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Differential developmental requirement and peripheral regulation for dermal V γ 4 and V γ 6T17 cells in health and inflammation

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Dermal IL-17-producing $\gamma\delta$ T cells have a critical role in skin inflammation. However, their development and peripheral regulation have not been fully elucidated. Here we demonstrate that dermal $\gamma\delta$ T cells develop from the embryonic thymus and undergo homeostatic proliferation after birth with diversified TCR repertoire. V γ 6T cells are *bona fide* resident, but precursors of dermal V γ 4T cells may require extrathymic environment for imprinting skin-homing properties. Thymic V γ 6T cells are more competitive than V γ 4 for dermal $\gamma\delta$ T cell reconstitution and TCR $\delta^{-/-}$ mice reconstituted with V γ 6 develop psoriasis-like inflammation after IMQ-application. Although both IL-23 and IL-1 β promote V γ 4 and V γ 6 proliferation, V γ 4 are the main source of IL-17 production that requires IL-1 signalling. Mice with deficiency of IL-1RI signalling have significantly decreased skin inflammation. These studies reveal a differential developmental requirement and peripheral regulation for dermal V γ 6 and V γ 4 $\gamma\delta$ T cells, implying a new mechanism that may be involved in skin inflammation.

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The skin has a unique composition of immune cells. In addition to adaptive $\alpha\beta$ T cells, many innate immune cells including dermal dendritic cells and $\gamma\delta$ T cells reside in the skin to establish a skin immune network that has a critical role in host defence and tissue repair¹. In mice, V γ 5V δ 1T cells, named dendritic epidermal T cells (DETCs), uniquely reside in the epidermis during fetal development². These cells have been shown to recognize a putative antigen (Ag) expressed on the keratinocytes (KCs) and are involved in the skin immunosurveillance³. Recently, a new subset of $\gamma\delta$ T cells has been identified in the skin^{4–6}. In comparison with DETCs, this subset of $\gamma\delta$ T cells resides mainly in the dermis under the steady condition. They bear different V γ usage and are the major interleukin (IL)-17 producers in the skin upon IL-23 or toll-like receptor-7/8 agonist imiquimod (IMQ) stimulation^{4,7,8}. However, their development, trafficking and peripheral regulation are not fully understood.

Previous studies have shown that DETCs are derived from early fetal thymic precursor cells⁹. DETCs are home to the skin between embryonic days 16 and 18 before birth. In addition, IL-17-producing $\gamma\delta$ T ($\gamma\delta$ T17) cells in the periphery such as lymph nodes (LN) also develop in the thymus after birth through a transforming growth factor- β -dependent mechanism¹⁰. It appears that different subsets of $\gamma\delta$ T17 cells migrate from the thymus into the periphery in a functional wave manner¹¹. At the molecular level, a thymic epithelial cell determinant, Skint-1, has a critical role in the development of interferon (IFN)- γ -producing versus IL-17-producing $\gamma\delta$ T cells¹². Transcriptional factor Sox13 is essential for all IL-17-committed V γ 4 T-cell development and function including dermal V γ 4 T cells^{13,14}. Previous studies have also identified that scavenger receptor SCART2 is uniquely expressed in IL-17-producing $\gamma\delta$ T cells homing to the peripheral LN and dermis¹⁵. Furthermore, studies have shown that $\gamma\delta$ T cells can traffick between LN and skin^{13,16}, posing the question whether dermal $\gamma\delta$ T17 cells develop similarly as other peripheral $\gamma\delta$ T cells. Through bone marrow (BM) chimeras where BM cells were transplanted into lethally irradiated host mice, it showed that 90% of dermal $\gamma\delta$ T cells were from host origin, whereas ~10% of dermal $\gamma\delta$ T cells were from donor BM⁶, suggesting that BM cells may contain precursor cells that give rise to dermal $\gamma\delta$ T cells. Although early studies from Gray *et al.*⁵ suggested that dermal $\gamma\delta$ T cells could not be reconstituted by BM cells, their later studies showed that IL-17-producing V γ 4 T cells could be reconstituted by BM¹³. However, a recent study demonstrated that IL-17-producing $\gamma\delta$ T cells develop before birth and maintain in adult mice as self-renewing cells¹¹, thus leaving the role of BM in the generation of dermal $\gamma\delta$ T cells uncertain. Furthermore, the detailed information for mature dermal $\gamma\delta$ T cell migration into skin is lacking. Previous studies have shown that embryonic trafficking of DETCs to skin requires E/P selectin ligands and CCR4 (ref. 17). CCR10 also has a critical role in the migration and location of DETCs^{18,19}. When and where dermal $\gamma\delta$ T cells develop and migrate into the skin are poorly understood.

Here we demonstrate that dermal $\gamma\delta$ T cells developed from fetal thymus and undergo homeostatic proliferation after birth, with diversified TCR repertoire. IL-17-producing V γ 6 T cells are *bona fide* resident in dermis and are reconstituted from fetal thymus, whereas thymic V γ 4 T cells may require extrathymic environment for imprinting of their skin-homing properties. Chemokine receptor CCR6 is critical for dermal V γ 4 but not for V γ 6 T-cell migration. It appears that thymic V γ 6 T cells are more competitive than V γ 4 for dermal $\gamma\delta$ T cell reconstitution. In addition, V γ 6 T cells are pathogenic and can induce skin inflammation, whereas V γ 4 T cells are preferentially expanded and are the major IL-17 producers in the IMQ model of psoriasis-like skin inflammation. Although IL-23 and IL-1 β are capable of

driving dermal V γ 4 and V γ 6 T-cell proliferation, IL-17 is mainly produced by V γ 4, for which IL-1 signalling is essential. Deficiency of IL-1 receptor (IL-1R) signalling pathway significantly decreases both IL-23 and IMQ-induced skin inflammation. These results demonstrate the importance of IL-1 β in the regulation of the proliferation and IL-17 production by different subsets of dermal $\gamma\delta$ T cells when interplaying with IL-23, implying a new mechanism that may be involved in skin inflammation.

