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Authors
Laidlaw, Brian J
Gray, Elizabeth E
Zhang, Yang
et al.

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BRIEF DEFINITIVE REPORT

Sphingosine-1-phosphate receptor 2 restrains egress of γδ T cells from the skin

Brian J. Laidlaw[1,2*], Elizabeth E. Gray[1,2*], Yang Zhang[1,2], Francisco Ramírez-Valle[1,2], and Jason G. Cyster[1,2]

Maintenance of a population of IL-17–committed γδ T cells in the dermis is important in promoting tissue immunity. However, the signals facilitating γδ T cell retention within the dermis remain poorly understood. Here, we find that sphingosine-1-phosphate receptor 2 (S1PR2) acts in a cell-intrinsic manner to oppose γδ T cell migration from the dermis to the skin draining lymph node (dLN). Migration of dermal γδ T cells to the dLN under steady-state conditions occurs in an S1PR1-dependent manner. S1PR1 and CD69 are reciprocally expressed on dermal γδ T cells, with loss of CD69 associated with increased S1PR1 expression and enhanced migration to the dLN. γδ T cells lacking both S1PR2 and CD69 are impaired in their maintenance within the dermis. These findings provide a mechanism for how IL-17$^+$ γδ T cells establish residence within the dermis and identify a role for S1PR2 in restraining the egress of tissue-resident lymphocytes.

Introduction

Protection of the skin against physical insults and microbial invasion is critical to the long-term health of the host. The skin contains a diverse array of immune cells that function cooperatively to facilitate tissue repair and host defense, along with a multitude of microorganisms that are essential in regulating skin immunity and inflammation (Lai et al., 2009; Naik et al., 2012; Ridaura et al., 2018). Recently, a population of dermal γδ T cells has been identified in both mice and humans (Cai et al., 2011; Gray et al., 2011; Sumaria et al., 2011). Most dermal γδ T cells are CCR6$^+$, arise from Sox13-expressing progenitors, and are precommitted to express IL-17 (Gray et al., 2013; Spidale et al., 2018). They maintain themselves within the skin and are dependent on IL-7, but not IL-15, for their self-renewal with mouse dermal γδ T cells expressing a TCR containing either Vγ4 or Vγ6 (Gray et al., 2011, 2013; Sumaria et al., 2011). Dermal γδ T cells are a primary source of IL-17 following skin infection with pathogens such as *Mycobacterium bovis* and are critical for neutrophil recruitment to the skin and eventual pathogen clearance (Sumaria et al., 2011; Nakamizo et al., 2015; Ramírez-Valle et al., 2015). They are also a major source of IL-17 in psoriatic skin lesions with increased IL-17 expression correlating with disease progression (Gatzka et al., 2013; Gray et al., 2013). Acute depletion of γδ T cells results in protection in an induced psoriasis model (Sandrock et al., 2018).

While γδ T cells are motile and largely resident within the dermis, they undergo a low rate of trafficking to the skin draining LN (dLN) under steady-state conditions (Gray et al., 2011, 2013; Jiang et al., 2017). Flux of γδ T cells from the dermis to the dLN increases under conditions of inflammation, with CCR2 contributing to the migration of γδ T cells expanded in the dLN back to the inflamed sites (Gray et al., 2013; Ramírez-Valle et al., 2015; McKenzie et al., 2017). Within the dLN, γδ T cells migrate in close association with the subcapsular sinus in a CCR6-dependent manner (Zhang et al., 2016). γδ T cells can also travel to noninflamed dermis and distant LNs, where they are maintained at elevated numbers for months and display enhanced responsiveness upon stimulation (Ramírez-Valle et al., 2015; McKenzie et al., 2017). γδ T cells expanded in the dLN are important for protection against *Staphylococcus aureus* skin reinfection (Dillen et al., 2018).

The constant motility of dermal γδ T cells facilitates surveillance of the dermis for commensals and invading pathogens (Gray et al., 2011; Ridaura et al., 2018). Maintaining a sufficient density of γδ T cells within the dermis is likely to be essential to allow patrolling cells to rapidly detect invaders. Therefore, while migration of dermal γδ T cells to the dLN may be useful to establish a γδ T cell population in the LN that can protect against pathogens that bypass the skin, it is important that sufficient cells are retained in the dermis to maintain barrier immunity

[1]Department of Microbiology and Immunology, Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA; [2]Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA.

*B.J. Laidlaw and E.E. Gray contributed equally to this paper; Correspondence to Jason G. Cyster: jason.cyster@ucsf.edu; E.E. Gray's present address is Seattle Genetics, Bothell, WA; Y. Zhang's present address is ZXBio Co., Ltd., Huzhou, China; F. Ramírez-Valle's present address is Translational Development and Clinical Pharmacology, Celgene Corp., Summit, NJ.

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(Nakamizo et al., 2015; Davies et al., 2017). The signals mediating γδ T cell retention in the dermis are not yet defined.

Skin lymphatics produce CCL21, sphingosine-1-phosphate (S1P), and other chemoattractants such as CXCL12 (Gunn et al., 1998; Kabashima et al., 2007; Pappu et al., 2007). Cell exit from skin via lymphatics can be mediated by CCR7 in the case of dendritic cells and naive T cells or S1P receptor 1 (S1PR1) in the case of effector or memory T cells (Ohl et al., 2004; Debes et al., 2005; Skon et al., 2013). Tissue-resident memory CD8⁺ T (T_{RM}) cells are often characterized by expression of CD69, a repressor of S1PR1, and removal of CD69 from the cells can result in their S1PR1-mediated loss from the tissue (Shiow et al., 2006; Bankovich et al., 2010; Lee et al., 2011; Mackay et al., 2013, 2015; Skon et al., 2013). Whether CD69 has a role in γδ T cell retention in the skin is not known.

Here, we discover a role for S1PR2, a Rho-activating migration inhibitory S1PR (Takuwa et al., 2011; Green and Cyster, 2012), in restraining egress of γδ T cells from the dermis. We also find that CD69 restricts expression of S1PR1 on dermal γδ T cells and accordingly opposes migration of these cells from the dermis. We propose a model in which S1PR2 functions to prevent CD69 expressing γδ T cells from exiting the dermis, thereby limiting egress under steady-state conditions to the small proportion of cells that are able to sufficiently express S1PR1 to escape S1PR2-mediated confinement.

Results and discussion

Accumulation of CCR6⁺ γδ T cells in the dLN of mice lacking sphingosine kinase (Sphk) activity

To examine the role of S1P in γδ T cell positioning, we intercrossed *Mx1*^{Cre} mice with animals carrying floxed and null alleles of sphingosine kinase 1 (*Sphk1*) and null alleles of *Sphk2*. Neonatal poly(I:C) treatment of these mice induces Cre expression and ablation of the remaining *Sphk1*^{f} allele, causing plasma S1P to decrease to undetectable levels (Pappu et al., 2007). These *Sphk1/2*-deficient mice (referred to henceforth as *Sphk1/2*$^{-/-}$ mice) displayed a marked increase in the frequency and number of CCR6⁺, but not CCR6⁻, γδ T cells present in the skin dLN (Fig. 1 a). *Sphk1/2*$^{-/-}$ mice had a decreased number of TCRβ⁺ cells in the dLN, possibly due to the role of S1P in promoting naive T cell survival and mitochondrial function (Mendoza et al., 2017; Fig. 1 a). While the frequency of CCR6⁺ γδ T cells was similar in the dermis of *Sphk1/2*$^{-/-}$ and control mice, we found that there was increased proliferation of dermal CCR6⁺ γδ T cells in the *Sphk1/2*$^{-/-}$ mice (Fig. 1, b and c; gating shown in Fig. S1 a). Increased proliferation was not evident in the dLN CCR6⁺ γδ T cells or in dermal TCRβ⁺ cells or dendritic epidermal T cells (Fig. 1 c and Fig. S1 b). Mice in which Sphk activity was ablated in the lymphatic endothelium similarly displayed an increased frequency of CCR6⁺ γδ T cells in the dLN but not dermis, consistent with the notion that lymphatic endothelial cells are an important in vivo source of S1P (Pham et al., 2010; Fig. S1, c and d). Together, these data indicate that S1P may oppose the accumulation of CCR6⁺ γδ T cells in the skin dLN, with the increased flux of cells from the dermis compensated by an increased proliferation rate. It is also possible that this result could be due to a role for S1P in facilitating the egress of CCR6⁺ γδ T cell from the skin dLN.

