Inflammation induces dermal Vγ4$^+$ γδT17 memory-like cells that travel to distant skin and accelerate secondary IL-17–driven responses

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Gamma delta (γδ) T cells represent a major IL-17 committed T-cell population (γδT17 cells) in the mouse dermis. Following exposure to the inflammatory agent imiquimod (IMQ) the Vγ4$^+$ subset of γδT cells produce IL-17 in the skin and expand rapidly in draining lymph nodes (LNs). Local IMQ treatment in humans is known to exacerbate psoriasis skin lesion activity at distant sites. Whether expanded γδT17 cells sensitize distant sites to inflammation has been unknown. Here we show that expanded Vγ4$^+$ γδT17 cells egress from LNs in a fingolimod (FTY720)-sensitive manner and use C-C chemokine receptor type 2 to accumulate in inflamed skin where they augment neutrophil recruitment and inflammation. They also travel to noninflamed skin and peripheral LNs and remain in elevated numbers at these distant sites for at least 3 mo. Sensitized mice show more rapid skin inflammation and greater proliferation and IL-17 production by Vγ4$^+$ γδT cells upon imiquimod challenge. Transfer experiments confirm that memory-like Vγ4$^+$ γδT17 cells respond more rapidly. Memory-like Vγ4$^+$ γδT17 cells are distinguished by greater IL-1R1 expression and more proliferation in response to IL-1β. These findings establish that local skin inflammation leads to faster and stronger secondary responses to the same stimulus through long-term and systemic changes in the composition and properties of the dermal γδT-cell population.

immunological memory | γδT cells | inflammation

The skin is a crucial barrier organ that protects us from infection while also harboring a diverse commensal microbial population (1). The immunological properties of the skin that allow responses against pathogens but limit reactions against innocuous agents, or provide responses that are supportive for commensals, are incompletely understood (1). Recent studies identified a population of migratory γδT cells in the mouse dermis (2–4). The majority of dermal γδT cells express C-C chemokine receptor type 6 (CCR6) and are precommitted to interleukin 17 (IL-17) production (referred to as γδT17 cells) (3–5). In C57BL/6 mice roughly half of these cells express a T-cell receptor (TCR) containing Vγ4 (Heilig and Tonegawa nomenclature is used throughout) (6), and some of these cells are also Vδ4$^+$ (7–9). Vγ4$^+$Vδ4$^+$ γδT17 cells were first identified in studies of the lymph node (LN) response to intradermal injection with collagen in complete Freund's adjuvant (CFA) (10). Studies in mice expressing photoconvertible proteins have provided evidence that a fraction of the steady-state γδT17 cells in skin-draining LNs are derived from the skin (7, 11). However, γδT17 cells are rare in blood in the steady state (7). Their production in the thymus peaks in the late embryonic and perinatal period and it is thought that in adult mice the cells are largely replenished locally in the skin (2, 7, 8, 12). During inflammation, additional types of γδT cells can be recruited to the skin, in particular CD27$^+$ interferon-γ–secreting cells (13).

IL-17 family cytokines have well-established roles in antibacterial and antifungal defense. A prominent condition in IL-17–deficient humans is mucocutaneous candidiasis (14). IL-17 also has an established role in chronic inflammatory conditions in humans, including psoriasis (3, 15, 16). Treatment of murine skin with the Toll-like receptors 7 and 8 and purinergic receptor agonist-containing cream imiquimod induces skin lesions with features similar to human psoriasis (17). Studies in mice lacking γδT cells or deficient in Sox13, a transcription factor crucial for Vγ4$^+$ γδT-cell development, have established that dermal γδT17 cells contribute to the development of these lesions (5, 7, 8, 18). This involves an early phase of IL-17 production by skin resident cells and marked expansion of Vγ4$^+$ γδT cells in skin-draining LNs, after which these expanded LN cells can home efficiently to the inflamed skin (3, 5, 7). Dermal γδT cells are also activated to produce cytokines [IL-17, IL-22, tumor necrosis factor (TNF)] following treatment with IL-1β and IL-23, during bacterial infection, and in an atopic dermatitis model (2, 3, 11, 19, 20).

A central feature of adaptive immunity is the ability to mount responses that are faster and of greater magnitude on secondary challenge with the same agent. Memory responses are a defining feature of the conventional αβT-cell compartment, yet whether γδT cells develop immune memory has been investigated less. Recent studies of Vγ6$^+$ intestinal and peritoneal cells have begun to provide support for γδT-cell memory responses (21, 22). However, it is not yet clear whether dermal γδT17 cells can take on memory characteristics.

Here we show that the Vγ4$^+$ γδT17 cells that expand in draining LNs following skin imiquimod (IMQ) sensitization travel via the blood to distant skin sites and LNs where they persist for months. Upon secondary challenge at a distant skin site, memory-like Vγ4$^+$ γδT17 cells increase in number more rapidly and produce more IL-17 than upon primary challenge, leading to a faster skin inflammatory response. In addition to

Significance

An attribute of adaptive immunity is the generation of memory cells that mount enhanced responses after rechallenge. While memory is best understood for αβT cell receptor-bearing lymphocytes, the properties of γδT cells in the context of a secondary challenge have been less examined. After murine skin inflammation with imiquimod, IL-17–secreting γδT cells expand in lymph nodes and traffic to skin where they persist as memory-like cells capable of rapid activation upon rechallenge. This enhanced secondary response is in part mediated by increased IL-1R1 expression. Expansion of potentially pathogenic memory cells in lymph nodes and redistribution throughout normal and inflamed skin may help explain the generalized worsening of psoriasis reported in patients undergoing localized skin treatment with imiquimod.