Results

Dermal $\gamma\delta$ T cell development in mice before and after birth. To investigate the development of dermal $\gamma\delta$ T cells, skin tissues from embryonic or neonatal pups were used to track dermal $\gamma\delta$ T cell appearance. $\gamma\delta$ TCR^{hi} epidermal $\gamma\delta$ T cells were readily seen on E20 and day 1 (Supplementary Fig. 1a and Fig. 1a), consistent with a previous report⁹. In contrast, $\gamma\delta$ TCR^{int} dermal $\gamma\delta$ T cells were scarce on E20 or day 1. Dermal $\gamma\delta$ T cells became obvious on day 3 and had similar frequency as adult mice on day 14 (Fig. 1a). Next we examined the TCR repertoire of developing dermal $\gamma\delta$ T cells. TCR repertoire of dermal $\gamma\delta$ T cells on days 3–5 showed almost exclusive V γ 6 (Fig. 1b). Dermal V γ 4 became apparent on day 7. In adult mice, dermal $\gamma\delta$ T cells expressed TCRs mainly containing V γ 6 and V γ 4 (Fig. 1b). In contrast, TCR repertoire of thymic $\gamma\delta$ T cells on days 1–3 showed that V γ 1, V γ 4 and V γ 6 while V γ 6 was gradually decreased over the time (Fig. 1b). Side-by-side comparison studies indicated that V γ 1 and V γ 4 were the predominant $\gamma\delta$ T cells in the BM. Ki-67 staining suggested that both dermal V γ 6 and V γ 4 T cells had a significant more homeostatic proliferation at early days and then maintained lower proliferation rate in adult mice (Fig. 1c). In addition, dermal $\gamma\delta$ T cells were capable of producing IL-17 (Fig. 1d). We also examined $\gamma\delta$ T cells in the lung. Lung $\gamma\delta$ T cells were apparent on E20 and contained both V γ 6 and V γ 4. In contrast to dermal $\gamma\delta$ T cells, V γ 6 T cells were more than V γ 4 in the lung even after birth and in adult mice (Supplementary Fig. 1b). Taken together, these results suggest that dermal $\gamma\delta$ T cells migrate into the skin before birth and are further expanded after birth with more diversified TCR repertoire.

Fetal thymus is required for dermal $\gamma\delta$ T cell development. We next examined if development of dermal $\gamma\delta$ T cells was confined to the fetal thymus. To this end, we transplanted BM cells or BM cells plus neonatal thymocytes from wild-type (WT) C57Bl/6 mice (CD45.2) into CD45.1 congenic SJL mice. Mice transplanted with BM cells alone had significantly lower dermal $\gamma\delta$ T cells as compared with mice transplanted with BM plus neonatal thymocytes (Fig. 2a). In mice transplanted with BM alone, the majority of CCR6⁺ dermal $\gamma\delta$ T cells were host resident, whereas ~15% were donor origin. Dermal $\gamma\delta$ T cells from both sources were capable of secreting IL-17 (Fig. 2b). In contrast, mice were transplanted with BM plus neonatal thymocytes, almost all dermal $\gamma\delta$ T cells were of donor origin (Fig. 2b). To further confirm that BM cells contain precursors of dermal $\gamma\delta$ T cells, TCR δ -deficient mice were used as host to receive BM cells from SJL mice. Indeed, dermal $\gamma\delta$ T cells could be reconstituted from donor BM and were able to secrete IL-17 (Fig. 2c). To determine whether the thymus is required for dermal $\gamma\delta$ T cell development, we examined dermal $\gamma\delta$ T cells in athymic nude mice. As shown in Supplementary Fig. 2, dermal $\gamma\delta$ T cells were lacking in nude mouse skin. In addition, $\gamma\delta$ T cells in BM were also scarce. We then transplanted BM cells from CD45.1 SJL mice into CD45.2 nude or WT mice. Consistent with Fig. 2a, dermal $\gamma\delta$ T cells were reconstituted in both WT and nude mice, and these cells were able to secrete IL-17 (Fig. 2d). Dermal $\gamma\delta$ T cells were significantly expanded in nude mice, compared with those in WT recipients

(Fig. 2d). Further TCR usage analysis indicated that V γ 4 and V γ 1 were predominant in WT mice, whereas nude mice reconstituted with WT BM had exclusively V γ 4. No V γ 6 was observed (Fig. 2d).

Since dermal $\gamma\delta$ T cells consist both V γ 4 and V γ 6 TCR repertoire in adult mice, we reasoned whether dermal V γ 6 T cells were from fetal thymus. To exclude precursors of dermal $\gamma\delta$ T cells from BM, BM cells from TCR δ -deficient mice were mixed with neonatal thymocytes (Fig. 2e). Dermal $\gamma\delta$ T cells were almost all donor origin and predominately expressed V γ 6. Since previous study showed that IL-17-producing $\gamma\delta$ T cells develop from embryonic thymus¹¹, we further used E18 embryonic thymocytes for reconstitution. Similar as neonatal thymocytes, dermal $\gamma\delta$ T cells from E18 thymocytes showed exclusively donor origin and were capable of producing IL-17 and predominantly expressed V γ 6 (Fig. 2f). These data suggest that IL-17-producing dermal $\gamma\delta$ T cells can be reconstituted from both fetal thymus and adult BM with different TCR repertoire, and thymus is absolutely required for dermal $\gamma\delta$ T cell development.

Thymic V γ 4 requires extrathymic environment for skin homing. E18 and neonatal thymocytes contain both V γ 4- and V γ 6-expressing $\gamma\delta$ T cells with similar frequency (Fig. 1b). However, mice transplanted with E18 or fetal thymocytes had exclusive V γ 6 T-cell reconstitution, whereas majority of V γ 4 T cells were reconstituted from BM (Fig. 2). To further validate this, we sorted V γ 4 or V γ 6 T cells from neonatal thymocytes (CD45.2) and mixed with BM cells (CD45.1) as a source of BM V γ 4. TCR δ -deficient mice were used as recipient mice. The ratio of V γ 4 from both sources was approximately at 1:1 as well as V γ 6 to V γ 4 (Fig. 3a). As shown in Fig. 3b, mice transplanted with V γ 4 from neonatal thymus plus BM cells had dermal $\gamma\delta$ T cells expressing V γ 4, predominately from BM (>80%). In contrast, mice transplanted with neonatal V γ 6 plus BM cells had dermal $\gamma\delta$ T cells expressing V γ 6, exclusively from neonatal thymus (Fig. 3b). Both V γ 4 and V γ 6 T cells were capable of secreting IL-17 (Fig. 3b). These data suggest that V γ 4 and V γ 6 dermal $\gamma\delta$ T cells may have a differential developmental requirement.

To gain insight into this difference, we examined maturation status of V γ 4 and V γ 6 T cells in thymus and BM. As shown in

Figure 1 | Development of dermal $\gamma\delta$ T cells. (a) Whole-skin cell suspensions prepared from C57Bl/6 WT mice at different days ($n = 3-5$) after birth were stained with CD3 and $\gamma\delta$ TCR, and the frequency of $\gamma\delta$ TCR^{int} dermal $\gamma\delta$ T cells was analysed by flow cytometry. Flow plots gated on CD3⁺ cells are representative of three independent experiments with similar results. (b) Thymocytes (Thy), whole-skin cells and BM cells from different days after birth were stained with CD3, $\gamma\delta$ TCR and V γ (V γ 1, 4, 6) mAbs. Percentages of V γ 1, V γ 4 and V γ 6 $\gamma\delta$ T cells were analysed by flow cytometry and summarized in pie chart. (c) Percentages of Ki-67 expression on dermal V γ 4 and V γ 6 T cells were analysed by flow cytometry. Cells were gated on CD3^{int} $\gamma\delta$ TCR^{int} V γ 4⁺ or V γ 6⁺ cells. Data are shown as mean $\pm$ s.e.m. (d) Whole-skin cells were stimulated with phorbol myristate acetate (PMA) plus ionomycin for 5 h, and the percentage of IL-17-producing $\gamma\delta$ T cells gated on CD3^{int} $\gamma\delta$ TCR^{int} cells was analysed by flow cytometry. Data are shown as mean $\pm$ s.e.m.