S1PR2 restrains CCR6⁺ γδ T cell migration from the skin

S1PR2 is a G protein–coupled receptor that signals through $G\alpha_{13}$ to promote migration inhibition in some cell types (Michaud et al., 2010; Green et al., 2011; Takuwa et al., 2011). The finding that S1P restrained the accumulation of γδ T cells in skin dLN led us to ask whether S1PR2 might control γδ T cell egress from the skin. Expression of *S1pr2* mRNA was detected in CCR6⁺ γδ T cells in the dermis and dLN, with analysis of Venus intensity in *S1pr2*$^{Venus/+}$ reporter mice showing higher expression of *S1pr2* in CCR6⁺ γδ T cells relative to their CCR6⁻ counterparts (Fig. 2 a). S1P inhibited the migration of dermal CCR6⁺ γδ T cells toward CXCL12 in a dose-dependent manner, with this inhibition reversed upon coincubation with the S1PR2 antagonist JTE-013 (Fig. 2 b). This inhibition was not evident in CCR6⁺ γδ T cells from the dLN (Fig. 2 b). Similarly, while S1P inhibited the migration of dermal CCR6⁺ γδ T cells from *S1pr2*$^{+/-}$ mice toward CXCL12, this inhibition was absent in cells from *S1pr2*$^{-/-}$ mice (Fig. 2 c).

S1pr2$^{-/-}$ mice displayed an increase in the frequency and number of CCR6⁺ γδ T cells present in the skin dLN (Fig. 2 d). This increase was evident as early as 7 wk and was maintained across all ages examined (Fig. S2 a). There was no difference in the number of CCR6⁻ γδ T cells or TCRβ⁺ cells in the dLN (Fig. 2 d). There was an increase in the number of IL-17⁺, but not IL-17⁻, γδ T cells in the dLN, with this accumulation not evident in the spleen, blood, adult thymus, or neonatal thymus (Fig. 2 e and Fig. S2 b). The increase in γδ T cell frequency in *S1pr2*$^{-/-}$ mice was evident in both Vγ4⁺ and Vγ4⁻ subsets (Fig. S2 c). We found that while the frequency and number of CCR6⁺ γδ T cells was similar in the dermis of *S1pr2*$^{-/-}$ and control mice, *S1pr2*$^{-/-}$ cells demonstrated an increased proliferation rate (Fig. 2, f and g; and Fig. S2 d). There was no difference in the frequency or number of dermal TCRβ⁺ cells or in the frequency of dermal CD45⁺ cells or CD11b⁺ cells (Fig. S2 d). Increased proliferation of CCR6⁺ γδ T cells was not apparent in the dLN (Fig. 2 g). The smaller increase in dLN CCR6⁺ γδ T cells in *S1pr2*$^{-/-}$ mice than in *Sphk1/2*$^{-/-}$ mice might be because γδ T cells in mice selectively lacking S1PR2 have some ability to exit the LN in response to S1P, whereas this cannot occur in S1P-deficient mice.

We next tested whether S1PR2 acts in a cell-intrinsic manner to regulate CCR6⁺ γδ T cell accumulation by reconstituting lethally irradiated mice with *S1pr2*$^{-/-}$ or *S1pr2*$^{+/-}$ neonatal thymocytes and an equal number of congenically distinct control neonatal thymocytes, as well as control bone marrow (BM). Neonatal thymocytes are capable of efficiently reconstituting the CCR6⁺ γδ T cell population (Gray et al., 2011). We found that *S1pr2*$^{-/-}$ cells displayed a competitive advantage in repopulating the CCR6⁺ γδ T cells found in the dLN, while being equivalently represented among dermal CCR6⁺ γδ T cells and dLN TCRβ⁺ cells (Fig. 2 h).

As an approach to test the sufficiency of S1PR2 to inhibit T cell egress from tissue into lymphatics, we used a method that

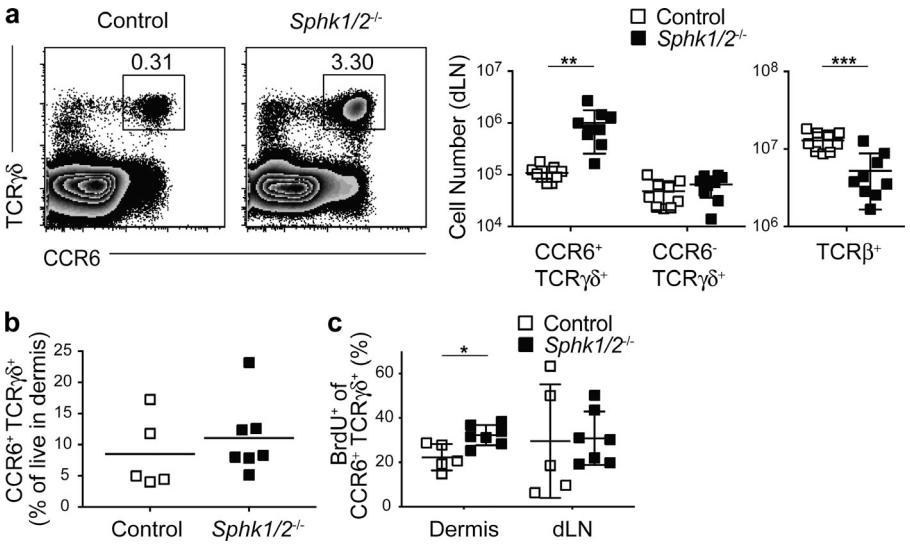

Figure 1. **CCR6⁺ γδ T cell accumulation in the skin dLN is enhanced in Sphk-deficient mice.** **(a)** Representative FACS plots of CCR6⁺ TCRγδ⁺ cells (left) and numbers of CCR6⁺ and CCR6⁻ TCRγδ⁺ cells (middle) in the dLN of control and *Sphk1/2*$^{-/-}$ mice. Numbers of TCRβ⁺ cells in the dLN are shown on the right. **(b)** Percentage of CCR6⁺ TCRγδ⁺ cells in the dermis of control and *Sphk1/2*$^{-/-}$ mice. Data are pooled from six independent experiments with one to three mice per group. **(c)** Percentage of BrdU⁺ cells among the CCR6⁺ TCRγδ⁺ population in the dermis (left) and dLN (right) of control and *Sphk1/2*$^{-/-}$ mice. Data are pooled from four independent experiments with one to three mice per group. Statistical analyses were performed using the unpaired two-tailed Student's *t* test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

measures the impact of receptor overexpression on egress from LNs (Pham et al., 2008). S1PR2 or empty vector (EV)–transduced T cells were transferred into congenically distinct mice. The recipient mice were treated with αL and α4 integrin–blocking antibodies the following day to inhibit further entry into LNs, and the frequency of transduced cells in the LNs was assessed at the time of treatment (t = 0 h) and 18 h later (t = 18 h; Pham et al., 2008). While EV-transduced cells displayed a similar percentage of reporter⁺ cells in the LNs at both time points, S1PR2-overexpressing cells were enriched in the LNs at t = 18 h, indicating that S1PR2 can inhibit T cell exit from tissue via lymphatics (Fig. S2 e). These findings also indicate that the accumulation of CCR6⁺ γδ T cells in the dLN of *S1pr2*$^{-/-}$ mice was not because S1PR2 contributes to egress of these cells from the dLN.

We next examined whether *S1pr2*$^{-/-}$ γδ T cells had an enhanced ability to migrate from the skin to the dLN using green-to-red photoconvertible KikGR protein-expressing transgenic mice. 10-min exposure of the ear skin of these mice to 415-nm violet light is sufficient to induce photoconversion specifically among ear skin cells with no measurable induction of inflammation (Nowotschin and Hadjantonakis, 2009; Gray et al., 2013). We analyzed the number of KikGR Red⁺ cells present in the dLN 24 h after photoconversion of the ear skin and found that *S1pr2*$^{-/-}$ γδ T cells displayed an enhanced ability to migrate from the dermis to the dLN (Fig. 2 i). γδ T cells that migrate from the skin to the dLN after photoconversion are largely Vg4⁺ cells expressing CCR6 (Nakamizo et al., 2015). The migrating γδ T cells in S1pr2$^{-/-}$ mice remained predominantly Vg4⁺ (Fig. S2 f). Together, these data indicate a model in which S1PR2 acts, in a cell intrinsic manner, to restrain dermal CCR6⁺ Vg4⁺ γδ T cells that sense S1P from egressing the skin.

CCR6⁺ γδ T cell migration from the skin is sensitive to S1PR1 functional antagonism

We next sought to explore the cues promoting CCR6⁺ γδ T cell egress from the skin under steady-state conditions. Unlike dendritic cells or naive TCRβ⁺ cells, dermal γδ T cell migration from the skin to the dLN occurs in a CCR7-independent manner (Vrieling et al., 2012; Nakamizo et al., 2015). These findings led us to consider a role for S1PRs. Although our initial studies had shown γδ T cells accumulated in the skin dLN of *Sphk1/2*$^{-/-}$ mice, it seemed possible that S1P might cooperate with another lymphatic cue, such as CXCL12 (Kabashima et al., 2007), to mediate skin egress. CCR6⁺ γδ T cells in the dermis and dLN express *S1pr1* and *S1pr4*, and have intermediate expression of *S1pr5* and low expression of *S1pr3* (Fig. 3 a). To examine the role of S1PRs in γδ T cell migration from skin to dLNs, KikGR-transgenic mice were treated with FTY720 or a vehicle control for 1 wk before exposure of the ear skin to 415-nm violet light. FTY720 is a functional antagonist of S1PR1 and has also been shown to engage S1PR2, S1PR3, S1PR4, and S1PR5 (Brinkmann et al., 2010; Sobel et al., 2015). FTY720-treated mice displayed a dramatic impairment in the frequency of KikGR Red⁺ γδ T cells present in the dLN 1 d later (Fig. 3 b).

