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Effector T cell egress via afferent lymph modulates local tissue inflammation[1]

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Abstract

Memory/effector T cells recirculate through extralymphoid tissues by entering from blood and egressing via afferent lymph. While T cell entry into effector sites is key to inflammation, the relevance of T cell egress to this process is unknown. Here we found that antigen recognition at the effector site reduced the tissue egress of pro-inflammatory Th1 cells in a mouse model of delayed hypersensitivity. Transgenic expression of 'tissue exit receptor' CCR7 enhanced lymphatic egress of antigen-sequestered Th1 cells from the inflamed site and ameliorated inflammation. In contrast, lack of CCR7 on Th1 cells diminished their tissue egress while enhancing inflammation. Lymph-borne Th1 and Th17 cells draining the inflamed skin of sheep migrated toward the CCR7 ligand CCL21, suggesting the CCR7-CCL21 axis as a physiological target in regulating inflammation. In conclusion, exit receptors can be targeted to modulate T cell dwell time and inflammation at effector sites, revealing T cell tissue egress as a novel control point of inflammation.

Introduction

During immunosurveillance and inflammation, effector/memory T cells, unlike naïve T cells, recirculate through extralymphoid tissues, entering from the blood and exiting via the afferent lymph (1). From the afferent lymph, T cells reach regional lymph nodes, which they leave via the efferent lymph, returning them back into blood. T cell migration into tissues is regulated by a multistep cascade involving adhesion and chemoattractant receptors on T cells interacting with their respective endothelial ligands (2). Similarly, T cell egress from lymph nodes is controlled by chemoattractant sphingosine-1 phosphate and its receptors (3). In the absence of inflammation, $CD4^+$ and $CD8^+$ T cells require expression of CCR7 to egress from extralymphoid tissues (4, 5). Congruently, lymphatic endothelial cells constitutively express the CCR7 ligand CCL21 in many organs (6).

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T cell entry into effector sites is critical for inflammation and a target of anti-inflammatory therapy (7). Given the large number of lymphocytes, including pro-inflammatory Th1 and Th17 cells, that egress from chronically inflamed sites (8, 9); T cell egress potentially determines effector T cell accumulation and their downstream effector functions *in situ*. During inflammation and infection, the CCR7-CCL21 receptor-ligand pair guides T cells out of affected tissues via afferent lymph (5, 9, 10), and CCR7 deficiency promotes the development of long-term tissue-resident memory T cells (T_{RM})[4] key to site-specific immunity (11). However, inflammation also facilitates CCR7-independent T cell tissue egress (9, 12) with contributions from additional chemoattractants, such as sphingosine-1 phosphate (9, 13).

Ccr7$^{-/-}$ mice demonstrate exacerbated inflammation in various models, such as cutaneous hypersensitivity (14), chronic arthritis (15), gastrointestinal inflammation (16, 17), and autoimmunity (reviewed in (18)), suggesting a role of T cell egress in regulating tissue inflammation. Conversely, mice with transgenic expression of *Ccr7* (*Ccr7*^{TG}) exhibit reduced effector responses (19). Nevertheless, in these models it is impossible to dissect the role of T cell egress in inflammation, in particular as other anomalies contribute to the observed phenotypes, *e.g.* regulatory T cell defects in *Ccr7*$^{-/-}$ mice (14, 20) or retention of *Ccr7*^{TG} T cells in splenic white pulp (19). As a result, although discussed (9, 16, 17, 21), the role of T cell egress in the modulation of tissue inflammation remains unresolved.

In this study, we revisited this question and demonstrate that targeting the tissue egress capacity of pro-inflammatory T cells modulates the local inflammatory response, enhancing our understanding of the pathogenesis of inflammation and laying the foundation for future therapies for inflammatory diseases.

Materials and Methods

Animals and surgical procedures in sheep

All mice were on C57BL/6 background, bred in our animal facility, and used between 8 and 16 weeks of age in male or female sex- and age-matched groups. CD45.1 and CD45.2 congenic mice, and OTII mice were obtained from The Jackson Laboratory, *Ccr7*$^{-/-}$ mice (22) were from Martin Lipp (Max Delbrück Center), *Ccr7*^{TG} mice (23) from Nigel Killeen (UCSF), and *plt* mice (24) from Avinash Bhandoola. OTII mice were crossed with *Ccr7*$^{-/-}$ mice and with *Ccr7*^{TG} mice to obtain *Ccr7*$^{-/-}$ OTII mice and *Ccr7*^{TG} OTII mice, respectively. *Ccr7*$^{-/-}$ OTII and *Ccr7*^{TG} OTII breeders, but not their offspring, were maintained on an antibiotic diet (Mouse Helicobacter MD's™ 4 Drug Combo, Bio-Serv). For sheep experiments, 5–10 month-old female mixed breed or Dorset sheep were purchased from Animal Biotech Industries or Pine Ridge Dorsets, respectively. Skin draining 'pseudoafferent' lymph vessels were induced by lymphectomy of the subiliac (prefemoral) lymph nodes as detailed (25). Pseudoafferent (prenodal) skin draining lymph vessels were cannulated with heparin-coated sterile catheters (Carmeda) in a surgical procedure under isoflurane anesthesia as described (9, 25). Lymph was collected from unanesthetized