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persisting in greater numbers, the cells have elevated interleukin 1 receptor, type I (IL-1R1) expression and respond more vigorously to IL-1β.

Results

IMQ-Activated Vγ4$^+$ γδT17 Cells Egress from LNs and Accumulate in Inflamed Skin in an Fingolimod-Sensitive Manner. Previous work established that Vγ4$^+$ γδT17 cells that also express Vδ4$^+$ expand markedly in LNs draining IMQ-treated skin and when transferred into the blood they home to inflamed skin (7). In this study, as before (7), quiescent γδT17 cells were identified as Vγ4$^+$ cells expressing high levels of CCR6; however, IMQ-activated γδT17 cells down-regulate CCR6 (see below) and were therefore tracked by coexpression of Vγ4 and Vδ4. To investigate whether IMQ-activated cells normally travel from LN to skin, we tested the effect of fingolimod (FTY720), a drug that causes S1PR1 down-modulation and inhibits LN egress of conventional T cells (23). As expected (7), Vγ4$^+$Vδ4$^+$ cells accumulated in draining LN after 5 d of IMQ treatment (d5) and decreased by d7. Vγ4$^+$Vδ4$^+$ cells also increased in the blood at d5 and d7, whereas only increasing in skin at d7 (Fig. 1*A*). FTY720 administration during the IMQ treatment led to an even more marked accumulation of Vγ4$^+$Vδ4$^+$ cells in responding LNs and blocked their increase both in blood and in inflamed ear skin (Fig. 1*A*). Consistent with direct action, Vγ4$^+$Vδ4$^+$ cells at d5 of the LN response expressed surface S1PR1 which was down-modulated following FTY720 treatment (Fig. 1*B*). These findings suggest that following expansion in the draining LN, γδT17 cells egress into the blood in an S1PR1-dependent manner and subsequently migrate to and accumulate in inflamed skin.

C-C Chemokine Receptor Type 2 Influences the Accumulation of Activated Vγ4$^+$ γδT17 Cells in Inflamed Skin. Movement of cells from blood into inflamed skin typically involves expression of cutaneous lymphocyte antigen (CLA) that serves as a ligand for E- and P-selectins (24). Activated (d5) Vγ4$^+$ cells had high CLA expression and showed strong binding of E- and P-selectin (Fig. 1*C* and *SI Appendix*, Fig. S1*A*). The chemoattractant requirements for cell homing to sites of skin inflammation are complex and are often thought to involve multiple chemokines. In an IL-23 model of psoriasis, CCR6 was important for the development of inflammation and trafficking of γδT cells from dermis to epidermis (25). Although Vγ4$^+$ γδT17 cells express CCR6 (5, 7), levels of this receptor were reduced on activated Vγ4$^+$ cells (*SI Appendix*, Fig. S1*B*). C-C chemokine receptor type 2 (CCR2) plays a role in homing of macrophages to inflamed skin and is present on dermal γδT cells (3, 26, 27). IMQ-expanded LN Vγ4$^+$ Vδ4$^+$ cells expressed high levels of CCR2 (Fig. 1*D*). Additionally, activated Vγ4$^+$Vδ4$^+$ cells recruited to inflamed skin expressed higher levels of CCR2 than those present in control skin (Fig. 1*D*). The ligand chemokine (C-C motif) ligand 2 (CCL2) is expressed in psoriatic skin in humans (28) and was up-regulated in IMQ-inflamed mouse skin (Fig. 1*E*). IMQ activated LN Vγ4$^+$ γδT cells migrated toward CCL2 in vitro (Fig. 1*F*). When cells were transferred from LNs of d5 IMQ-treated mice into congenically marked mice that were treated with IMQ on ear skin, *Ccr2*$^{-/-}$ Vγ4$^+$ cells showed reduced skin accumulation after 3–8 h compared with WT cells (Fig. 1*G*). By contrast, CCR2-deficient cells were present in slightly augmented numbers in the draining LNs of recipient mice (Fig. 1*G*). These data indicate that CCR2 contributes to Vγ4$^+$ γδT17 cell recruitment to or retention within inflamed skin.

LN Activated Vγ4$^+$ γδT17 Cells Accelerate Inflammation at a Distal Site. Given the efficient homing of LN-derived cells to the initiating site of skin inflammation, we next asked whether these cells are capable of accelerating lesion development at a distant site. Mice that had been IMQ or control treated for 5 d on their left ear were then IMQ treated on the right ear and ear thickness and cell numbers were monitored. This analysis revealed a strong augmentation in appearance of Vγ4$^+$Vδ4$^+$ cells in the newly inflamed site that was associated with heightened neutrophil recruitment (Fig. 2 *A* and *B*) and a trend to more rapid ear thickening (Fig. 2*C*). These findings indicate that LN-activated γδT cells responding to inflammation at one skin region are able to migrate to and accelerate the response to an inflammatory stimulus occurring at a new site.