Fig. 3c, almost all V γ 6 T cells in neonatal thymus had mature phenotype (CD24^{lo}). In contrast, most V γ 4 T cells were immature (CD24^{hi}) in neonatal or adult thymus. This was consistent with CD44 CD62L staining patterns where the majority of V γ 6 T cells in E20 or neonatal stage were CD44^{hi}CD62L^{lo} while most V γ 4 T cells were CD44^{lo} (Supplementary Fig. 3a). Strikingly, V γ 4 T cells from BM were almost all mature (CD24^{lo}) (Fig. 3c), suggesting that V γ 4 T cells may require extrathymic environment for maturation. To rule out the possibility that V γ 4 T cells may have delayed maturation in the thymus as previously reported¹³, we used thymocytes from different ages of mice ranging from days 2 to 17 for dermal $\gamma\delta$ T reconstitution studies. Consistent with previous results (Fig. 1b), V γ 6 gradually decreased in the thymus after birth and were scarce on day 17 (Supplementary Fig. 4a). Mature V γ 4 T cells were indeed increased over time and peaked at day 5 and then decreased, which is consistent with a previous study¹³. The ratios of matured V γ 4 versus V γ 6 were 1:5 at day 2, 1:1 at day 4, 4:1 at day 5. On day 17, almost no V γ 6 was found in the thymus (Supplementary Fig. 4a). After 8 weeks reconstitution, we examined $\gamma\delta$ T cell composition. Dermal $\gamma\delta$ T cells were fully reconstituted with thymocytes of days 2–5 mice (Supplementary Fig. 4b). V γ 6 T cells were predominant $\gamma\delta$ T cells in the skin although dermal V γ 4 T cells did increase using thymocytes of day 5 mice. However, this was not comparable with the ratio of matured V γ 4 versus V γ 6 in the thymus of day 5 mice (4:1). Both V γ 4 and V γ 6 were capable of producing IL-17 (Supplementary Fig. 4c). The unbalanced ratios of V γ 6 over V γ 4 were also shown in the lung but less striking in the peripheral blood, LN and spleen. V γ 4 and V γ 6 were almost at 1:1 ratio in the peripheral blood and LN using thymocytes from day 5 mice (Supplementary Fig. 4d). Dermal $\gamma\delta$ T cells were poorly reconstituted using thymocytes of day 17 mice, which predominantly constituted V γ 4. Taken together, these data suggest that thymic V γ 4 T cells may require an extrathymic environment to imprint their skin-homing properties, and that thymic V γ 6 T cells may be more competitive than V γ 4 for dermal $\gamma\delta$ T cell reconstitution.

We also examined CCR6 expression on V γ 4 T cells since dermal $\gamma\delta$ T cells constitutively express CCR6 (ref. 4). V γ 4 T cells from neonatal thymus had almost no CCR6 expression, whereas V γ 6 T cells expressed an appreciable level of CCR6 (Fig. 3d and Supplementary Fig. 3b). In addition, 15% of V γ 4 T cells from BM expressed CCR6 (Fig. 3d), suggesting that BM V γ 4 T cells may have gained skin-homing receptor expression. Along this line, dermal V γ 4 T cells were significantly lower in CCR6-deficient mice but not in CCR10-deficient mice compared with those from WT mice (Fig. 3e and Supplementary Fig. 3c), although total dermal $\gamma\delta$ T cell frequency was not altered. In addition, the

percentage of V γ 4 T cells in BM, LN or spleen was not significantly changed between WT and CCR6KO mice (Fig. 3f and Supplementary Fig. 3d), suggesting that CCR6 is not essential for V γ 4 T cell trafficking from fetal thymus to BM, LN or spleen but is critical for their homing to skin.

Mice with predominate V γ 6 T cells develop skin inflammation.

We have previously shown that dermal $\gamma\delta$ T cells are critical in skin inflammation such as psoriasis⁴. Mice lacking V γ 4 IL-17T cells had significantly less epidermal thickening and neutrophil infiltration¹⁵. To determine whether V γ 6 T cells also have a role in skin inflammation, we used mice reconstituted with neonatal thymocytes plus BM cells, which predominantly express V γ 6 T cells, for IMQ topical treatment. Mice reconstituted with BM alone containing mainly V γ 4 were used as control. Consistent with the previous experiment (Fig. 2a), mice reconstituted with neonatal thymocytes plus BM cells had threefold more dermal $\gamma\delta$ T cells than mice reconstituted with BM alone (Fig. 4a). Despite lower frequency of dermal $\gamma\delta$ T cells in BM alone reconstituted mice, histological analysis showed similar levels of epidermal thickening (acanthosis) in both groups (Fig. 4b). Daily application of IMQ to the back skin significantly increased neutrophil infiltration and mRNA levels of IL-17 and IL-22 in both groups (Fig. 4c). In addition, the frequencies of IL-17-producing dermal $\gamma\delta$ T cells were also similar (Fig. 4d). To specifically demonstrate whether V γ 6 T cells are able to induce skin inflammation, we sorted V γ 6 from neonatal thymocytes and co-transferred with BM cells from TCR $\delta^{-/-}$ mice into TCR $\delta^{-/-}$ mice to eliminate any resident dermal $\gamma\delta$ T cells or $\gamma\delta$ T cells from BM cells (Supplementary Fig. 5a). The reconstituted mice expressed exclusive V γ 6 and developed severe skin inflammation upon IMQ topical treatment. The epidermal thickness and neutrophil infiltration were significantly increased as compared with TCR $\delta^{-/-}$ mice (Supplementary Fig. 5b,c). In addition, mRNA levels of IL-17 and IL-22 were also significantly increased (Supplementary Fig. 5d). Taken together, these findings suggest that dermal V γ 6 T cells are pathogenic and are able to induce psoriasis-like dermatitis. However, V γ 4 T cells may be more prone to induce skin inflammation because reconstituted mice expressing V γ 4 develop similar magnitude of disease with significant fewer total dermal $\gamma\delta$ T cells.

Normal naive mice have similar frequencies of dermal V γ 6 and V γ 4 T cells. We sought to determine whether dermal V γ 4 and V γ 6 T cells respond to IMQ differently in naive mice. As expected, mice applied with IMQ cream developed massive neutrophil infiltration (Fig. 4e). Total dermal $\gamma\delta$ T cells were also increased. Among them, dermal V γ 4 T cells were preferentially

Figure 2 | Fetal thymus is required for dermal $\gamma\delta$ T cell development and V γ 6 T cell reconstitution while dermal V γ 4 T cells originate mainly from BM.

