^+$ cells among ILCs in spleens (N=5). (**b**) Flow cytometric analysis of EGFP$^+$ and EGFP$^-$ CD45.2$^+$CD3$^-$ Lin$^-$ ILCs of *Ccr10*$^{+/EGFP}$ (CD45.2$^+$) versus *Ccr10*$^{EGFP/EGFP}$ (CD45.1$^+$) donors in spleens, sLNs and the skin of *Ccr10*$^{+/EGFP}$ (CD45.1$^+$CD45.2$^+$) mice that were irradiated and injected with equal numbers of *Ccr10*$^{+/EGFP}$ and *Ccr10*$^{EGFP/EGFP}$ BM cells. Analyzed 2 months after transfer. (**c**) Relative contribution of *Ccr10*$^{+/EGFP}$ versus *Ccr10*$^{EGFP/EGFP}$ BM donors to total donor-derived ILCs in spleen, sLNs and skin of recipients, based on analyses of the panel (b). Normalized on total spleen cells of *Ccr10*$^{+/EGFP}$ and *Ccr10*$^{EGFP/EGFP}$ donor origins. N=4. (**d**) Relative migration efficiency of donor *Ccr10*$^{+/EGFP}$ versus *Ccr10*$^{EGFP/EGFP}$ EGFP$^+$ sLN ILCs into the skin of same wild-type recipients. Analyzed two days after transfer. N=3. $^*P<0.05$. $^{**}P<0.01$, $^{***}P<0.001$; NS: no significant difference.

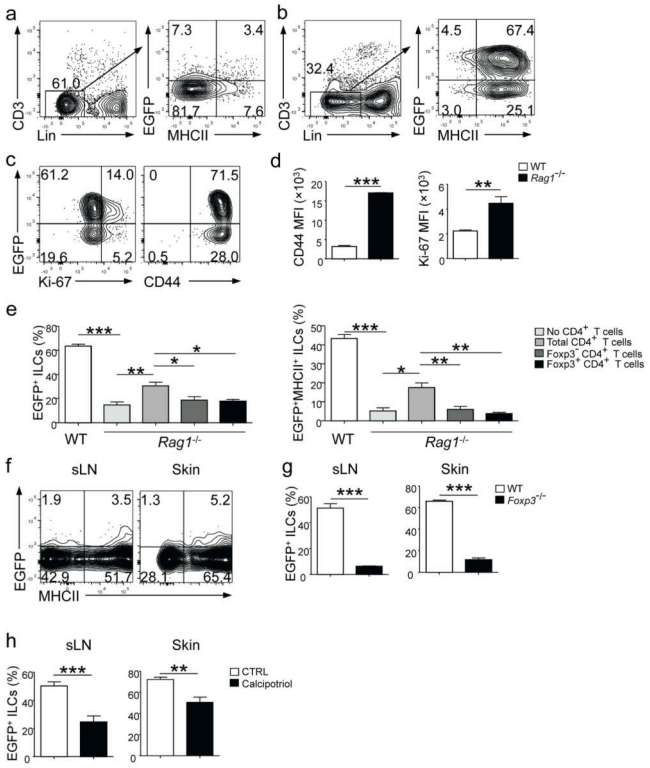

Figure 4.