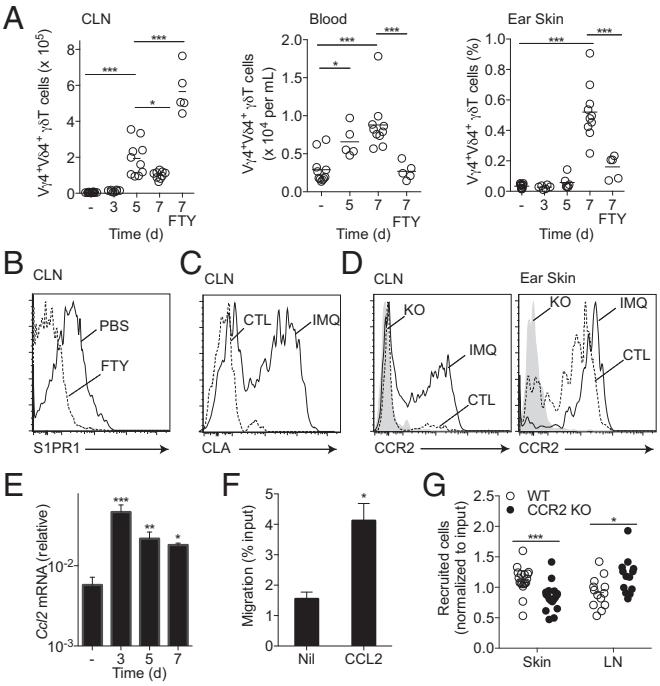

Fig. 1. Egress of IMQ-activated Vγ4$^+$ γδT17 cells from LNs and migration to inflamed skin. (*A*) Cell number or frequency of Vγ4$^+$Vδ4$^+$ cells in draining cervical LN (CLN) (*Left*), blood (*Middle*), or ear skin (*Right*) at indicated times after IMQ treatment. FTY720-treated mice are indicated by FTY. Data from at least two experiments with 2–3 mice of each type. (*B*) S1PR1 expression on Vγ4$^+$Vδ4$^+$ cells from draining LNs at d5 of IMQ treatment, mice received PBS or FTY720 24 h before analysis. Representative of 2 mice per condition. (*C*) CLA expression on Vγ4$^+$Vδ4$^+$ cells from CLN of mice treated with IMQ or control (CTL) cream for 5 d. Representative of at least 10 mice of each type. (*D*) CCR2 expression on Vγ4$^+$Vδ4$^+$ cells from CLN as in *C* (*Left*) or from ear skin of mice treated with IMQ for 7 d (*Right*). CCR2-deficient cells are depicted by the solid histogram. Representative of at least 4 mice of each type. (*E*) RT-PCR analysis of *Ccl2* mRNA in ear skin from control (–) or mice treated with IMQ for 3, 5, or 7 d. Data are pooled from at least two experiments with two mice of each type; bars indicate mean ± SEM. (*F*) Transwell migration of CLN Vγ4$^+$ γδT17 cells to medium or CCL2 (100 ng/mL) from mice treated with IMQ on ear skin for 5 d. Representative of three experiments in duplicate; bars indicate mean ± SEM. (*G*) Efficiency of CCR2 KO versus WT Vγ4$^+$Vδ4$^+$ cell homing to skin and LN of mice that had been IMQ treated for 2 d. Tissues were analyzed 3–12 h after cell transfer. Data represent six independent experiments with at least two recipients each. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

IMQ-Expanded Vγ4$^+$ γδT17 Cells Persist in Inflamed Skin and Peripheral LNs. We then asked whether Vγ4$^+$Vδ4$^+$ cell expansion in LN and recruitment to inflamed skin is transient or persistent. Ear skin of mice was treated with IMQ for 5 d, and Vγ4$^+$Vδ4$^+$ cell frequencies were determined at different times. Beginning at d7, there was an ∼20-fold increase in Vγ4$^+$Vδ4$^+$ cell frequency in the previously inflamed skin. Importantly, this increase in cell composition was persistent, declining only slightly in skin over a 3-mo period (Fig. 3*A*). The frequency of Vγ4$^+$Vδ4$^+$ cells was also increased in distant skin-draining LNs, blood, and spleen. This increase was preferential to skin-draining LNs, as there was only a minor increase in mesenteric LNs (Fig. 3*A* and *SI Appendix*, Fig. S2). Numbers declined modestly over time in skin-draining LNs and blood, but even here they remained above baseline at 3 mo (Fig. 3*A* and *SI Appendix*, Fig. S2).

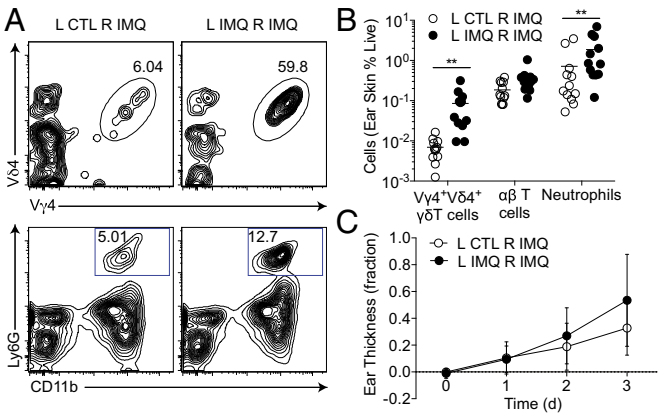

Fig. 2. Migration of LN expanded Vγ4$^+$ γδT17 cells to inflamed distal skin sites accelerates inflammation. (*A*) Representative plots of ear skin cells from mice that were control (CTL) or IMQ treated on the left (L) ear for 5 d (d−5 to d−1), followed by IMQ treatment of the right (R) ear for 3 d (d0–d2), after which the frequency of cells in R ear skin was analyzed (d3). (*Top*) Gated on CD45$^+$ γδ TCR intermediate cells. (*Bottom*) Gated on CD45$^+$ cells. (*B*) Quantification of indicated cell types from R ear skin treated as in *A*. (*C*) R ear skin thickness of mice treated as in *A* and measured daily (d0–d3), relative to baseline at day 0. Data are pooled from four experiments with three mice in each group. $^{**}P < 0.01$, $^{***}P < 0.001$.