BM cells or BM cells plus neonatal thymocytes (BM + neo Thy) from C57Bl/6 WT mice (CD45.2) were transplanted into lethally irradiated SJL mice (CD45.1, $n = 4-5$). (a) Eight weeks after reconstitution, percentage of dermal $\gamma\delta$ T cells gated on CD3⁺ cells from recipient mice was analysed by flow cytometry, and total frequency of dermal $\gamma\delta$ T cells was summarized. Data are shown as mean $\pm$ s.e.m. * $P < 0.05$ (unpaired Student's *t*-test). (b) Whole-skin cells were stimulated with PMA plus ionomycin for 5 h. CCR6⁺ and IL-17⁺CD3^{int} $\gamma\delta$ TCR^{int} cells were analysed by flow cytometry. Flow plots were gated on CD3^{int} $\gamma\delta$ TCR^{int} cells. Percentages of dermal $\gamma\delta$ T cells from donor are shown as mean $\pm$ s.e.m. * $P < 0.05$ (unpaired Student's *t*-test). Degree of chimerism of CCR6⁺ and IL-17-producing dermal $\gamma\delta$ T cells are also shown as open bars (donor) and filled bars (host). Data are representative of at least three independent experiments with similar results. (c) BM cells from SJL mice (CD45.1) were transplanted into lethally irradiated TCR δ KO mice (CD45.2). After 8 weeks of reconstitution, CCR6⁺ and IL-17-producing dermal $\gamma\delta$ T cells were analysed by flow cytometry. Flow plots gated on CD3⁺ $\gamma\delta$ TCR⁺ cells are representative of two independent experiments with similar results. (d) BM cells from SJL mice (CD45.1) were transplanted into lethally irradiated C57Bl/6 WT mice or nude mice (CD45.2). After 8 weeks of reconstitution, percentage of dermal $\gamma\delta$ T cells gated on CD3⁺ cells was detected by flow cytometry. Furthermore, CCR6 expression and V γ usage (V γ 1, 4, 6) of dermal $\gamma\delta$ T cells and intracellular IL-17 by dermal $\gamma\delta$ T cells after PMA plus ionomycin stimulation were also analysed by flow cytometry. Flow plots were gated on CD3^{int} $\gamma\delta$ TCR^{int} cells. (e,f) Thymocytes from C57Bl/6 WT neonatal (e) or E18 (f) pups plus BM cells from TCR δ KO mice were transplanted into lethally irradiated SJL mice. After 8 weeks of reconstitution, CCR6 expression and V γ usage (V γ 1, 4, 6) of dermal $\gamma\delta$ T cells and intracellular IL-17 after PMA plus ionomycin stimulation were analysed by flow cytometry. Flow plots were gated on CD3^{int} $\gamma\delta$ TCR^{int} cells.

expanded as compared with Vγ6 (Fig. 4e). Ki-67 staining indicated that Vγ4 had significantly more proliferation than Vγ6 (Fig. 4f). To assess IL-17 production by dermal γδT cells *ex vivo*, skin tissues were digested and stained intracellularly for IL-17. Both Vγ4 and Vγ6 dermal T cells produced IL-17. However, Vγ4 T cells produced significantly more IL-17 than Vγ6 T cells (Fig. 4g). Thus, dermal Vγ4 T cells appear to have a more critical role compared with Vγ6 T cells in IMQ-induced psoriasis-like dermatitis.

IL-23 and IL-1β differently regulate dermal Vγ4 and Vγ6. We reason that the differential responses to IMQ by dermal Vγ4 and Vγ6 T cells could be because of cytokine regulations, as the IMQ-induced psoriasis-like dermatitis model is dependent of the IL-23/IL-17 pathway²⁰. To determine the role of IL-23 and IL-1β in the regulation of dermal γδT cells, whole-skin cells were labelled with carboxyfluorescein succinimidyl ester (CFSE) and stimulated with IL-23, IL-1β or IL-23 plus IL-1β. IL-23 alone or IL-1β alone was capable of driving dermal γδT cell proliferation (Fig. 5a).

Figure 3 | Thymic V γ 4 T cells require extrathymic environment for imprinting of skin-homing properties. (a,b) Sorted V γ 4 or V γ 6 neonatal thymocytes (Thy) from C57Bl/6 WT mice (CD45.2) mixed with BM cells from SJL mice (CD45.1) were transferred into lethally irradiated TCR δ KO mice ($n = 3-5$). The ratio of V γ 4 from both sources was around 1:1 as well as V γ 6 to V γ 4 (based on V γ 4 frequency in BM). After 8 weeks of reconstitution, total dermal $\gamma\delta$ T cells (a) and the sources of V γ 4 or V γ 6 T cells as well as intracellular IL-17 stimulated with PMA plus ionomycin (b) were determined by flow cytometry. Flow plots were gated on CD3⁺ $\gamma\delta$ TCR⁺ cells. (c,d) CD24 (c) and CCR6 (d) expression by V γ 4 or V γ 6 cells on neonatal Thy, adult Thy, BM cells (Day 16) and adult BM cells were determined by flow cytometry. Flow plot gated on CD3⁺ $\gamma\delta$ TCR⁺ cells are representative of three independent experiments with similar results. (e,f) Whole-skin cells (e) or BM cells (f) from C57Bl/6 WT mice or CCR6KO mice ($n = 3$) were stained with CD3, $\gamma\delta$ TCR, V γ 4 and V γ 6. Percentages of total dermal $\gamma\delta$ T cells as well as dermal V γ 4 and V γ 6 cells or BM V γ 4 cells were analysed by flow cytometry. Data are shown as mean $\pm$ s.e.m. and are representative of three independent experiments with similar results. * $P < 0.05$ (unpaired Student's t -test).

Combination of IL-23 and IL-1 β did not significantly increase dermal $\gamma\delta$ T cell proliferation. In contrast, IL-17 production was largely induced upon IL-23 stimulation by both proliferated and unproliferated dermal $\gamma\delta$ T cells. IL-1 β alone induced minimal IL-17 production. Combined IL-1 β with IL-23 significantly enhanced IL-17 production. In addition, we found that either IL-23 or IL-1 β was able to stimulate V γ 4 and V γ 6 proliferation. Strikingly, dermal V γ 4 T cells produced significantly more IL-17 than V γ 6 T cells upon IL-23 or IL-23 plus IL-1 β stimulation (Fig. 5b).

Whole-skin cells contain endogenous IL-1 β presumably produced by skin resident cells^{21,22}. We next determined whether the proliferation of dermal $\gamma\delta$ T cells induced by IL-23 is dependent on IL-1 β and conversely whether IL-1 β -induced $\gamma\delta$ T cell proliferation is dependent on IL-23. Using neutralizing monoclonal antibodies (mAbs) against IL-23 and IL-1 β and corresponding isotype control mAbs, we found that the proliferation and IL-17 production by dermal $\gamma\delta$ T cells in response to IL-23 stimulation were significantly decreased when blocking endogenous IL-1 β (Fig. 5c). In contrast, IL-1 β -induced dermal $\gamma\delta$ T cell proliferation and IL-17 production were not significantly influenced by IL-23 (Fig. 5c). Thus, IL-1 β appears to be critical in both dermal $\gamma\delta$ T cell proliferation and IL-17 production induced by IL-23.