S1pr1 mRNA was more highly expressed in CCR6⁺ γδ T cells from the dLN relative to the dermis (Fig. 3 a). Moreover, while S1PR1 protein was expressed by most CCR6⁺ γδ T cells in the dLN, it was expressed on the surface of only a small subset of dermal CCR6⁺ γδ T cells (Fig. 3 c). These data provided correlative evidence that S1PR1 was involved in the skin egress of CCR6⁺ γδ T cells. To test for a specific role for S1PR1 in CCR6⁺ γδ T cell trafficking, KikGR-transgenic mice were treated with AUY954, a selective S1PR1 functional antagonist (Pan et al., 2006), 1 d before exposure of the skin to violet light. AUY954-treated mice displayed a marked impairment in the frequency of KikGR Red⁺ γδ T cells present in the dLN 1 d later (Fig. 3 d). Thus, while other lymphatic cues may be able to facilitate skin egress when S1PR2-mediated confinement is absent, our findings reveal that S1PR1 is critical for γδ T cell egress from the dermis under normal conditions. The most straightforward explanation for these findings is that S1PR1 is functioning intrinsically with the γδ T cells, though we cannot fully exclude the possibility that some actions of the S1PR1-modulating drugs are through other S1PR1-expressing cell types.

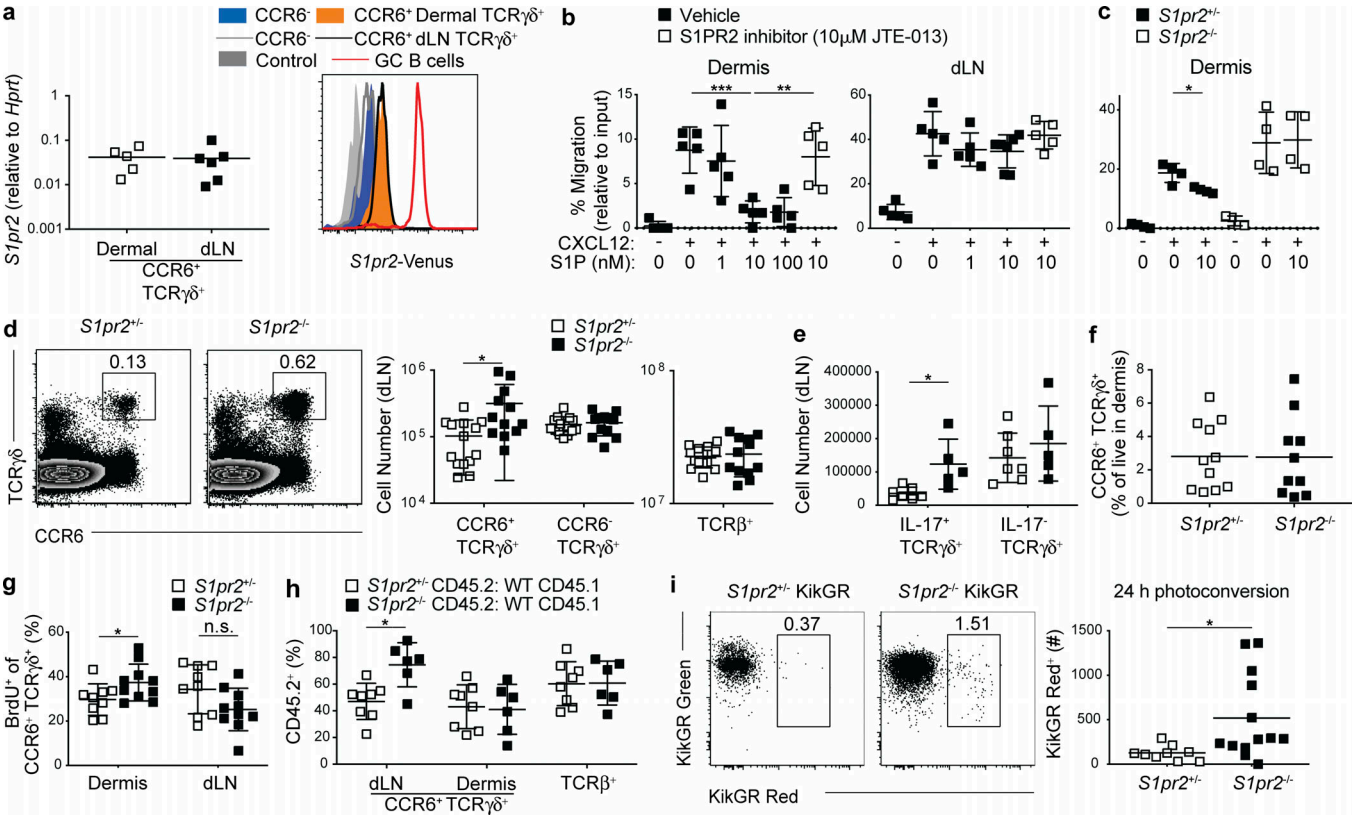

Figure 2. **S1PR2 acts in a cell-intrinsic manner to restrain CCR6⁺ γδ T cell migration from the skin. (a)** Expression of *S1pr2* in CCR6⁺ TCRγδ⁺ cells from the dermis or dLN quantified by RT-PCR (sorted cells, left) or in CCR6⁺ and CCR6⁻ TCRγδ⁺ cells by flow cytometry using *S1pr2*$^{Venus/+}$ mice (right). Venus expression in *S1pr2*$^{+/+}$ mice was used as a negative control. Venus expression in germinal center B cells (B220⁺IgD^{lo}CD95⁺) is shown for comparison. mRNA expression is pooled from two independent experiments with two or three mice per group. Venus expression is representative of five experiments with one or two mice per experiment. **(b)** Percentage migration of CCR6⁺ TCRγδ⁺ cells from the dermis (left) or dLN (right) toward the indicated agents using a transwell system. Data are pooled from three independent experiments. **(c)** Percentage migration of CCR6⁺ TCRγδ⁺ cells from the dermis of *S1pr2*$^{+/-}$ or *S1pr2*$^{-/-}$ mice toward the indicated agents using a transwell system. Data are pooled from two independent experiments with two mice per group. **(d)** Representative FACS plots of CCR6⁺ TCRγδ⁺ cells (left) and numbers of CCR6⁺ and CCR6⁻ TCRγδ⁺ cells (middle) in the dLN of *S1pr2*$^{+/-}$ and *S1pr2*$^{-/-}$ mice. Numbers of TCRβ⁺ cells in the dLN are shown on right. Data are pooled from eight independent experiments with one to three mice per group. **(e)** Number of IL-17⁺ (left) and IL-17⁻ (right) TCRγδ⁺ cells in the dLN of *S1pr2*$^{+/-}$ and *S1pr2*$^{-/-}$ mice. Data are pooled from five independent experiments with one to three mice per group. **(f)** Percentage of CCR6⁺ TCRγδ⁺ in the dermis of *S1pr2*$^{+/-}$ and *S1pr2*$^{-/-}$ mice. Data are pooled from six independent experiments with one to three mice per group. **(g)** Percentage of BrdU⁺ cells among the CCR6⁺ TCRγδ⁺ population in the dermis (left) and dLN (right) of *S1pr2*$^{+/-}$ and *S1pr2*$^{-/-}$ mice. Data are pooled from four independent experiments with one to three mice per group. **(h)** Percentage of CD45.2⁺ cells of the indicated cell populations in irradiated CD45.1⁺ mice reconstituted using neonatal thymocytes from CD45.2⁺ *S1pr2*$^{+/-}$ or *S1pr2*$^{-/-}$ mice. Data are pooled from five independent experiments with one to three mice per group. **(i)** Representative FACS plots (left) and numbers (right) of KikGR Red⁺ cells among the TCRγδ⁺ cells in the dLN of *S1pr2*$^{+/-}$ or *S1pr2*$^{-/-}$ KikGR mice 24 h after photoactivation of the ear tissue. Data are pooled from four independent experiments with two to four mice per group. Statistical analyses were performed using the unpaired two-tailed Student's *t* test. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

CD69 restricts S1PR1 expression and promotes CCR6⁺ γδ T cell retention in the dermis