IMQ-Expanded Vγ4$^+$ γδT17 Cells Sensitize Distant Skin Sites to Secondary Responses. To determine whether expanded Vγ4$^+$ γδT17 cells were able to reach noninflamed skin and, if so, whether they could persist, we treated back skin of mice with IMQ for 5 d, and analyzed uninvolved ear skin at different times. Remarkably, 30 d after IMQ treatment of back skin, there was a ∼20-fold increase in the Vγ4$^+$Vδ4$^+$ cell numbers in uninvolved healthy ear skin, which persisted for at least 7 mo (Fig. 3*B*). The increase in Vγ4$^+$Vδ4$^+$ cells did not appear to be at the expense of the other major T-cell types in the skin (Fig. 3*B*). As seen with imiquimod treatment of ear skin above, expanded Vγ4$^+$Vδ4$^+$ cells persisted in peripheral LNs (*SI Appendix*, Fig. S3). These data demonstrate that after local IMQ-mediated skin inflammation, expanded LN Vγ4$^+$ γδT17 cells not only migrate to inflamed skin, but also colonize previously uninflamed skin and skin-draining LNs where they persist for months.

To test whether the persisting Vγ4$^+$ γδT17 cell population could mount a memory-type response, mice that had been IMQ sensitized on back skin were IMQ challenged 1 mo later on previously untreated ear skin. In line with the results in untreated skin (Fig. 3*B*), analysis at day 3 of IMQ treatment revealed significantly more Vγ4$^+$Vδ4$^+$ cells in the ear skin of sensitized compared with nonsensitized mice (Fig. 4*A*). Compared with controls, sensitized WT mice displayed a more rapid increase in the extent of ear thickening and neutrophil accumulation (Fig. 4 *B* and *C*). The accelerated response in sensitized WT mice was due to the increase in Vγ4$^+$Vδ4$^+$ cells, as this worsening was not seen in sensitized *Sox13*-mutant mice that lack the Vγ4$^+$ γδT17 cell population (7) (Fig. 4 *B* and *C*). Indeed, the response to IMQ in sensitized *Sox13*-mutant mice did not approach even that of control WT mice, further emphasizing the role of Vγ4$^+$ γδT17 cells in this model. Transcript analysis of WT whole ear skin at d3 showed higher levels of *Il17a*, *Il17f*, defensin genes *Defb3* and *Defb4*, and chemokine (C-X-C motif) ligand 2 (*Cxcl2*) mRNA in sensitized compared with control ear skin (Fig. 4*D*). Histological analysis demonstrated more acanthosis and neutrophil infiltration in the epidermis of IMQ-treated ear skin of sensitized compared with control mice (*SI Appendix*, Fig. S4). There was a greater frequency of IL-17–producing cells in d3 inflamed skin of sensitized mice (Fig. 4 *E* and *F*). In contrast to control skin, where the Vγ4$^+$Vδ4$^+$ cell population was a minor IL-17 source, Vγ4$^+$Vδ4$^+$ cells were the major producers of this cytokine in sensitized mice (Fig. 4*G*). Moreover, in skin of sensitized mice, the IL-17A level per cell was elevated (Fig. 4*H*). IMQ treatment of sensitized mice resulted in expansion of Vγ4$^+$Vδ4$^+$ cells in the draining LN at d3, a time point before any proliferation of cells in draining LNs of control mice (Fig. 4*I*). Consistent with the earlier expansion, more of the Vγ4$^+$Vδ4$^+$ cells in the draining but not the nondraining LNs of sensitized and challenged mice were enlarged (*SI Appendix*, Fig. S5).

To determine whether previously activated Vγ4$^+$Vδ4$^+$ cells had intrinsic properties allowing for more rapid activation and proliferative response, we isolated d5 IMQ-expanded cells from draining LNs and transferred them to untreated recipients. After allowing IMQ-expanded cells to home to unperturbed skin and LNs of recipients and become quiescent for 2–4 wk, hosts were IMQ treated on ear skin daily for 3 d. At d3, a higher fraction of transferred Vγ4$^+$Vδ4$^+$ cells were producing IL-17A compared with host cells (Fig. 5 *A* and *B*) and the donor cells that responded in the ear-draining LNs were larger and more rapidly incorporated BrdU (Fig. 5 *C* and *D*).

Increased Cell Surface Expression of IL-1R1 on Memory-Like Vγ4$^+$ γδT17 Cells. The i.p. administration of mannan was recently reported to result in psoriasis-like skin inflammation over the course of 5–6 d, at least in part through activation of γδT17 cells (29). Interestingly, whereas treatment with mannan resulted in skin inflammation as measured by ear thickening, sensitization with IMQ 1 mo before mannan injection did not worsen this process (Fig. 6*A*). We next examined the response by Vγ4$^+$Vδ4$^+$ cells in LNs 5 d after mannan dosing. This time point represents the height of the