We next used IL-1RI knockout (KO) mice to examine the regulatory role of IL-1 β in dermal $\gamma\delta$ T cells. There was a significant lower basal level of proliferated dermal $\gamma\delta$ T cells in IL-1RI KO mice compared with WT mice (Fig. 5d). Dermal $\gamma\delta$ T cell proliferation was still observed upon IL-23 stimulation in IL-1RI KO mice, although the overall level was significantly lower than WT mice (Fig. 5d). IL-17 production was completely abrogated in IL-1RI KO mice. Further dermal $\gamma\delta$ T cell subset studies indicated that dermal V γ 6 but not V γ 4 T cells had IL-23-induced proliferation independent of IL-1 β signalling (Fig. 5e). However, IL-17 production by dermal V γ 4 and V γ 6 both required IL-1R signalling. Thus, IL-23-induced dermal V γ 6 T-cell proliferation has both IL-1 β -dependent and -independent pathways. However, IL-1 β signalling is essential for IL-23-induced dermal $\gamma\delta$ T cell IL-17 production.

IL-1 β stimulates KCs for chemokine production. IL-1 β has been identified as a key inflammatory cytokine in the pathogenesis of cutaneous inflammation including psoriasis²³. Elevated IL-1 β levels were reported in psoriatic skin lesions²⁴. To further delineate the role of IL-1 β on KC activation, we treated primary murine KC cells with IL-1 β , and found that mRNA levels of chemokines including CCL2, CCL5, CCL20, CXCL9 and CXCL10 were increased (Fig. 6a), suggesting that IL-1 β may modulate dermal $\gamma\delta$ T cell trafficking. CCL20-CCR6 axis has been implicated in dermal $\gamma\delta$ T cell trafficking and psoriasis pathogenesis^{25,26}. To investigate whether $\gamma\delta$ T cells could migrate from peripheral lymphoid organs into dermis in addition to already established resident $\gamma\delta$ T cells, we sorted $\gamma\delta$ T cells from spleen and LN and then adoptively transferred them into SJL mice. As depicted in Fig. 6b, $\gamma\delta$ T cells migrated into dermis and expressed CCR6. $\gamma\delta$ T cells from CCR6-deficient mice had significantly lower frequency in dermis, suggesting that CCR6 is critical in peripheral $\gamma\delta$ T cell trafficking into skin. In addition, no difference was observed in other anatomical sites (Fig. 6b).

IL-1R signalling is critical in skin inflammation. We next examined whether IL-1 β is involved in the skin inflammation such as psoriasis. As previously reported, both IL-23 dermal injection and IMQ induce human psoriasis-like skin inflammation and pathology^{20,27}. The epidermal hyperplasia and

neutrophil infiltration induced by IMQ or IL-23 were markedly decreased in IL-1RI KO mice compared with WT mice (Fig. 7a and Supplementary Fig. 6a). In addition, the mRNA levels of IL-17 and IL-22 were also significantly decreased in IL-1RI KO mice (Fig. 7b and Supplementary Fig. 6b). Further analysis of IL-17-producing dermal $\gamma\delta$ T cells from treated and untreated skin tissues indicated that the basal levels of IL-17-producing dermal $\gamma\delta$ T cells were significantly lower in IL-1RI KO mice compared with those in WT mice (Fig. 7c and Supplementary Fig. 6c). Upon IL-23 or IMQ treatment, dermal $\gamma\delta$ T cells secreted large amounts of IL-17 in WT mice, and IL-17 levels were significantly more in WT mice as compared with IL-1RI KO mice (Fig. 7c and Supplementary Fig. 6c). Taken together, these data suggest that IL-1RI expression is critical in both IL-23 and IMQ-induced skin inflammation and acanthosis.

Discussion

In this study, we examine dermal $\gamma\delta$ T cell development and find that these cells are scarce on E20 and neonatal pups but become obvious at day 3. They predominately express V γ 6 TCR, suggesting that V γ 6 T cells are *bona fide* resident dermal $\gamma\delta$ T cells. After birth, dermal $\gamma\delta$ T cells undergo haemostatic proliferation with diversified TCR repertoire. V γ 4 T cells gradually increase over time in the dermis. This striking ontogeny profile may imply differential functions for dermal $\gamma\delta$ T cells with a distinct TCR repertoire. This is also in sharp contrast with lung $\gamma\delta$ T cells, which appear on E20 and maintain similar ratios of V γ 4 and V γ 6 in different ages of mice. Interestingly, conventional $\alpha\beta$ T cells occur in a significantly delayed fashion, suggesting that one of the primary contributions of dermal $\gamma\delta$ T cells is neonatal skin protection.

Previous studies suggest that DETCs or innate-like CD27⁻ IL-17-producing cells cannot be generated from the BM²⁸. However, it is not definitive whether dermal $\gamma\delta$ T cells can be reconstituted by BM cells^{5,6,11,13}. Our study clearly shows that dermal $\gamma\delta$ T cells can be reconstituted from the BM, although thymus is absolutely required. This suggests that the precursors of dermal $\gamma\delta$ T cells in the BM are probably re-circulated from the thymus. The precursors of dermal $\gamma\delta$ T cells in the BM contain mainly V γ 4 and V γ 1 but not V γ 6. As shown in our study, V γ 6 T cells can be directly reconstituted from fetal thymus. It appears that this extra detour for thymic V γ 4 T cells is to gain skin-homing properties. It is possible that transferred neonatal thymic V γ 4 T cells may require a longer time for skin seeding as compared with adult BM V γ 4. Using thymocytes from different ages of mice, we showed that matured V γ 4 T cells are significantly increased and are more than matured V γ 6 T cells in the thymus of day 5 mice. However, V γ 6 T cells are still the predominant dermal $\gamma\delta$ T cells in the reconstituted mice. This is different from $\gamma\delta$ T cell composition in the peripheral blood and LN, suggesting that thymic V γ 4 T cells need extrathymic environment for imprinting their skin-homing properties, such as gaining an activated status or CCR6 expression that enables them home to the skin or sites of inflammation. It also suggests that thymic V γ 6 T cells may be more competitive than V γ 4 for dermal $\gamma\delta$ T cell reconstitution. Although it is unclear which factor(s) stimulates thymic egressed V γ 4 T cells for imprinting of their skin-homing capability, it is conceivable that Ag stimulation may be one of driving forces for their maturation and functional competency. Indeed, a recent study showed that $\gamma\delta$ T cells recognize a microbial-encoded B-cell Ag for activation and IL-17 production²⁹. These distinct developmental requirements between dermal V γ 4 and V γ 6 T cells are also supported by two recent studies showing their differential transcription factors for programming such as SOX4 and SOX13, respectively^{13,14}. It is thus proposed that dermal $\gamma\delta$ T

cells contain *bona fide* resident V γ 6 T cells that are non-migratory, mature $\gamma\delta$ T cells with IL-17-producing ability and are the major force in the network of neonatal skin immunosurveillance. In contrast, dermal V γ 4 T cells, which require extrathymic environment for imprinting their skin-homing properties, are probably migratory and the inducible IL-17-producing cells. V γ 4 T cells can be Ag-specific and produce large amounts of IL-17 in the presence of TCR engagement, thus perpetuating the IL-17 response in inflammation³⁰.