Immune homeostatic conditions and T cells regulate the programming and maintenance of CCR10$^+$ ILCs in sLNs and the skin. (**a–c**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on CD45.2$^+$CD3$^-$Lin$^-$ skin and sLN ILCs (**a,b**), and expression of Ki-67 and CD44 (**c**) on sLN ILCs of *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice. N=3 each. (**d**) Comparison of mean fluorescent intensity (MFI) of Ki-67 and CD44 expressed on EGFP$^-$ sLN ILCs of *Ccr10*$^{+/EGFP}$ (WT) and *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ (*Rag1*$^{-/-}$) mice. N=3. (**e**) Comparison of percentages of EGFP$^+$ and EGFP$^+$MHCII$^+$ cells in skin ILCs of *Ccr10*$^{+/EGFP}$ (WT) (N=6), *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice (No CD4$^+$ T cells, N=4) and *Rag1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice transferred with total CD4$^+$ cells (Total CD4$^+$ T cells, N=5), T_{reg}-depleted CD4$^+$ cells (Foxp3$^-$ CD4$^+$ T cells, N=5) or T_{reg} cells (Foxp3$^+$ CD4$^+$ T cells, N=3) of spleens of *Ccr10*$^{+/EGFP}$ mice. T cell-transferred mice were analyzed 6 weeks after transfer. (**f**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on CD45.2$^+$CD3$^-$Lin$^-$ ILCs of sLNs and the skin of *Foxp3*$^{-/-}$*Ccr10*$^{+/EGFP}$ (*Foxp3*$^{-/-}$) mice. (**g**) Comparison of percentages of EGFP$^+$ sLN and skin ILCs between *Ccr10*$^{+/EGFP}$ (WT) and *Foxp3*$^{-/-}$*Ccr10*$^{+/EGFP}$ (*Foxp3*$^{-/-}$) mice. N=4 each. (**h**) Comparison of percentages of EGFP$^+$ cells among CD45.2$^+$CD3$^-$Lin$^-$ ILCs of sLNs and the skin of untreated (CTRL, N=6) versus calcipotriol-treated mice (Calcipotriol, N=7). $^*P$<0.05. $^{**}P$<0.01, $^{***}P$<0.001.