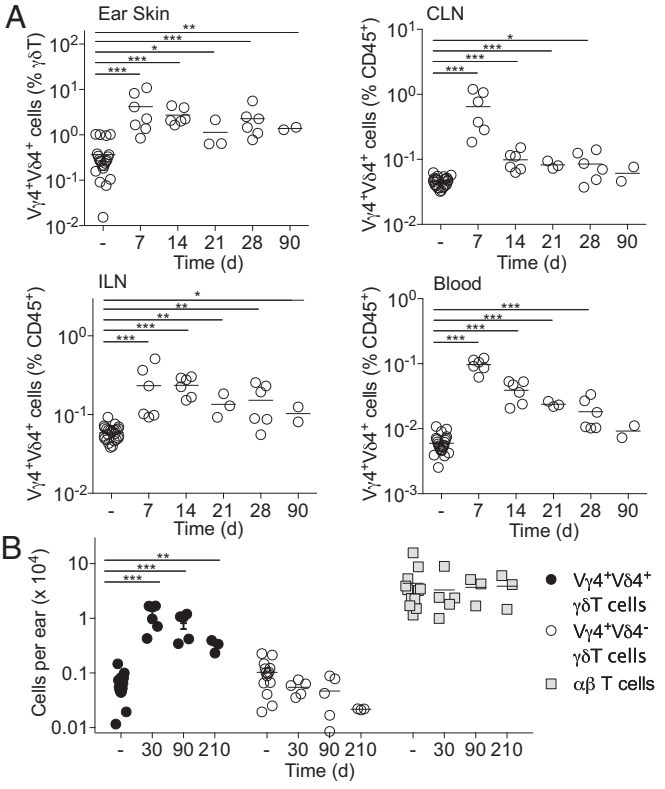

Fig. 3. Expanded Vγ4$^+$ γδT17 cells redistribute to inflamed and intact skin as well as peripheral LNs and persist for months. (*A*) Frequency of Vγ4$^+$Vδ4$^+$ cells in the indicated tissues in control (–) mice or animals that were treated with IMQ on ear skin daily for 5 d (d0–d4) and harvested at the indicated times. Frequency of cells in ear skin is depicted as fraction of total γδT cells to account for different digestion protocols used in some of the time points. ILN, inguinal LN. (*B*) Cell numbers in ear skin of mice that were treated only on back skin with control cream or IMQ for 5 d (d0–d4) and analyzed at the indicated times. Data are pooled from at least one experiment at each time point, with at least two mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

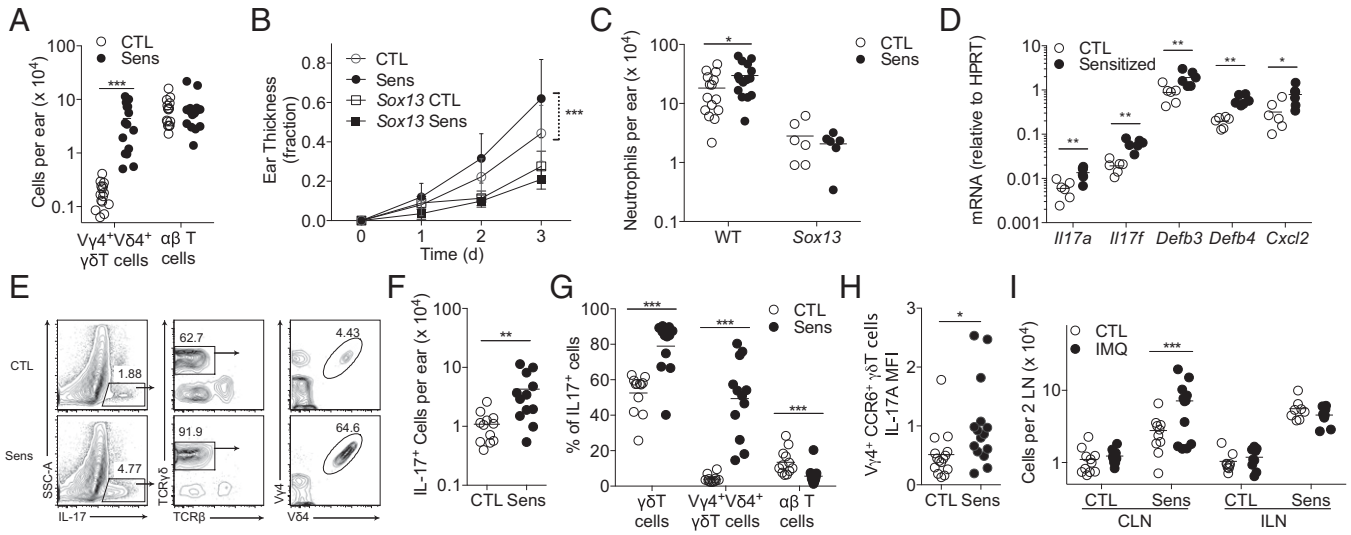

Fig. 4. Local IMQ-induced inflammation results in the redistribution of Vγ4$^+$ γδT17 cells that sensitize previously intact skin to IMQ-triggered inflammation. (*A*) Cell numbers in ear skin treated with IMQ for 3 d from mice that had been control treated or IMQ sensitized for 5 d on back skin 1 mo prior. Data are pooled from five experiments with three mice in each group. (*B*) Ear thickness of mice treated as in *A*. WT data as in *A* (circles); *Sox13*$^{mut/mut}$ data represent two experiments with three mice of each group (squares). (*C*) Quantification of Ly6G$^+$CD11b$^+$ neutrophils in ear skin of mice treated as in *A*. (*D*) RT-PCR analysis of mRNA in ear skin of WT mice treated as in *A*. Data represent two experiments with three mice. (*E*) Intracellular IL-17A staining of ear skin cells from mice treated as in *A*, gated on CD45$^+$ cells. (*F*) Quantification of IL-17A$^+$ cells in ear skin obtained as in *E*. (*G*) Frequency of indicated cells among total IL-17A$^+$ cells obtained from ear skin as in *E*. (*H*) IL-17A mean fluorescence intensity (MFI) of Vγ4$^+$ CCR6$^+$ cells obtained from ear skin as in *E*. Data are pooled from at least four experiments with three mice in each group. (*I*) Vγ4$^+$Vδ4$^+$ cell number in draining CLN or nondraining inguinal LN (ILN) from control or sensitized mice treated as in *A*. Data are pooled from four (CLN) or three (ILN) experiments with three mice in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

IMQ-induced proliferative response. In contrast to the IMQ model, there was only limited proliferation of Vγ4$^+$Vδ4$^+$ cells in response to systemic mannan, as evidenced by a small increase in the number of Vγ4$^+$Vδ4$^+$ cells in control mice treated with mannan and by an increase in cellular size (Fig. 6*B*). Importantly, proliferation of Vγ4$^+$Vδ4$^+$ cells in response to mannan was not enhanced in the IMQ-sensitized mice (Fig. 6*B*). These data suggest a context specificity to the secondary response of previously activated memory-like Vγ4$^+$Vδ4$^+$ cells.