We next probed how S1PR1 function was regulated on dermal CCR6⁺ γδ T cells. CD69 is a known repressor of S1PR1, with deficiency of CD69 resulting in S1PR1-mediated loss of CD8⁺ T_{RM} cells from the tissue (Shiow et al., 2006; Bankovich et al., 2010; Lee et al., 2011; Mackay et al., 2013, 2015; Skon et al., 2013). CCR6⁺ γδ T cells in the dermis expressed increased *Cd69* mRNA, and CD69 protein expression was only notable on dermal CCR6⁺ γδ T cells (Fig. 4, a and b). Suggestively, CD69 and S1PR1 were reciprocally expressed on dermal CCR6⁺ γδ T cells (Fig. 4 b). CCR6⁺ γδ T cells from the dermis, but not dLN, of *Cd69*$^{-/-}$ mice displayed increased expression of S1PR1, leading us to hypothesize that CD69 promotes CCR6⁺ γδ T cell retention in the dermis (Fig. 4 c). Indeed, *Cd69*$^{-/-}$ mice displayed a marked increase in the frequency and number of CCR6⁺ γδ T cells present in the skin dLN (Fig. 4 d). The increase in γδ T cell frequency in *Cd69*$^{-/-}$ mice was present in both Vγ4⁺ and Vγ4⁻ subsets, although it only reached statistical significance in the Vγ4⁻ subset (Fig. S2 g). There was no difference in the number of CCR6⁻ γδ T cells or TCRβ⁺ cells in the dLN (Fig. 4 d). The frequency and number of CCR6⁺ γδ T cells was similar in the dermis of *Cd69*$^{-/-}$ and control mice, with CCR6⁺ γδ T cells in the dermis, but not dLN, undergoing increased proliferation (Fig. 4, e and f; and Fig. S2 h). There was no difference in the frequency or number of dermal TCRβ⁺ cells or in the frequency of dermal CD45⁺ cells or CD11b⁺ cells (Fig. S2 h). We exposed the ear skin of *Cd69*$^{-/-}$ and control KikGR-transgenic mice to violet light and observed an increase

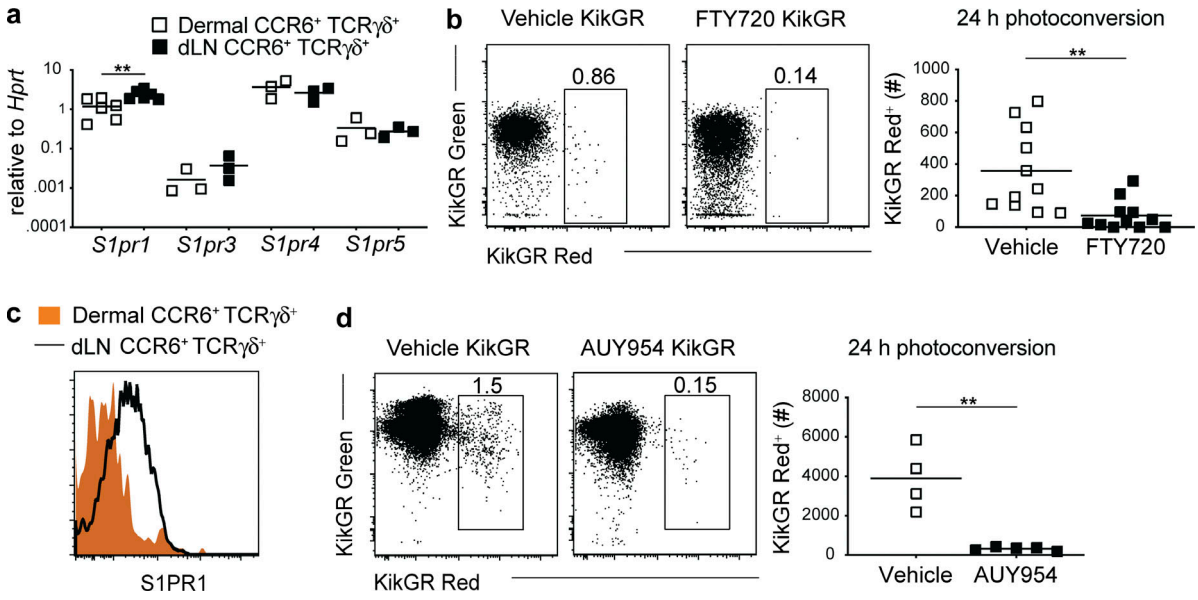

Figure 3. **S1PR1 promotes CCR6⁺ γδ T cell migration from the skin. (a)** Expression of *S1pr1, S1pr3, S1pr4,* and *S1pr5* in sorted CCR6⁺ TCRγδ⁺ cells from the dermis or dLN. mRNA expression is pooled from two independent experiments with two or three mice per group. **(b)** Representative FACS plots (left) and numbers (right) of KikGR Red⁺ cells among the TCRγδ⁺ cells in the dLN of vehicle and FTY720-treated KikGR mice 24 h after photoactivation of the ear tissue. Data are pooled from three independent experiments with three or four mice per group. **(c)** Expression of S1PR1 on CCR6⁺ TCRγδ⁺ cells from the dermis or dLN by flow cytometry. Data are representative of six mice. **(d)** Representative FACS plots (left) and numbers (right) of KikGR Red⁺ cells among the TCRγδ⁺ cells in the dLN of vehicle and AUY954-treated KikGR mice 24 h after photoactivation of the skin. Data are pooled from three independent experiments with one or two mice per group. Statistical analyses were performed using the unpaired two-tailed Student's *t* test. **, $P < 0.01$.

in the frequency and number of KikGR Red⁺ γδ T cells in the dLN of the *Cd69*$^{-/-}$ mice (Fig. 4 g). Collectively, these data indicate that CD69 acts to promote CCR6⁺ γδ T cell retention in the dermis through regulation of S1PR1 expression and activity.

S1PR2 and CD69 cooperate to promote CCR6⁺ γδ T cell retention in the dermis

Finally, we wanted to explore whether S1PR2 and CD69 cooperate to restrain CCR6⁺ γδ T cell egress from the skin. CCR6⁺ γδ T cells from the dLN of *S1pr2*$^{-/-}$ mice displayed a marked decrease in S1PR1 expression and increase in the proportion of CD69-expressing cells relative to control groups (Fig. 5 a). This suggested that S1PR2 promotes retention of CD69⁺S1PR1^{lo} cells in the dermis. CD69 does not directly regulate S1PR2 function, as WEHI231 cells overexpressing CD69 displayed comparable S1PR2-mediated migration inhibition to EV-transduced cells (Fig. 5 b). To test whether S1PR2 and CD69 cooperate to mediate dermal CCR6⁺ γδ T cell retention, we generated *Cd69*$^{-/-}$*S1pr2*$^{-/-}$ mice. We found that there was a decrease in the frequency and number of CCR6⁺ γδ T cells in the dermis relative to littermate controls lacking either *Cd69* or *S1pr2* (Fig. 5 c). The frequency and number of CCR6⁺ γδ T cells in the dLN was similar between both groups (Fig. 5 c). These results indicate that while mice can maintain a similarly sized population of CCR6⁺ γδ T cells in the dermis in the absence of either S1PR2 or CD69, compound loss of both proteins overwhelms the ability of the host to compensate for the loss of dermal CCR6⁺ γδ T cells caused by the increased egress. The lack of double-deficient γδ T cell accumulation in the dLN might be explained by enhanced S1PR1-mediated egress from the LN in the absence of inhibition by CD69 and S1PR2.

Concluding remarks

Recent studies have highlighted the importance of T_{RM} cells in peripheral tissues for protection from disease (Muruganandah et al., 2018). A principal active mechanism of tissue retention so far defined has been expression of CD69 and antagonism of S1PR1-mediated egress (Lee et al., 2011; Mackay et al., 2013; Skon et al., 2013). Here we define a new mechanism of tissue residence involving expression of the migration inhibitory S1PR2 receptor. S1P was recently found to inhibit the in vitro migration of human CD4⁺ and CD8⁺ T_{RM} cells in an S1PR2-dependent manner (Drouillard et al., 2018). Taking these in vitro observations and our in vivo findings together, it can be proposed that S1PR2 will contribute to the tissue residence of some T cell types in humans. Our work suggests a model in which S1PR2 and CD69 cooperate to restrain afferent lymphatic entry of CCR6⁺ γδ T cells, with neither system alone being sufficient to achieve stable tissue residence of the cells (Fig. S3). Some CCR6⁺ γδ T cells down-regulate CD69, perhaps in response to local signals in the dermis, and preferentially reach the skin dLN. The insufficiency of S1PR2 to antagonize this migration may be a consequence of the relatively low level of S1PR2 expression on dermal CCR6⁺ γδ T cells compared, for example, to germinal center B cells (Fig. 2 a). Alternatively, while *S1pr2* mRNA expression is maintained in dLN CCR6⁺ γδ T cells, it is possible that S1PR2 protein has been down-regulated in the migratory subset of CD69^{lo}S1PR1^{hi} γδ T cells. Despite the increased flux of CCR6⁺ γδ T cells to the dLN in *S1pr2*$^{-/-}$ (and *Cd69*$^{-/-}$) mice, there is no change in the total number of cells in the dermis, likely due to the increased cell proliferation compensating for the loss due to egress. How dermal CCR6⁺ γδ T cells are able to sense this