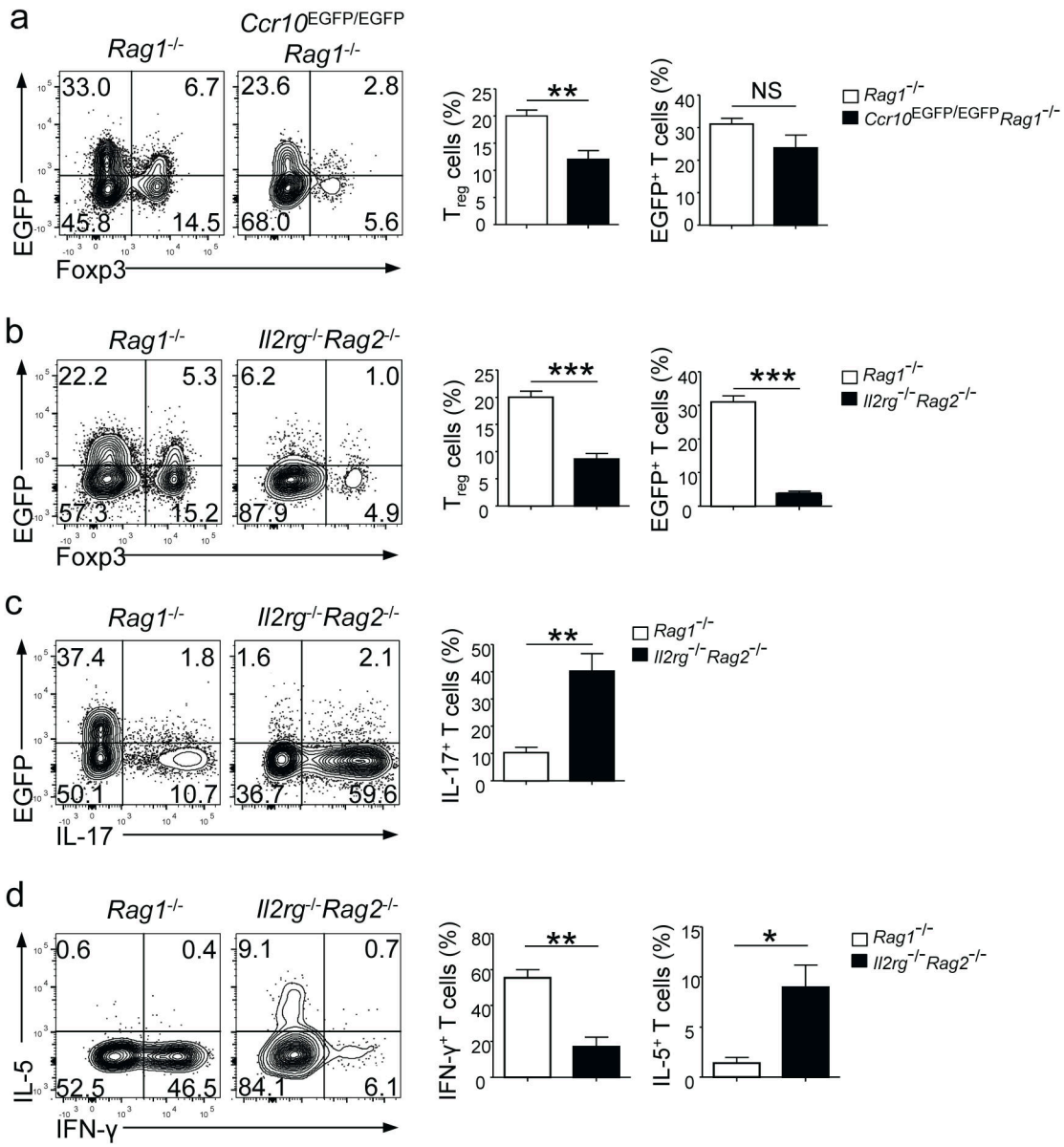

Figure 5.

CCR10$^+$ ILCs regulates T_H cell homeostasis in the skin. (**a**) Flow cytometric analysis of donor CD3$^+$CD4$^+$ T cells for Foxp3$^+$ and EGFP$^+$ subsets in *Rag1*$^{-/-}$ and *Ccr10*$^{EGFP/EGFP}$*Rag1*$^{-/-}$ mice that were transferred with *Ccr10*$^{+/EGFP}$ splenic CD3$^+$CD4$^+$EGFP$^-$ T cells. Analyzed 6 weeks after transfer. Bar graphs show percentages of Foxp3$^+$ and EGFP$^+$ subpopulations among donor CD4$^+$ T cells in the skin of *Rag1*$^{-/-}$ (N=8) and *Ccr10*$^{EGFP/EGFP}$*Rag1*$^{-/-}$ (N=9) recipients. (**b–d**) Flow cytometric analysis of gated CD3$^+$CD4$^+$ donor T cells for Foxp3$^+$ and EGFP$^+$ (b), IL-17-producing (c) and IL-5 and IFN-γ-producing (d) subsets in *Rag1*$^{-/-}$ and *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ mice that were transferred with *Ccr10*$^{+/EGFP}$ splenic CD3$^+$CD4$^+$EGFP$^-$ T cells. Analyzed 6 weeks after transfer. Bar graphs show percentages of Foxp3$^+$ and EGFP$^+$ (b), IL-17$^+$ (c), and IL-5$^+$ and IFN-γ$^+$ (d) subsets of

total donor CD4^{+} T cells in the skin of *Rag1*$^{-/-}$ and *Il2rg*$^{-/-}$*Rag2*$^{-/-}$ recipients. N=5 for b and c, and 3 for d. $^{*}P<0.05$. $^{**}P<0.01$, $^{***}P<0.001$, NS: no significant difference.