To test whether the IMQ stimulation involved TCR signaling we attempted to use Nur77-GFP reporter mice (30). Increased Nur77-GFP was observed in skin Vγ4$^+$ γδT17 cells, although not in draining LN cells, after 2 d of treatment (*SI Appendix*, Fig. S6 *A* and *B*). However, in vitro culture experiments showed that whereas TCR signaling induced Nur77-GFP in Vγ4$^+$ γδT17 cells most strongly, it was also induced by IL-1β and IL-23 signaling (*SI Appendix*, Fig. S6*C*). Therefore, further studies will be needed to determine whether Vγ4$^+$ γδT17 cell activation following IMQ treatment involves TCR signaling.

IL-1β and IL-23 are sufficient to promote proliferation of dermal Vγ4$^+$ γδT17 cells, and IL-1R1 is important in the activation of dermal γδT17 cells in response to IMQ (8). Conversely, mannan-induced IL-17 expression and skin inflammation can be prevented by TNF but not IL-1β blockade (29). We hypothesized that enhanced responsiveness to specific cytokines may form the basis for the more rapid in vivo proliferative response of Vγ4$^+$Vδ4$^+$ cells in sensitized mice following IMQ rechallenge. Indeed, compared with controls, we found elevated IL-1R1 surface levels in Vγ4$^+$ γδT17 cells from LNs of 1 mo IMQ-sensitized mice (Fig. 6*C*). Following intradermal injection of IL-1β into the ear, there was greater accumulation of Vγ4$^+$Vδ4$^+$ cells in the draining LN of sensitized compared with control mice (Fig. 6*D*). When tested in vitro, the cells underwent greater amounts of proliferation in response to IL-1β (Fig. 6 *E* and *F*). IL-1β activation of Vγ4$^+$ γδT17 cells results in increased CD25 surface expression, which was enhanced in cells from sensitized compared with control mice (Fig. 6 *G* and *H*). These data suggest that memory-like Vγ4$^+$Vδ4$^+$ γδT17 cells have an intrinsically increased sensitivity to IL-1β, an adaptation that likely contributes to their ability to respond more rapidly during secondary exposure to inflammatory stimuli.

Discussion

The above findings establish that Vγ4$^+$Vδ4$^+$ γδT17 cells expanded by IMQ-induced inflammation travel to and persist in healthy skin and skin-draining LNs and support more rapid secondary responses. This memory feature of skin exposure to IL-17–inducing inflammatory agents is likely to enhance protection against repeat exposure to skin-penetrating bacteria or fungal pathogens. The strong ability of γδT17 cells to expand and contribute to inflammation at a distant skin site may also provide an explanation for the ability of IMQ treatment to induce flares of disease at nontreated sites in some psoriasis patients (31).

Whereas immunological memory is well established for αβT cells, relatively little is known about the ability of γδT cells to mount memory responses. A recent study of the intestinal response against *Listeria monocytogenes* showed that expanded Vγ6$^+$ γδT cells homed to and persisted in the intestine and responded more effectively to a secondary mucosal infection by *Listeria* (21). In another study, peritoneal infection with *Staphylococcus aureus* led to the persistence of an expanded population of Vγ6$^+$ γδT cells. Again the expanded cells showed restricted tissue tropism, persisting in the peritoneum and draining LNs (22). Our findings for Vγ4$^+$Vδ4$^+$ cells provide further evidence for the ability of γδT cells to acquire memory characteristics including accelerated activation and proliferation after reencountering a stimulus. Importantly, each type of "trained" γδT-cell appears to retain the central properties of its precursor in terms of homing and cytokine secretion profiles.

The mechanism of IMQ-induced expansion of γδT17 cells involves induction of IL-1β and IL-23 by skin cells and possibly also by cells in the draining LNs. Both cytokines can promote dermal γδT17 cell activation and proliferation (3, 4, 8). IL-7 can also induce Vγ4$^+$ γδT17 cell proliferation (2, 32). Although we did not observe increased IL-7 transcripts in IMQ-exposed skin or LNs (*SI Appendix*, Fig. S7), we do not rule out the possibility that γδT17 cells are exposed to more IL-7 under inflammatory conditions. It remains unclear whether γδT17 cell expansion following IMQ