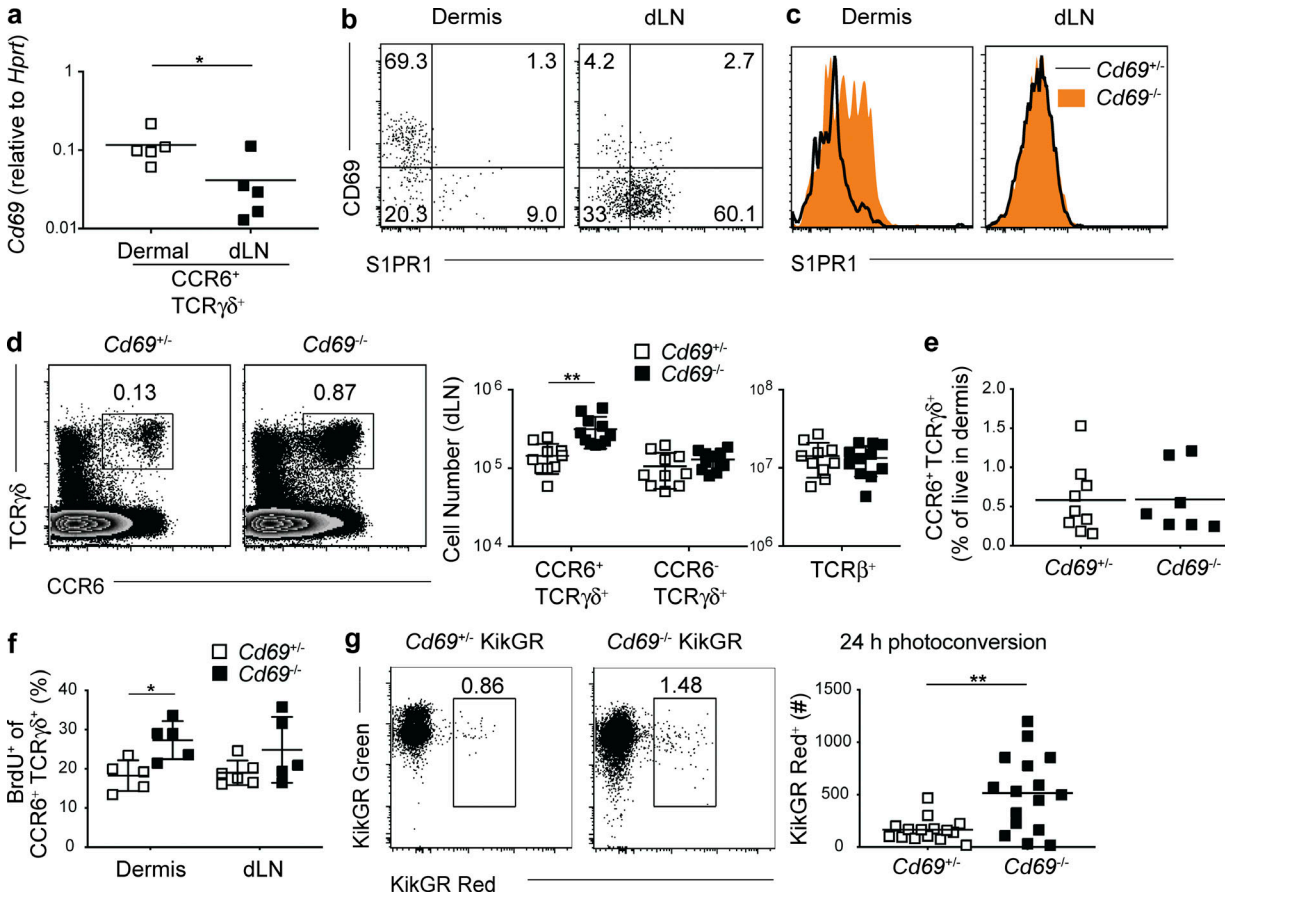

Figure 4. **CD69 restrains S1PR1 expression and limits CCR6⁺ γδ T cell migration from the skin. (a)** Expression of *Cd69* in sorted CCR6⁺ TCRγδ⁺ cells from the dermis or dLN. mRNA expression is pooled from two independent experiments with two or three mice per group. **(b)** Expression of S1PR1 and CD69 on CCR6⁺ TCRγδ⁺ cells from the dermis or dLN by flow cytometry. Data are representative of four independent experiments with one to three mice per experiment. **(c)** Expression of S1PR1 on CCR6⁺ TCRγδ⁺ cells from the dermis or dLN in *Cd69*$^{+/-}$ and *Cd69*$^{-/-}$ mice by flow cytometry. Data are representative of three independent experiments with one to three mice per experiment. **(d)** Representative FACS plots of CCR6⁺ TCRγδ⁺ cells (top) and numbers of CCR6⁺ and CCR6⁻ TCRγδ⁺ cells (bottom left) in the dLN of *Cd69*$^{+/-}$ and *Cd69*$^{-/-}$ mice. Numbers of TCRβ⁺ cells in the dLN are shown on bottom right. Data are pooled from five independent experiments with one to three mice per group. **(e)** Percentage of CCR6⁺ TCRγδ⁺ in the dermis of *Cd69*$^{+/-}$ and *Cd69*$^{-/-}$ mice. Data are pooled from three independent experiments with one to three mice per group. **(f)** Percentage of BrdU⁺ cells among the CCR6⁺ TCRγδ⁺ population in the dermis (left) and dLN (right) of *Cd69*$^{+/-}$ and *Cd69*$^{-/-}$ mice. Data are pooled from two independent experiments with one or two mice per group. **(g)** Representative FACS plots (left) and numbers (right) of KikGR Red⁺ cells among the TCRγδ⁺ cells in the dLN of *Cd69*$^{+/-}$ or *Cd69*$^{-/-}$ KikGR mice 24 h after photoactivation of the ear tissue. Data are pooled from five independent experiments with one to three mice per group. Statistical analyses were performed using the unpaired two-tailed Student's *t* test. *, P < 0.05; **, P < 0.01.

increased flux remains to be determined, with the increased proliferation in the dermis but not dLN suggesting a local signal might be important. Future work will also be needed to reveal how generalizable the role of S1PR2 is in promoting leukocyte retention in peripheral tissues. It will also be of interest to determine whether local administration of an S1PR2 antagonist may be an effective treatment for diseases such as psoriasis that are induced by an overactive tissue-resident lymphocyte response (Blankenbach et al., 2016).

Materials and methods

Mice

Adult C57BL/6 CD45.1⁺ (stock number 564) mice ≥6 wk of age were purchased from the National Cancer Institute (NCI) or NCI at Charles River. *Sphk1*$^{f/-}$ *Sphk2*$^{-/-}$ mice were provided by Dr. Shaun Coughlin (University of California, San Francisco, San Francisco, CA) and crossed to *Mx1*^{Cre} mice (Kühn et al., 1995; Pappu et al., 2007). *Lyve1*^{Cre}, *S1pr2*$^{Venus/+}$, *Cd69*$^{-/-}$, *S1pr2*$^{-/-}$ mice were generated as described (Lauzurica et al., 2000; Kono et al., 2004; Shiow et al., 2006; Pham et al., 2010; Moriyama et al., 2014). *CAG::KikGR*$^{Tg/+}$ mice were obtained from Jackson Laboratories (013753) and backcrossed to C57BL/6 for at least six generations (Nowotschin and Hadjantonakis, 2009). Mice were housed in a specific pathogen–free environment in the Laboratory Animal Research Center at the University of California, San Francisco (UCSF). All animal procedures were approved by the UCSF Institutional Animal Care and Use Committee.