Dermal V γ 4 and V γ 6 T cells also use differential chemokine receptors for their skin homing. Deficiency of CCR6 or CCR10 alone does not significantly alter dermal V γ 6 T-cell skin migration. However, dermal V γ 4 T cells are significantly lower in CCR6-deficient mice but not in CCR10-deficient mice. CCR6 is critical for V γ 4 T cells in the BM homing to skin but not essential for V γ 4 thymic egress, as frequencies of V γ 4 T cells in the BM of WT or CCR6 KO mice are similar. Although V γ 4 and V γ 6 dermal $\gamma\delta$ T cells have differential developmental requirement, it appears that both subsets are functional competent in terms of IL-17 production and induction of psoriasis-like skin inflammation. This is different from previous studies using Sox13-deficient mice or Sox13 spontaneous mutant mice^{13,14}. The discrepancy may arise from the overall frequency of dermal $\gamma\delta$ T cells. IL-17-producing V γ 4 T cells are selectively deficient in Sox13-mutated mice¹³ and T-Sox4-deficient mice¹⁴, thereby leading to reduced skin inflammation. In our study, mice transplanted with fetal thymus or sorted V γ 6 from neonatal thymocytes have exclusively IL-17-producing V γ 6 T cells with the overall frequency similar as WT mice and develop severe skin inflammation upon IMQ treatment. Thus, it appears that the different results could be in part owing to the quantity of total IL-17-producing $\gamma\delta$ T cells in dermis. However, in naive mice where both dermal V γ 4 and V γ 6 T cells exist, V γ 4 T cells are preferentially expanded and are the major IL-17 producers upon IMQ topical treatment, suggesting that a peripheral regulation programme could be different for dermal V γ 4 and V γ 6 T cells.

Although $\gamma\delta$ T cells are considered as pre-committed functional competent cells, dermal $\gamma\delta$ T cells are subject to peripheral regulation. We found that IL-1 β and IL-23 are both capable of stimulating V γ 4 and V γ 6 T-cell proliferation. IL-23-stimulated dermal $\gamma\delta$ T cell proliferation is largely driven by IL-1 β signalling but also has a IL-1 independent pathway, mainly with V γ 6. It is possible that endogenous levels of IL-18 could effect in conjunction with IL-23 to stimulate minimal dermal $\gamma\delta$ T cell proliferation in the absence of IL-1 β , as IL-18 is critical in human $\gamma\delta$ T cell expansion and is independent of IL-1R signalling³¹. We also found that dermal V γ 4 cells are the major IL-17 producer as compared with dermal V γ 6 upon IL-23 or IL-23 plus IL-1 β stimulation. This *in vitro* study is consistent with *in vivo* IMQ-induced psoriasis model in naive mice where IL-17-producing V γ 4 T cells are

significantly expanded. However, the molecular mechanism accounting for these differences between V γ 4 and V γ 6 is not presently known. Both dermal V γ 4 and V γ 6 T cells are CD44^{hi}CD62L^{lo}CD24^{lo}CD27⁺ and constitutively express CCR6 and ROR γ t (Supplementary Fig. 7). They do not express NK1.1 as most NK1.1⁺ $\gamma\delta$ T cells produce IFN- γ ³². IL-23R expression level is also low in both subsets, which may be related to their activation status³³. Previous studies also identify scavenger receptor SCART2 is uniquely expressed in IL-17-producing $\gamma\delta$ T cells¹⁵. Nevertheless, we found that IL-1 β is essential in both V γ 4 and V γ 6 T cell IL-17 production. Thus, IL-1 β is not only critical in regulating dermal $\gamma\delta$ T cell expansion under inflammatory conditions but also is essential for dermal $\gamma\delta$ T cell effector function.

IL-1 β also stimulates KC to secrete many chemokines including CCR6 ligand CCL20. Since CCL20 expression is higher in skin inflammation such as psoriasis³⁴, this raises the possibility that IL-1 β signalling may be involved in dermal $\gamma\delta$ T cell trafficking via chemokine secretion. We show that peripheral $\gamma\delta$ T cells can be trafficked into the skin in a CCR6-dependent manner. Previous studies show that only CCR6⁺ $\gamma\delta$ T cells produce IL-17 (ref. 32). CCR6⁺ $\gamma\delta$ T cells are also found in the cerebrospinal fluid in multiple sclerosis patients³⁵ and in other inflammatory conditions³⁶. However, CCR6 may be specific for V γ 4 T-cell peripheral trafficking into skin because V γ 6 T cells are minimal in draining lymph nodes (DLNs). This corroborates with a recent study showing two ways of V γ 4 dermal $\gamma\delta$ T cell migration from skin to draining LNs and back to inflamed skin¹³. This may also be related to previous studies showing that CCR6 KO mice have decreased skin inflammation in a murine psoriasis model^{25,37,38}. IL-1 β has also been demonstrated to have an important role in the course of cutaneous inflammation such as psoriasis^{39,40}. Psoriatic lesions are found to have increased IL-1 β mRNA expression and caspase-1 activation, which is responsible for cleaving the inactive precursor of IL-1 β into active form^{24,41}. Indeed, mice with excessive IL-1 β expression or the lack of IL-1R antagonist (*Il1rn*^{-/-}) develop a psoriasis-like skin inflammation⁴²⁻⁴⁴. In contrast, neutrophil infiltration and epidermal thickness are significantly decreased in IL-1RI-deficient mice. This is consistent with the overall significantly decreased IL-17-producing $\gamma\delta$ T cells in the skin. In addition, mRNA levels of IL-17 and IL-22 in IL-1RI KO mice were significantly lower than WT mice. Although there is still controversy over the treatment of psoriasis patients with IL-1R antagonist, a promising therapeutic efficacy of targeting IL-1 pathway has been reported from a small clinical trial, indicating the importance of IL-1 signalling in the development of human psoriasis^{45,46}.

Methods

Mice. C57Bl/6 WT, B6.SJLWT, *TCRd*^{-/-}, *CCR6*^{-/-} and *Il1r1*^{-/-} on C57Bl/6 background (female, 6–8 weeks old) were purchased from Jackson Laboratory. Nude mice (female, 6–8 weeks old) on C57Bl/6 background were purchased from Taconic.