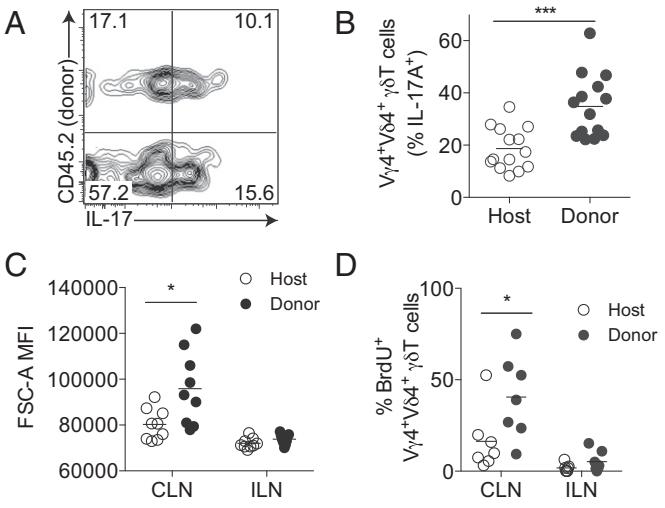

Fig. 5. Previously activated Vγ4$^+$ γδT17 cells demonstrate intrinsic features of a memory response. (*A*) Representative plot of intracellular IL-17A staining in ear skin cells from WT CD45.1$^+$ mice that received d5 IMQ-activated WT CD45.2$^+$ LN cells 2–4 wk before IMQ application on ear skin daily for 3 d. (*B*) Frequency of IL-17A$^+$ cells among donor (CD45.2$^+$ or Thy1.2$^+$) or host (CD45.1$^+$ or Thy1.1$^+$) cells from mice treated as in *A*. (*C*) Cell size of Vγ4$^+$Vδ4$^+$ cells in draining CLN or nondraining ILN from host or recipient mice as in *B*. (*D*) BrdU incorporation in Vγ4$^+$Vδ4$^+$ cells in draining CLN or nondraining ILN from host or recipient mice as in *B*. (*A* and *B*) Data are pooled from two to three experiments with at least three mice each. *$P < 0.05$, ***$P < 0.001$.

treatment involves TCR engagement. Although the selective expansion of Vγ4$^+$Vδ4$^+$ cells would be consistent with this possibility, it could also reflect signaling through other surface molecules unique to these cells.

The high expression of CLA on activated Vγ4$^+$Vδ4$^+$ cells is consistent with the cells engaging P- and E-selectins to gain access to skin. In addition to being present on inflamed endothelium, E-selectin is constitutively expressed on dermal microvessels making it likely that LN emigrant Vγ4$^+$Vδ4$^+$ γδT cells are able to undergo CLA–E-selectin–supported rolling interactions with healthy dermal endothelium (33). The contribution of CCR2 to γδT-cell accumulation in the skin adds to data showing a role for CCR2 in γδT-cell homing to the pleural cavity (34).

It is notable that γδT17 cells are locally maintained in the skin (2, 7, 8, 12); yet population representation in adult mice can be strongly influenced by input from circulating precursors. Consistent with local maintenance during homeostasis, the frequency of γδT17 cells was similar in the skin of adult control and LTβR-deficient mice, which lack LNs (*SI Appendix*, Fig. S8) (35). Still to be addressed is whether in the memory-like condition the shifted cell representation is again locally maintained or depends on ongoing input from LNs.

In germ-free mice, dermal γδT17 cell numbers are reduced (36). It seems possible that the marked expansion and persistence of dermal γδT17 cells observed here in response to a strong inflammatory stimulus may represent an exaggerated form of the response occurring to skin commensals. That is, commensal colonization of skin may provide low grade inflammatory signaling that causes expansion and an increase in the number of dermal γδT17 cells at all skin sites. The elevated frequency of γδT17 cells observed in mice with defects in skin barrier function (37, 38) or skin immunity (16) could in turn be a consequence of skin commensal dysbiosis.

The pathogenesis of psoriasis is not understood and likely involves genetic as well as environmental factors. In a subset of patients, psoriasis is triggered or exacerbated after streptococcal throat or skin infections, and, in some patients with recurrent streptococcal pharyngitis and psoriasis, improvement has been found after tonsillectomy (39). We speculate that local skin or oral inflammation may result in expansion of IL-17–producing cells in responding LNs, which could then home to the skin and