Tissue preparation

LNs and ear skin were digested as described previously (Gray et al., 2013). LNs were digested with rotation for

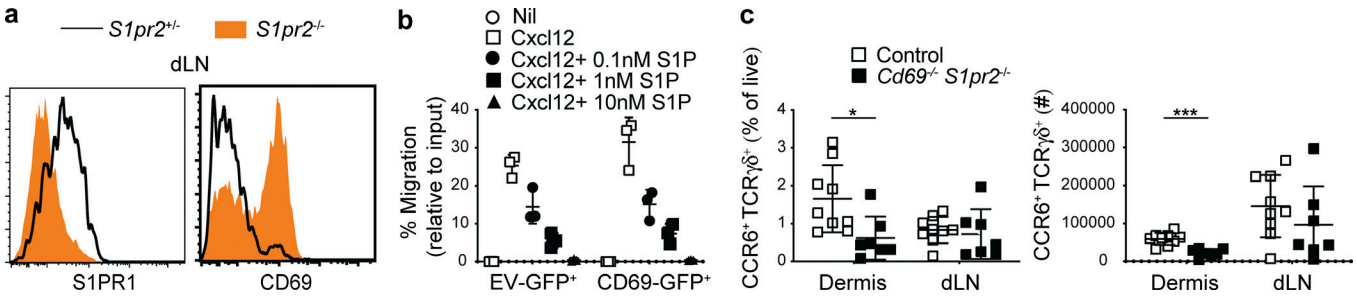

Figure 5. **CD69 and S1PR2 cooperate to restrain CCR6⁺ γδ T cell migration from the skin. (a)** Expression of S1PR1 and CD69 on CCR6⁺ TCRγδ⁺ cells from the dLN of *S1pr2*$^{+/-}$ and *S1pr2*$^{-/-}$ mice by flow cytometry. Data are representative of two independent experiments with one to three mice per experiment. **(b)** Percentage migration of S1PR2-Thy1.1⁺ WEHI231 cells transduced with control (EV-GFP) or CD69-GFP expressing retrovirus, to the indicated stimuli. Data are representative of two to four independent experiments with three wells per condition for experiment. **(c)** Percentage of CCR6⁺ TCRγδ⁺ cells in the dermis (left) and dLN (right) of control and *Cd69*$^{-/-}$*S1pr2*$^{-/-}$ mice. Data are pooled from five independent experiments with one to three mice per group. Statistical analyses were performed using the unpaired two-tailed Student's *t* test. *, P < 0.05; ***, P < 0.001.

20–30 min at 37°C with 67 µg/ml Liberase TM (Roche Applied Science) and 20 µg/ml DNaseI (Sigma-Aldrich). Ears were split into dorsal and ventral halves and were digested in 2 mg/ml Dispase (Gibco). Separated epidermal and dermal sheets were digested for 60–120 min at 37°C, with rotation, in DMEM containing penicillin–streptomycin, Hepes buffer, 85 µg/ml Liberase TM, 100 µg/ml DNaseI, 0.5 mg/ml hyaluronidase (Sigma-Aldrich), and 2% FCS. Alternatively, whole ear skin was minced and digested with liberase, DNase I, and hyaluronidase as described above. Digestion enzymes were quenched by the addition of 5 mM EDTA and 1% serum. All tissues were disaggregated by passage through a 70-µm or 100-µm nylon sieve (BD Bioscience).

Transwell migration assay

LN and ear skin were prepared as described above and resuspended in RPMI medium containing 10 mM Hepes and 2% fatty acid–free BSA. Lymphocytes from ear skin samples were further isolated using Lympholyte-M Separation Media (Cedarlane Labs) and were rested for 40 min in media containing 1 ng/ml recombinant mouse IL-7. Cells were then tested for transmigration across uncoated 5-µm transwell filters for 3 h to medium alone or to media containing 0.1 µg/ml CXCL12. In some cases, cells were incubated with medium containing 0.1 µg/ml CXCL12 and 1 nM, 10 nM, or 100 nM S1P with or without 10 µM of the S1PR2 antagonist JTE-013 (Tocris Bioscience). WEHI231 cells in which a fraction of cells expressed S1PR2-Thy1.1 were transduced with EV-GFP or CD69-GFP retroviruses, rested for 1 d, and then tested for migration across uncoated 5-µm transwell filters for 3 h to the agents described above. The response of Thy1.1⁺ GFP⁺ cells was studied.

Neonatal thymocyte transfer and BM chimeras

A total of 5–10 × 10^6 thymocytes harvested within 48 h of birth were transferred i.v. to congenic recipients lethally irradiated with a split dose of 1,300 rad. The next day, 3–6 × 10^6 congenic BM cells were transferred. Recipient mice were analyzed ≥8 wk later.

FTY720 and AUY954 treatment and in vivo BrdU labeling

Mice were treated with FTY720 (or saline) at 1 mg/kg i.v. every 2–3 d and sacrificed 1 wk after first treatment. Mice were treated with AUY954 (Cellagen Technology) at 3 mg/kg orally at time 0 and 12 h and analyzed 1 d after first treatment. For experiments involving BrdU, mice were maintained on drinking water containing BrdU (0.7 mg/ml) and glucose (1%) until analysis.

LN entry blockade

25 × 10^6 T cells transduced with EV-Thy1.1 or S1PR2-Thy1.1 retroviruses were transferred to CD45 congenically distinct recipient mice. The next day, mice were treated with integrin-neutralizing antibodies administered i.p. at 100 µg per mouse. The anti-αL (clone M17/4, rat IgG2a) and anti-α4 (clone PS/2, rat IgG2b) neutralizing antibodies were from Bio X Cell. Mice were analyzed at the time of treatment or 18 h later.

Photoconversion

A 415-nm Silver LED light source with a 1.5-mm (core diameter) light guide and collimating adaptor (Prizmatix) was used for photoconversion. Mice were anesthetized with ketamine, and ventral ear skin was exposed for 5–10 min to 415-nm violet light (~1 cm diameter). Ear draining (cervical) LNs were analyzed 1 d later. For some experiments, clipped abdominal skin was also exposed to 415-nm violet light (~1 cm diameter) for 5 min. In these cases, the inguinal, axillary, and brachial LNs were pooled with the auricular LN for the analysis. Flow cytometry was used to assess photoconversion as previously described (Gray et al., 2013).

Antibodies for flow cytometry

The following antibodies were used for flow: PE-indotricarbocyanine (PE-Cy7) anti-CD69 (H1.2F3), Biotin anti-CD69 (H1.2F3), Pacific Blue anti-TCRβ (H57-597; all from BioLegend); allophycocyanin (APC) anti-CCR6 (140706), PE-Cy7 anti-TCRγδ (GL3), FITC anti-TCRγδ (GL3), FITC anti-Vγ4 (UC3-10A6), PE-Cy7 anti-Vγ4 (UC3-10A6), Brilliant Violet 605 (BV605), streptavidin (110738; all from BD Biosciences); FITC anti-BrdU (BU20A; from eBioscience); and PerCP-Cy5.5 anti-CD45.2 (65-0454-U100; Tonbo Biosciences). For

S1PR1 staining, cells were incubated with a rat monoclonal antibody raised against an S1PR1–N-terminal peptide as previously described (R&D Systems; Arnon et al., 2011). For intracellular staining of IL-17A, cells were stimulated for 2 h with 50 ng/ml PMA (Sigma-Aldrich) and 1 µg/ml ionomycin (EMD Biosciences) in brefeldin A (BD Biosciences). Cells were stained with the fixable viability dye eFluor 780 according to the manufacturer's instructions (eBioscience) for the exclusion of dead cells, nonspecific binding was blocked with anti-CD16/32 (clone 2.4G2; University of California, San Francisco, Hybridoma Core) in 5% normal mouse and rat serum, and then cells were stained for surface antigens and fixed with BD Cytofix Buffer. After permeabilization with Perm/Wash reagent (BD Biosciences), cells were stained with anti–IL-17A (eBio17B7; eBioscience). Flow cytometry data were acquired on a BD LSRII with FACSDiva software and were analyzed with FlowJo software (TreeStar).

Real-time PCR

Total RNA was isolated and converted to cDNA as described previously (Gray et al., 2011). A StepOnePlus real-time PCR system (Applied Biosystems) with iTaq SYBR Green Supermix (Bio-Rad) and the appropriate primer pairs (Integrated DNA Technologies) were used for real-time PCR. The following primers were used: *Cd69* forward, 5′-TGGTCCTCATCACGTCCTTAATAA-3′; reverse, 5′-TCCAACTTCTCGTACAAGCCTG-3′. *S1pr1* forward, 5′-GTGTAGACCCAGAGTCCTGCG-3′; reverse, 5′-AGCTTTTCCTTGGCTGGAGAG-3′. *S1pr2* forward, 5′-GGCCTAGCCAGTGCTCAGC-3′; reverse, 5′-CCTTGGTGTAATTGTAGTGTTCCAGA-3′. *S1pr3* forward, 5′-GGAGCCCCTAGACGGGAGT-3′; reverse, 5′-CCGACTGCGGGAAGAGTGT-3′. *S1pr4* forward, 5′-CCTGGAACTCACTTTATAGACCAGG-3′; reverse, 5′-AGAAAGCGTGCCATAGGCAG-3′. *S1pr5* forward, 5′-GAGTGCCGGTTACAGGAGACTT-3′; reverse, 5′-CGCTGCTGTGTCCTGCC-3′; *Hprt* forward, 5′-AGGTTGCAAGCTTGCTGGT-3′; reverse, 5′-TGAAGTACTCATTATAGTCAAGGGCA-3′.