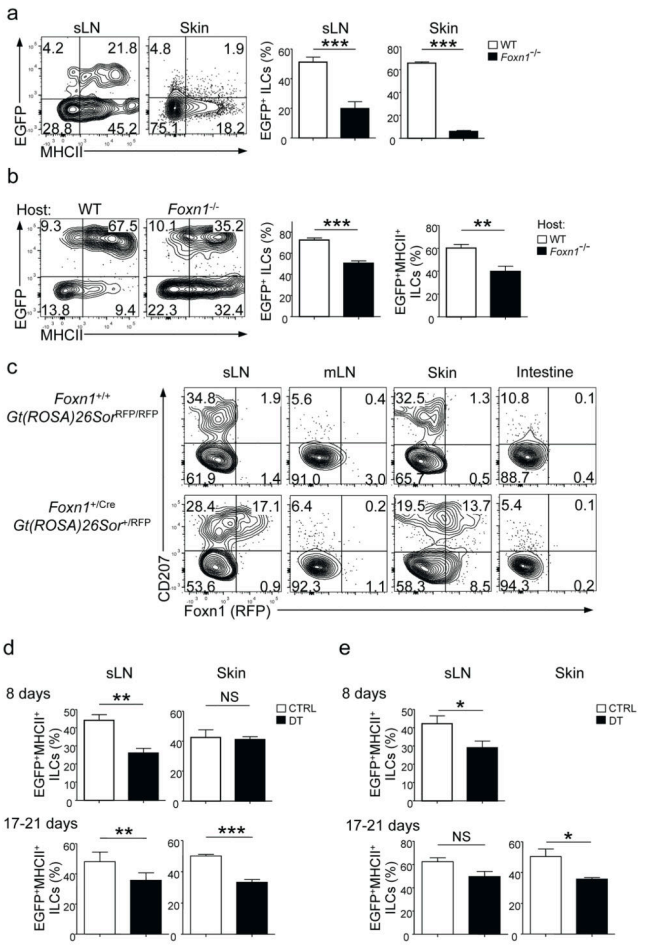

Figure 6.

Transcription factor Foxn1 and CD207$^+$ dendritic cells are involved in programming CCR10$^+$ ILCs in sLNs. (**a**) Flow cytometric analysis of expression of CCR10 (EGFP) and MHCII on CD45.2$^+$CD3$^-$Lin$^-$ ILCs of sLNs and the skin of *Foxn1*$^{-/-}$*Ccr10*$^{+/EGFP}$ mice. Bar graphs show percentages of EGFP$^+$ subpopulations of total ILCs in the sLNs and skin of *Foxn1*$^{-/-}$*Ccr10*$^{+/EGFP}$ (*Foxn1*$^{-/-}$) mice compared to *Ccr10*$^{+/EGFP}$ (WT) controls. N=4. (**b**) Flow cytometric analysis of EGFP$^+$ and EGFP$^-$ CD45.1$^+$CD45.2$^+$CD3$^-$Lin$^-$ ILCs of *Ccr10*$^{+/EGFP}$ donor BM cells in sLNs of *Foxn1*$^{-/-}$ and wild-type mice (CD45.2$^+$) that were irradiated and transferred with total BM cells of *Ccr10*$^{+/EGFP}$ mice (CD45.1$^+$CD45.2$^+$). Analyzed eight weeks after transfer. Bar graphs show percentages of EGFP$^+$ and EGFP$^+$MHCII$^+$ subpopulations of donor ILCs in the sLNs of recipients. N=5 for WT and 4 for *Foxn1*$^{-/-}$ mice. (**c**) Flow cytometric analysis of Foxn1 (RFP) expression in CD207$^+$ and CD207$^-$ CD45$^+$CD3$^-$CD11c$^+$ DCs of the sLNs, mLNs, skin and intestine of *Foxn1*$^{+/Cre}$*Gt(ROSA)26Sor*$^{+/RFP}$ mice. Cells of *Foxn1*$^{+/+}$*Gt(ROSA)26Sor*$^{RFP/RFP}$ mice were used as negative control for RFP signals. Representative of two independent experiments. (**d,e**) Comparison of percentages of EGFP$^+$MHCII$^+$ subsets of total CD45.2$^+$CD3$^-$Lin$^-$ ILCs in sLNs and the skin of MuLanDTR*Ccr10*$^{+/EGFP}$ (d) or HuLanDTR*Ccr10*$^{+/EGFP}$ (e) mice treated with diphtheria toxin (DT) for indicated periods of time. CTRL: untreated controls. N=5 for CTRL and 7 for DT for the 8-day sLNs, 5 each for the 8-day skin, 3 for

CTRL and 6 for DT for the 17–21-day sLNs and skin samples of MuLanDTR*Ccr10*$^{+/EGFP}$ mice (d). N=7 for CTRL and 6 for DT for the 8-day sLNs, 4 each for the 17–21-day sLNs and skin samples of HuLanDTR*Ccr10*$^{+/EGFP}$ mice (e). $^{*}P<0.05$. $^{**}P<0.01$, $^{***}P<0.001$, NS: no significant difference.