Figure 4 | Dermal V γ 6 T cells induce skin inflammation but dermal V γ 4 are preferentially expanded and are the major IL-17 producers. (a) BM cells or BM cells plus neonatal thymocytes (BM + Thy) from C57Bl/6 WT mice (CD45.2) were transplanted into lethally irradiated SJL mice (CD45.1, $n = 5$). After 8 weeks of reconstitution, frequency of dermal $\gamma\delta$ T cells was detected by flow cytometry. Mice receiving BM cells alone predominantly reconstituted V γ 4, whereas mice receiving BM plus neonatal thymocytes predominantly reconstituted V γ 6. **(b)** Reconstituted mice were treated daily for 5 days with IMQ or control cream (control). Representative H&E-stained sections are shown and epidermal thickness were measured at day 5. Scale bar, 100 μ m. Data are shown as mean $\pm$ s.e.m. $^{**}P < 0.01$, $^{***}P < 0.001$ (unpaired Student's *t*-test). **(c)** Percentage of CD45⁺Gr-1⁺ cells after IMQ treatment was analysed by flow cytometry. IL-17 and IL-22 mRNA levels were measured by qPCR. Data are shown as mean $\pm$ s.e.m. $^{*}P < 0.05$ (unpaired Student's *t*-test). **(d)** Intracellular IL-17 production by skin dermal $\gamma\delta$ T cells from IMQ-treated or control mice was determined by flow cytometry (without stimulation). Flow plots were gated on CD3⁺ cells. Data are shown as mean $\pm$ s.e.m. $^{*}P < 0.05$ (unpaired Student's *t*-test). **(e)** C57Bl/6 WT mice ($n = 5$ –6) were treated daily for 5 days with IMQ or control cream (control). Percentages of CD45⁺Gr-1⁺ cells, total dermal $\gamma\delta$ T cells, dermal V γ 4 and V γ 6 cells were analysed by flow cytometry. Data are shown as mean $\pm$ s.e.m. and are representative of two independent experiments with similar results. $^{**}P < 0.01$ (unpaired Student's *t*-test). **(f,g)** Ki-67 expression **(f)** and intracellular IL-17 production **(g)** by dermal V γ 4 and V γ 6 cells were determined by flow cytometry (without stimulation). Flow plots were gated on CD3^{int} $\gamma\delta$ TCR^{int} cells. Data are shown as mean $\pm$ s.e.m. and are representative of two independent experiments with similar results. $^{*}P < 0.05$, $^{**}P < 0.01$ (unpaired Student's *t*-test).

CCR10 KO mice were described previously¹⁹. All animals were housed and treated in accordance with institutional guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Louisville.

Tissue preparation and cell stimulation. Whole-skin cells were prepared from mouse back skin⁴. In brief, skin tissues were digested with a buffer containing

collagenase IV, hyaluronidase and DNase I. Skin cells were labelled with CFSE and stimulated with rIL-23 (1 ng ml⁻¹, eBioscience), rIL-1β (1 ng ml⁻¹, eBioscience) or rIL-23 plus rIL-1β for 3 days. Cell proliferation and intracellular IL-17 were measured by flow cytometry. For blocking experiment, neutralizing mAbs for IL-1β (2 μg ml⁻¹, eBioscience), IL-23 (12.5 μg ml⁻¹, eBioscience) or matched isotype control mAbs were added. In addition, primary mouse KCs (Cellntec) were

Figure 5 | IL-23 and IL-1 β regulate dermal V γ 4 and V γ 6 $\gamma\delta$ T cell proliferation and IL-17 production. (a,b) Whole-skin cell suspensions were labelled with CFSE and then stimulated with IL-23, IL-1 β or IL-23 plus IL-1 β for 3 days. CFSE dilution and intracellular IL-17 production by dermal $\gamma\delta$ T cells (a) or dermal V γ 4 and V γ 6 $\gamma\delta$ T cells (b) were determined by flow cytometry. Flow plots gated on CD3^{int} $\gamma\delta$ TCR^{int} cells are representative of at least three independent experiments with similar results. Data are shown as mean $\pm$ s.e.m. ($n=10$). ** $P<0.01$, *** $P<0.001$ (unpaired Student's t -test). (c) Whole-skin cells suspensions were labelled with CFSE and then stimulated with IL-23, IL-1 β , IL-23 plus anti-mouse IL-1 β , IL-1 β plus anti-mouse IL-23 or isotype control mAb in WT mice for 3 days. CFSE dilution and intracellular IL-17 production by dermal $\gamma\delta$ T cells were determined by flow cytometry. Flow plots gated on CD3^{int} $\gamma\delta$ TCR^{int} cells are representative of at least three independent experiments with similar results. Summarized data ($n=8-9$) are shown as mean $\pm$ s.e.m. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ (unpaired Student's t -test). (d,e) Whole-skin cells suspensions from IL-1RI KO mice labelled with CFSE were stimulated with IL-23 for 3 days. CFSE dilution and intracellular IL-17 production by dermal $\gamma\delta$ T cells (d) or dermal V γ 4 and V γ 6 $\gamma\delta$ T cells (e) were determined by flow cytometry. Flow plots gated on CD3^{int} $\gamma\delta$ TCR^{int} cells are representative of at least three independent experiments with similar results. Data are shown as mean $\pm$ s.e.m. ($n=8-9$). ** $P<0.01$, *** $P<0.001$ (unpaired Student's t -test).

stimulated with IL-1 β for 6 h and RNA were extracted for analysis of chemokine expression by real-time quantitative PCR (qPCR). For $\gamma\delta$ T cell development study, skin, thymus and BM were taken from pups before (E18 or E20) or after birth.

Flow cytometry analysis and intracellular cytokine staining. Fluorochrome-labelled mAbs including mouse $\gamma\delta$ TCR (GL3, 1:100), V γ 4 (UC3-10A6, 1:500), V γ 1 (2.11, 1:500), CD24 (M1/69, 1:500), CD27 (LG.3A10, 1:500), CD62L (MEL-14,

Figure 6 | CCR6 is essential for $\gamma\delta$ T cell trafficking from periphery to dermis. (a) Primary murine KC were stimulated with IL-1 β for 6 h. CCL2, CCL5, CCL20, CXCL9 and CXCL10 mRNA levels were measured by qPCR. The figure shows fold changes normalized for β -MG mRNA versus medium alone. Data are shown as mean $\pm$ s.e.m. (b) Sorted $\gamma\delta$ T cells from spleen and LN from C57Bl/6 WT mice or CCR6KO mice were adoptively transferred into SJL mice ($n = 4$). After 5–7 days, cells from skin, peripheral blood, LN and spleen were stained with CD3, $\gamma\delta$ TCR and CCR6. Frequency of total $\gamma\delta$ T cells and CCR6 $^{+}$ $\gamma\delta$ T cells from donor (CD45.2) were determined by flow cytometry. Flow plots were gated on skin CD3 int $\gamma\delta$ TCR int cells. Data are shown as mean $\pm$ s.e.m. ** $P < 0.01$, NS, not significant (unpaired Student's t -test).