Statistical analysis

Results represent the mean ± SEM unless indicated otherwise. Statistical significance was determined by the unpaired Student's *t* test. Statistical analyses were performed using Prism GraphPad software v5.0. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Online supplemental material

Fig. S1 shows an example flow-cytometric gating scheme to identify dermal CCR6$^+$ γδ T cells and the percentage of CCR6$^+$ γδ T cells in the dermis and dLN of mice lacking lymphatic endothelial cell–derived S1P. Fig. S2 shows additional phenotyping of *S1pr2*$^{-/-}$ and *Cd69*$^{-/-}$ mice and includes data revealing a role for S1PR2 in opposing T cell egress from the LN. Fig. S3 shows a model of how S1PR2 and CD69 mediated regulation of CCR6$^+$ γδ T cell migration from the skin.

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J.G. Cyster is an investigator of the Howard Hughes Medical Institute. E.E. Gray is affiliated with Seattle Genetics. The authors declare no other competing financial interests.

Author contributions: Conceived and designed the experiments: B.J. Laidlaw, E.E. Gray, Y. Zhang, F. Ramírez-Valle, and J.G. Cyster. Performed the experiments: B.J. Laidlaw, E.E. Gray, Y. Zhang, and F. Ramírez-Valle. Analyzed the data: B.J. Laidlaw, E.E. Gray, Y. Zhang, F. Ramírez-Valle, and J.G. Cyster. Wrote the manuscript: B.J. Laidlaw and J.G. Cyster.

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References

Arnon, T.I., Y. Xu, C. Lo, T. Pham, J. An, S. Coughlin, G.W. Dorn, and J.G. Cyster. 2011. GRK2-dependent S1PR1 desensitization is required for lymphocytes to overcome their attraction to blood. *Science.* 333: 1898–1903. https://doi.org/10.1126/science.1208248

Bankovich, A.J., L.R. Shiow, and J.G. Cyster. 2010. CD69 suppresses sphingosine 1-phosophate receptor-1 (S1P1) function through interaction with membrane helix 4. *J. Biol. Chem.* 285:22328–22337. https://doi.org/10.1074/jbc.M110.123299

Blankenbach, K.V., S. Schwalm, J. Pfeilschifter, and D. Meyer Zu Heringdorf. 2016. Sphingosine-1-Phosphate Receptor-2 Antagonists: Therapeutic Potential and Potential Risks. *Front. Pharmacol.* 7:167. https://doi.org/10.3389/fphar.2016.00167

Brinkmann, V., A. Billich, T. Baumruker, P. Heining, R. Schmouder, G. Francis, S. Aradhye, and P. Burtin. 2010. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat. Rev. Drug Discov.* 9:883–897. https://doi.org/10.1038/nrd3248

Cai, Y., X. Shen, C. Ding, C. Qi, K. Li, X. Li, V.R. Jala, H.-G. Zhang, T. Wang, J. Zheng, and J. Yan. 2011. Pivotal role of dermal IL-17-producing γδ T cells in skin inflammation. *Immunity.* 35:596–610. https://doi.org/10.1016/j.immuni.2011.08.001

Davies, B., J.E. Prier, C.M. Jones, T. Gebhardt, F.R. Carbone, and L.K. Mackay. 2017. Cutting Edge: Tissue-Resident Memory T Cells Generated by Multiple Immunizations or Localized Deposition Provide Enhanced Immunity. *J. Immunol.* 198:2233–2237. https://doi.org/10.4049/jimmunol.1601367

Debes, G.F., C.N. Arnold, A.J. Young, S. Krautwald, M. Lipp, J.B. Hay, and E.C. Butcher. 2005. Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. *Nat. Immunol.* 6:889–894. https://doi.org/10.1038/ni1238

Dillen, C.A., B.L. Pinsker, A.I. Marusina, A.A. Merleev, O.N. Farber, H. Liu, N.K. Archer, D.B. Lee, Y. Wang, R.V. Ortines, et al. 2018. Clonally expanded γδ T cells protect against Staphylococcus aureus skin reinfection. *J. Clin. Invest.* 128:1026–1042. https://doi.org/10.1172/JCI96481

Drouillard, A., A. Neyra, A.-L. Mathieu, A. Marçais, M. Wencker, J. Marvel, A. Belot, and T. Walzer. 2018. Human Naive and Memory T Cells Display Opposite Migratory Responses to Sphingosine-1 Phosphate. *J. Immunol.* 200:551–557. https://doi.org/10.4049/jimmunol.1701278

Gatzka, M., A. Hainzl, T. Peters, K. Singh, A. Tasdogan, M. Wlaschek, and K. Scharffetter-Kochanek. 2013. Reduction of CD18 promotes expansion of inflammatory γδ T cells collaborating with CD4+ T cells in chronic murine psoriasiform dermatitis. *J. Immunol.* 191:5477–5488. https://doi.org/10.4049/jimmunol.1300976

Gray, E.E., K. Suzuki, and J.G. Cyster. 2011. Cutting edge: Identification of a motile IL-17-producing gammadelta T cell population in the dermis. *J. Immunol.* 186:6091–6095. https://doi.org/10.4049/jimmunol.1100427

Gray, E.E., F. Ramírez-Valle, Y. Xu, S. Wu, Z. Wu, K.E. Karjalainen, and J.G. Cyster. 2013. Deficiency in IL-17-committed Vγ4(+) γδ T cells in a spontaneous Sox13-mutant CD45.1(+) congenic mouse substrain provides protection from dermatitis. *Nat. Immunol.* 14:584–592. https://doi.org/10.1038/ni.2585

Green, J.A., and J.G. Cyster. 2012. S1PR2 links germinal center confinement and growth regulation. *Immunol. Rev.* 247:36–51. https://doi.org/10.1111/j.1600-065X.2012.01114.x

Green, J.A., K. Suzuki, B. Cho, L.D. Willison, D. Palmer, C.D.C. Allen, T.H. Schmidt, Y. Xu, R.L. Proia, S.R. Coughlin, and J.G. Cyster. 2011. The sphingosine 1-phosphate receptor $S1P_2$ maintains the homeostasis of germinal center B cells and promotes niche confinement. *Nat. Immunol.* 12:672–680. https://doi.org/10.1038/ni.2047

Gunn, M.D., K. Tangemann, C. Tam, J.G. Cyster, S.D. Rosen, and L.T. Williams. 1998. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 95:258–263. https://doi.org/10.1073/pnas.95.1.258

Jiang, X., C.O. Park, J. Geddes Sweeney, M.J. Yoo, O. Gaide, and T.S. Kupper. 2017. Dermal γδ T Cells Do Not Freely Re-Circulate Out of Skin and Produce IL-17 to Promote Neutrophil Infiltration during Primary Contact Hypersensitivity. *PLoS One.* 12:e0169397. https://doi.org/10.1371/journal.pone.0169397

Kabashima, K., N. Shiraishi, K. Sugita, T. Mori, A. Onoue, M. Kobayashi, J. Sakabe, R. Yoshiki, H. Tamamura, N. Fujii, et al. 2007. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. *Am. J. Pathol.* 171:1249–1257. https://doi.org/10.2353/ajpath.2007.070225

Kono, M., Y. Mi, Y. Liu, T. Sasaki, M.L. Allende, Y.-P. Wu, T. Yamashita, and R.L. Proia. 2004. The sphingosine-1-phosphate receptors S1P1, S1P2, and S1P3 function coordinately during embryonic angiogenesis. *J. Biol. Chem.* 279:29367–29373. https://doi.org/10.1074/jbc.M403937200

Kühn, R., F. Schwenk, M. Aguet, and K. Rajewsky. 1995. Inducible gene targeting in mice. *Science.* 269:1427–1429. https://doi.org/10.1126/science.7660125

Lai, Y., A. Di Nardo, T. Nakatsuji, A. Leichtle, Y. Yang, A.L. Cogen, Z.-R. Wu, L.V. Hooper, R.R. Schmidt, S. von Aulock, et al. 2009. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat. Med.* 15:1377–1382. https://doi.org/10.1038/nm.2062

Lauzurica, P., D. Sancho, M. Torres, B. Albella, M. Marazuela, T. Merino, J.A. Bueren, C. Martínez-A, and F. Sánchez-Madrid. 2000. Phenotypic and functional characteristics of hematopoietic cell lineages in CD69-deficient mice. *Blood.* 95:2312–2320.