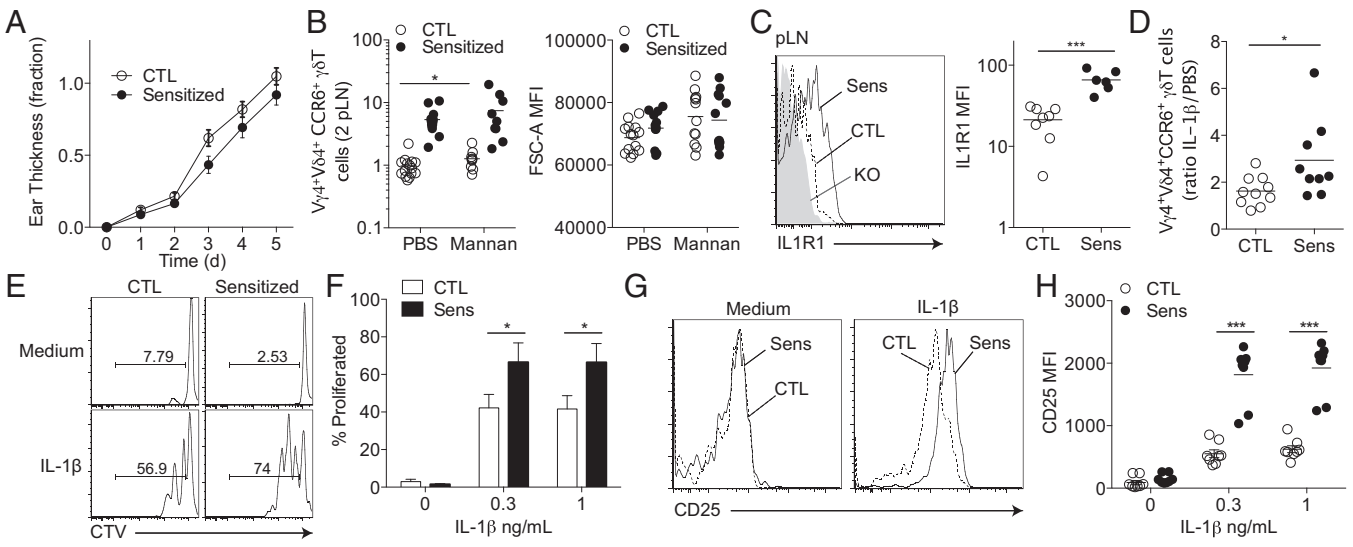

Fig. 6. Up-regulation of IL-1R1 and enhanced response to IL-1β in memory-like Vγ4$^+$ γδT17 cells. (*A*) Ear thickness after one dose of mannan at d0 in mice that, 1 mo prior, were control treated or IMQ sensitized for 5 d on back skin. (*B*) Quantification of cell number (*Left*) and cell size (*Right*) of peripheral LN (ILN) Vγ4$^+$Vδ4$^+$ cells from mice treated as in *A*. (*A* and *B*) Data are pooled from three to four experiments with three mice of each type. (*C*) IL-1R1 expression (*Left*) and MFI (*Right*) in Vγ4$^+$ CCR6$^+$ cells in peripheral lymph node (pLN) of mice 1 mo after control or IMQ sensitization of back skin. (*D*) Ratio of Vγ4$^+$Vδ4$^+$ cell number in *Left* (IL-1β draining) to *Right* (PBS draining) CLN in mice treated as in *C* that received two intradermal injections of IL-1β in the left ear and PBS in the right ear (d0 and d2), and harvested at d3. (*C* and *D*) Data are pooled from three experiments with at least two mice of each group. (*E*) Representative histograms of CellTrace Violet (CTV)-labeled mixed LN cells from WT CD45.1$^+$ (control) or WT CD45.2$^+$ (IMQ sensitized 1 mo prior) mice, cultured in medium alone or stimulated with IL-1β (0.3 ng/mL) for 3 d, gated on Vγ4$^+$ CCR6$^+$ cells. (*F*) Proliferation (CTV dilution) of Vγ4$^+$ CCR6$^+$ cells in mixed cultures as in *E*; bars indicate mean ± SEM. (*G*) CD25 expression on Vγ4$^+$ CCR6$^+$ cells from LNs of mice that were control or IMQ sensitized for 5 d on back skin 1 mo before analysis, after mixed culture for 3 d in medium only (*Left*) or IL-1β (0.3 ng/mL, *Right*). (*H*) CD25 MFI on Vγ4$^+$ CCR6$^+$ cells from LNs of mice treated as in *G*. (*F–H*) Data are pooled from four experiments, performed at least in duplicate. *$P < 0.05$, ***$P < 0.001$.

act to generalize cutaneous inflammation. In this regard, γδT cells capable of producing IL-17 have been identified in the blood and skin of patients with psoriasis (3, 15, 16).

Whereas expanded $V\gamma4^+V\delta4^+$ γδT17 cells may preferentially migrate to skin given CLA expression, it is possible that they may be recruited to other inflamed tissues where they could aggravate disease. In an arthritis model, intradermal injection of collagen in CFA led to an accumulation of $V\gamma4^+V\delta4^+$ cells in both the LN and joints (10). Whether the accrual of γδT17 cells in the joint resulted from recruitment or local expansion was not examined. Of note, a subset of patients with psoriasis develop inflammatory arthritis, and γδT cells are an important source of IL-17 in a murine model of this joint disease (29). In addition, $IL\text{-}17^+V\gamma4^+$ γδT cells infiltrate the CNS during the development of experimental autoimmune encephalomyelitis (19). Thus, redistribution of γδT17 cells through their recirculation may aggravate inflammation in multiple tissues. Our finding that the FDA-approved drug FTY720 inhibits $V\gamma4^+V\delta4^+$ γδT17 cell migration from LNs to skin highlights a possible therapeutic strategy to limit involvement of these cells in autoimmune processes.

In summary, we show that in response to local inflammation, $V\gamma4^+V\delta4^+$ γδT17 cells expand in responding LNs and redistribute in the skin and peripheral LNs where they persist as memory-like cells capable of altering the set point for induction of inflammation. We hypothesize that similar responses may occur in patients with chronic diseases such as psoriasis and speculate that inhibition of this redistribution may alter the natural history or the complications of this disease.

Materials and Methods

Mice, Treatments, and Tissue Preparation. All experiments conformed to the ethical principles and guidelines approved by the University of California San Francisco Institutional and Animal Care and Use Committee. Induction of psoriasis-like inflammation was done as described (7). Lymph nodes and spleen were dissected free of fat and fasciae and disaggregated by passing through a 100-μm nylon sieve (BD Bioscience) in DMEM containing penicillin, streptomycin, and Hepes buffer, pH 7.2. Ear skin preparation and flow cytometry of cell suspensions were performed as described (7). Additional details, including mice strains, adoptive transfers, and in vivo BrdU labeling, are provided in *SI Appendix, SI Materials and Methods*.

In Vitro Assays and Molecular Methods. For proliferation assays, LN cells from WT $CD45.1^+$ control or IMQ-sensitized WT $CD45.2^+$ mice were mixed, CTV labeled, and cocultured in medium alone or medium plus different concentrations of IL-1β for 3 d. Additional details, including chemotaxis assays and real-time PCR, are provided in *SI Appendix, SI Materials and Methods*.

Statistical Analysis. Prism software (GraphPad) was used for all statistical analysis. Ear-thickness changes were compared by two-way analysis of variance. The two-tailed, unpaired *t* test was used for chemotaxis comparisons. The Mann–Whitney *u* test was used for comparisons of all other datasets.

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