1:500) and IL-17A (TC11-18H10.1, 1:500, Biolegend), CD44 (IM7, 1:200), ROR γ t (AFKJS-9, 1:200), and Ki-67 (SolA15, 1:200, eBioscience), IL-23R (753317, 1:10) and CCR6 (140706, 1:50, R&D Systems) were used. Anti-mouse V γ 6 (17D1, 1:500) was kindly provided by Dr. Tigelaar (Department of Dermatology, Yale University). For intracellular cytokine staining, cells were first blocked with anti-CD16/32 and then stained with different cell surface antibodies (Abs). Cells were then fixed, permeabilized and stained intracellularly for IL-17, ROR γ t or Ki-67. The relevant isotype control mAbs were also used. Samples were harvested with BD FACS Calibur or Canto (Becton Dickinson, San Jose, CA, USA) and analysed with FlowJo software (TreeStar).

BM chimeras. The BM chimeras were generated as previously reported⁵. In brief, recipient mice were lethally irradiated with 950 cGy and then were intravenously transferred with 5×10^6 thymocytes. After 24 h, the recipient mice received 5×10^6 BM cells. In some experiments, the lethally irradiated WT, nude or $TCRd^{-/-}$ mice were transferred with BM cells alone. In addition, sorted V γ 4 or V γ 6 $\gamma\delta$ T cells from C57Bl/6 neonatal thymocytes mixed with BM cells from SJL mice were transferred into irradiated $TCRd^{-/-}$ mice. In some experiments, sorted V γ 6 cells from neonatal thymocytes plus BM cells from $TCRd^{-/-}$ mice were transferred into irradiated $TCRd^{-/-}$ mice. Neonatal thymocytes used in all experiments were taken from pups born within 48 h. All chimeric mice were allowed to reconstitute for at least 8 weeks before use in experiments.

Peripheral $\gamma\delta$ T cells trafficking. $\gamma\delta$ T cells were sorted from LN and spleens from C57Bl/6 or $CCR6^{-/-}$ mice and then intravenously transferred to SJL mice. After 5–7 days, recipient mice were killed. Peripheral blood, skin, LN and spleen samples were collected for further analysis of the expression of $\gamma\delta$ T cells from donor by flow cytometry.

Establishment of psoriasis-like mouse models. IL-23-induced psoriasis-like mouse model was established as previously described²⁷. In brief, IL-23 (1 μ g) or vehicle control was daily intradermally injected on the back skin of WT or $Il1r1^{-/-}$ mice for 4 days. For IMQ-induced psoriasis-like model²⁰, WT or $Il1r1^{-/-}$ mice

were applied daily with IMQ cream (5%; Aldara; 3M Pharmaceuticals) for 5 consecutive days. In some experiments, BM chimera mice were also applied with IMQ. Mice were killed and the skin samples were embedded and frozen in (optimal cutting temperature (OCT) for hematoxylin and eosin (H&E) and immunohistochemistry (IHC) staining. Skin samples were also excised in TRIzol (Invitrogen) for RNA extraction. Skin cell suspensions were stained for Gr-1 expression and IL-17 production.

Skin histology and IHC staining. Skin sections were stained with H&E and the epidermal thickness was determined by measuring the average interfollicular distance under the microscope in a blinded manner. For IHC staining, skin cryosections were fixed, blocked and stained with rat-anti-mouse Gr-1 mAb (1:50) followed by goat-anti-rat IgG secondary Ab (1:200, Southern Biotech)⁴. Slides were developed with 3-amino-9-ethylcarbazole substrate solution (Vector Laboratories) and counterstained with hematoxylin. Images were acquired at $\times 200$ magnification using Aperio ScanScope digital scanners.

RNA extraction and real-time qPCR. RNAs were isolated using a Qiagen RNeasy kit. After reverse transcription into cDNA, qPCR was performed on Bio-Rad MyiQ single colour real time (RT)-PCR detection system using SYBR Green Supermix (Bio-Rad) and gene-specific primers were listed as follows: all chemokines (Real Time Primers, LLC, Elkins Park, PA, USA); IL-17A (Mm_Il17a_SG, Qiagen); IL-22: 5'-ATACATCGTCAACCGCACCTTT-3' (forward), 5'-AgCCGGACATCTGTGTTGTTAT-3' (reverse); IL-6: 5'-GAGAAAGAGTTCAATGGC-3' (forward), 5'-CCA GTT TGG TAG CAT CCA TCA T-3' (reverse). We normalized gene expression level to β -2 microglobulin (β -MG) housekeeping gene and represented data as fold differences by the $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct = Ct_{\text{target}} - Ct_{\beta\text{-MG}}$ and $\Delta\Delta Ct = \Delta Ct_{\text{induced}} - \Delta Ct_{\text{reference}}$.

Statistical analysis. All quantitative data are shown as mean $\pm$ s.e.m. unless otherwise indicated. All samples were compared using two-tailed, unpaired Student's t -test. A P value < 0.05 was considered significant. Statistical analysis was performed with GraphPad Prism software.

Figure 7 | IL-1R signalling is essential for IMQ-induced skin inflammation and acanthosis. (a) C57Bl/6 WT and IL-1RI KO mice ($n = 6-8$) were treated daily for 5 days with IMQ or control cream (control). Representative H&E-stained sections and frozen sections stained with Gr-1 are shown. Gr-1 positive cells are brown. Skin tissues were also stained with CD45 and Gr-1 assessed by flow cytometry. Epidermal thickness and percentage of CD45⁺Gr-1⁺ cells were measured at day 5. Scale bar, 100 μm. Data are shown as mean $\pm$ s.e.m. $^{**}P < 0.01$, $^{***}P < 0.001$ (unpaired Student's t -test). (b) IL-17, IL-22 and IL-6 mRNA levels were measured by qPCR. The figure shows fold changes normalized for β -MG mRNA versus control skin from WT mice. Data are shown as mean $\pm$ s.e.m. $^{*}P < 0.05$, $^{**}P < 0.01$ (unpaired Student's t -test). (c) Intracellular IL-17 production by dermal $\gamma\delta$ T cells with or without PMA plus ionomycin stimulation was determined by flow cytometry. Flow plots were gated on CD3⁺ cells. Data are shown as mean $\pm$ s.e.m. $^{*}P < 0.05$, $^{***}P < 0.001$ (unpaired Student's t -test).

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Author contributions

Y.C. and F.X. designed and performed the experiments and wrote the manuscript; C.F., J.Y., C.D., Y.M., M.L. participated in experiments, H.-g.Z., J.Z., N.X. participated in experimental design; J.Y. designed the experiments and wrote the manuscript.

Additional information

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