Lee, Y.-T., J.E. Suarez-Ramirez, T. Wu, J.M. Redman, K. Bouchard, G.A. Hadley, and L.S. Cauley. 2011. Environmental and antigen receptor-derived signals support sustained surveillance of the lungs by pathogen-specific cytotoxic T lymphocytes. *J. Virol.* 85:4085–4094. https://doi.org/10.1128/JVI.02493-10

Mackay, L.K., A. Rahimpour, J.Z. Ma, N. Collins, A.T. Stock, M.-L. Hafon, J. Vega-Ramos, P. Lauzurica, S.N. Mueller, T. Stefanovic, et al. 2013. The developmental pathway for CD103(+)CD8+ tissue-resident memory T cells of skin. *Nat. Immunol.* 14:1294–1301. https://doi.org/10.1038/ni.2744

Mackay, L.K., A. Braun, B.L. Macleod, N. Collins, C. Tebartz, S. Bedoui, F.R. Carbone, and T. Gebhardt. 2015. Cutting edge: CD69 interference with sphingosine-1-phosphate receptor function regulates peripheral T cell retention. *J. Immunol.* 194:2059–2063. https://doi.org/10.4049/jimmunol.1402256

McKenzie, D.R., E.E. Kara, C.R. Bastow, T.S. Tyllis, K.A. Fenix, C.E. Gregor, J.J. Wilson, R. Babb, J.C. Paton, A. Kallies, et al. 2017. IL-17-producing γδ T cells switch migratory patterns between resting and activated states. *Nat. Commun.* 8:15632. https://doi.org/10.1038/ncomms15632

Mendoza, A., V. Fang, C. Chen, M. Serasinghe, A. Verma, J. Muller, V.S. Chaluvadi, M.L. Dustin, T. Hla, O. Elemento, et al. 2017. Lymphatic endothelial S1P promotes mitochondrial function and survival in naive T cells. *Nature.* 546:158–161. https://doi.org/10.1038/nature22352

Michaud, J., D.-S. Im, and T. Hla. 2010. Inhibitory role of sphingosine 1-phosphate receptor 2 in macrophage recruitment during inflammation. *J. Immunol.* 184:1475–1483. https://doi.org/10.4049/jimmunol.0901586

Moriyama, S., N. Takahashi, J.A. Green, S. Hori, M. Kubo, J.G. Cyster, and T. Okada. 2014. Sphingosine-1-phosphate receptor 2 is critical for follicular helper T cell retention in germinal centers. *J. Exp. Med.* 211:1297–1305. https://doi.org/10.1084/jem.20131666

Muruganandah, V., H.D. Sathkumara, S. Navarro, and A. Kupz. 2018. A Systematic Review: The Role of Resident Memory T Cells in Infectious Diseases and Their Relevance for Vaccine Development. *Front. Immunol.* 9:1574. https://doi.org/10.3389/fimmu.2018.01574

Naik, S., N. Bouladoux, C. Wilhelm, M.J. Molloy, R. Salcedo, W. Kastenmüller, C. Deming, M. Quinones, L. Koo, S. Conlan, et al. 2012. Compartmentalized control of skin immunity by resident commensals. *Science.* 337:1115–1119. https://doi.org/10.1126/science.1225152

Nakamizo, S., G. Egawa, M. Tomura, S. Sakai, S. Tsuchiya, A. Kitoh, T. Honda, A. Otsuka, S. Nakajima, T. Dainichi, et al. 2015. Dermal Vγ4(+) γδ T cells possess a migratory potency to the draining lymph nodes and modulate CD8(+) T-cell activity through TNF-α production. *J. Invest. Dermatol.* 135:1007–1015. https://doi.org/10.1038/jid.2014.516

Nowotschin, S., and A.-K. Hadjantonakis. 2009. Use of KikGR a photoconvertible green-to-red fluorescent protein for cell labeling and lineage analysis in ES cells and mouse embryos. *BMC Dev. Biol.* 9:49. https://doi.org/10.1186/1471-213X-9-49

Ohl, L., M. Mohaupt, N. Czeloth, G. Hintzen, Z. Kiafard, J. Zwirner, T. Blankenstein, G. Henning, and R. Förster. 2004. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. *Immunity.* 21:279–288. https://doi.org/10.1016/j.immuni.2004.06.014

Pan, S., Y. Mi, C. Pally, C. Beerli, A. Chen, D. Guerini, K. Hinterding, B. Nuesslein-Hildesheim, T. Tuntland, S. Lefebvre, et al. 2006. A monoselective sphingosine-1-phosphate receptor-1 agonist prevents allograft rejection in a stringent rat heart transplantation model. *Chem. Biol.* 13:1227–1234. https://doi.org/10.1016/j.chembiol.2006.09.017

Pappu, R., S.R. Schwab, I. Cornelissen, J.P. Pereira, J.B. Regard, Y. Xu, E. Camerer, Y.-W. Zheng, Y. Huang, J.G. Cyster, and S.R. Coughlin. 2007. Promotion of lymphocyte egress into blood and lymph by distinct sources of sphingosine-1-phosphate. *Science.* 316:295–298. https://doi.org/10.1126/science.1139221

Pham, T.H.M., T. Okada, M. Matloubian, C.G. Lo, and J.G. Cyster. 2008. S1P1 receptor signaling overrides retention mediated by G alpha i-coupled receptors to promote T cell egress. *Immunity.* 28:122–133. https://doi.org/10.1016/j.immuni.2007.11.017

Pham, T.H.M., P. Baluk, Y. Xu, I. Grigorova, A.J. Bankovich, R. Pappu, S.R. Coughlin, D.M. McDonald, S.R. Schwab, and J.G. Cyster. 2010. Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J. Exp. Med.* 207:17–27. https://doi.org/10.1084/jem.20091619

Ramírez-Valle, F., E.E. Gray, and J.G. Cyster. 2015. Inflammation induces dermal Vγ4+ γδT17 memory-like cells that travel to distant skin and accelerate secondary IL-17-driven responses. *Proc. Natl. Acad. Sci. USA.* 112:8046–8051. https://doi.org/10.1073/pnas.1508990112

Ridaura, V.K., N. Bouladoux, J. Claesen, Y.E. Chen, A.L. Byrd, M.G. Constantinides, E.D. Merrill, S. Tamoutounour, M.A. Fischbach, and Y. Belkaid. 2018. Contextual control of skin immunity and inflammation by *Corynebacterium*. *J. Exp. Med.* 215:785–799. https://doi.org/10.1084/jem.20171079

Sandrock, I., A. Reinhardt, S. Ravens, C. Binz, A. Wilharm, J. Martins, L. Oberdörfer, L. Tan, S. Lienenklaus, B. Zhang, et al. 2018. Genetic models reveal origin, persistence and non-redundant functions of IL-17–producing γδ T cells. *J. Exp. Med.* 215:3006–3018. https://doi.org/10.1084/jem.20181439

Shiow, L.R., D.B. Rosen, N. Brdicková, Y. Xu, J. An, L.L. Lanier, J.G. Cyster, and M. Matloubian. 2006. CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. *Nature.* 440:540–544. https://doi.org/10.1038/nature04606

Skon, C.N., J.-Y. Lee, K.G. Anderson, D. Masopust, K.A. Hogquist, and S.C. Jameson. 2013. Transcriptional downregulation of S1pr1 is required for the establishment of resident memory CD8+ T cells. *Nat. Immunol.* 14:1285–1293. https://doi.org/10.1038/ni.2745

Sobel, K., L. Monnier, K. Menyhart, M. Bolinger, R. Studer, O. Nayler, and J. Gatfield. 2015. FTY720 Phosphate Activates Sphingosine-1-Phosphate Receptor 2 and Selectively Couples to Gα12/13/Rho/ROCK to Induce Myofibroblast Contraction. *Mol. Pharmacol.* 87:916–927. https://doi.org/10.1124/mol.114.097261

Spidale, N.A., K. Sylvia, K. Narayan, B. Miu, M. Frascoli, H.J. Melichar, W. Zhihao, J. Kisielow, A. Palin, T. Serwold, et al. 2018. Interleukin-17-Producing γδ T Cells Originate from SOX13$^+$ Progenitors that Are Independent of γδTCR Signaling. *Immunity.* 49:857–872.e5. https://doi.org/10.1016/j.immuni.2018.09.010

Sumaria, N., B. Roediger, L.G. Ng, J. Qin, R. Pinto, L.L. Cavanagh, E. Shklovskaya, B. Fazekas de St Groth, J.A. Triccas, and W. Weninger.

2011. Cutaneous immunosurveillance by self-renewing dermal gammadelta T cells. *J. Exp. Med.* 208:505–518. https://doi.org/10.1084/jem.20101824

Takuwa, N., W. Du, E. Kaneko, Y. Okamoto, K. Yoshioka, and Y. Takuwa. 2011. Tumor-suppressive sphingosine-1-phosphate receptor-2 counteracting tumor-promoting sphingosine-1-phosphate receptor-1 and sphingosine kinase 1 - Jekyll Hidden behind Hyde. *Am. J. Cancer Res.* 1: 460–481.

Vrieling, M., W. Santema, I. Van Rhijn, V. Rutten, and A. Koets. 2012. γδ T cell homing to skin and migration to skin-draining lymph nodes is CCR7 independent. *J. Immunol.* 188:578–584. https://doi.org/10.4049/jimmunol.1101972

Zhang, Y., T.L. Roth, E.E. Gray, H. Chen, L.B. Rodda, Y. Liang, P. Ventura, S. Villeda, P.R. Crocker, and J.G. Cyster. 2016. Migratory and adhesive cues controlling innate-like lymphocyte surveillance of the pathogen-exposed surface of the lymph node. *eLife.* 5:e18156. https://doi.org/10.7554/eLife.18156