[4]Abbreviations used: dLN, draining lymph node; eF670, eFluor670; MAC, monoclonal antibody core; *Ccr7*^{TG}, *Ccr7*-transgenic; T_{RM}, resident memory T cells; WT, wild-type.

animals into sterile collection bottles containing heparin (APP Pharmaceuticals, LLC). All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Cell isolation, culture, and labeling, and chemotaxis assay

Lymphocytes were isolated from lymph nodes and spleens by passage through 40 μm cell strainers (BD Biosciences), as described (10). Red blood cells were lysed with 160 mM ammonium chloride and cells washed in RPMI 1640 with 10% fetal bovine serum. Th1 cells were generated from microbead-sorted (Miltenyi Biotec) $CD4^+$ T cells cultured on plate-bound anti-CD3 (145-2C11; UCSF Monoclonal Antibody Core (MAC)) and anti-CD28 (37.51; UCSF MAC) in the presence of IL-12 (R&D Systems), IFN-γ (R&D Systems), and anti-IL-4 (11B11, BioXCell) as described (9). On day 5 of culture, dead cells were removed using a Nycodenz gradient (Axis-Shield). Th1 cells were labeled with 0.5 mM CFSE (Life Sciences) or 5 mM eFluor670 (eF670; eBioscience) in HBSS with 25 mM HEPES (Gibco) for 10 minutes at 37°C. Bovine serum was added to stop the reaction, and cells were washed 3 times. Femurs and tibias were flushed to isolate bone marrow (BM) cells. BM-derived dendritic cells (BMDCs) were generated as a source of APCs by culture of BM cells with 20 ng/ml of GM-CSF (PeproTech) for 8–9 days. BMDCs were pulsed overnight with 500 ng/ml LPS (Sigma-Aldrich), 10 ng/ml mouse TNFα (R&D Systems) and 1 mg/ml of BSA (Sigma-Aldrich) or OVA protein (Sigma-Aldrich). Cells were isolated from footpad skin by mincing mouse feet followed by two 30-minute enzymatic digestion steps in HBSS at 37°C with 0.1 mg/ml DNase I (Roche) and 12.5 μg/ml Liberase TM (Roche). Subsequently, samples were passed through a 100-μm cell strainer. The chemotaxis assay was carried out and analyzed as described (26). Briefly, 5×10^5 lymph-borne cells in RPMI 1640 containing 0.5% BSA were added to the upper chamber of 5-μm pore size Transwell inserts (Corning). Mouse CCL21 (R&D Systems) was added to the lower chamber at 100 nM, its optimal concentration to attract ovine $CD4^+$ T cells (4, 27). $CD4^+$ T cells that migrated to the lower chamber during the 90 minute incubation at 37°C were quantified by flow cytometry using a bead standard (15 μm polystyrene beads, Polysciences Inc). Frequencies of cytokine producing $CD4^+$ T cells were determined in input and migrated wells by intracellular staining (described below) to calculate percent migration of total IFN-γ^+ and IL-17^+ $CD4^+$ T cells.

Induction of skin inflammation in sheep and mice, and T cell egress assay

Chronic skin inflammation was induced in sheep by subcutaneous injection of 0.3–0.5 ml of CFA (Sigma-Aldrich), emulsified at 1:1 with saline, into two sites in the area of the flank (9). ≥ 3 weeks later, lymph vessels draining the inflamed skin and the control side were cannulated and lymph-borne $CD4^+$ T cells analyzed. To elicit inflammation in mice and to test T cell egress from the site, we modified a model of delayed hypersensitivity by Streilein (28). Unless otherwise indicated, responder (OTII) Th1 cells and antigen-pulsed BMDCs generated from wild-type (WT) or CCL19-deficient *plt* mice were co-cultured at a 2:1 ratio, keeping APCs constant at 2×10^5 cells per recipient. After 1 h, the APC-T cell mixture was injected subcutaneously in 10 μl of PBS into the footpads of recipient mice, and tissue swelling was blindly measured over time using an engineer's caliper (Fisher Scientific). Δ Swelling was defined as the footpad thickness after injection of cells subtracted by pre-

injection values for the same foot. In T cell egress assays, 20–70 h post-injection of $4–6\times10^6$ responder Th1 cell subsets and 1×10^6 antigen-loaded APCs, the draining and non-draining (control) popliteal lymph nodes and spleens of recipient mice were analyzed for donor cells based on fluorescent and/or congenic labels as well as CD4 expression as described (9). Enumeration of migrated Th1 cell subsets was done by flow cytometry using a fixed number of beads (15 μm polystyrene beads) added to each sample. Cell counts and ratios were corrected for differences in input populations.

Flow cytometry

To reduce non-specific staining, mouse cells were pre-incubated with rat IgG (Jackson ImmunoResearch), anti-CD16/CD32 (2.4G2; UCSF MAC), and if necessary, with donkey and mouse IgG (Jackson ImmunoResearch). Sheep cells were pre-incubated with mouse and sheep IgG. Cells were stained for surface markers and analyzed as described (9), using fluorochrome-conjugated (fluorescein isothiocyanate, Pacific Blue, eFluor450, phycoerythrin, and/or allophycocyanin) anti-mouse antibodies from eBioscience: CD4 (RM4-5), CD45.1 (A20), and CD45.2 (104) or anti-ovine CD4 (44.38) from Serotec. CCR7 was stained with a CCL19-human Ig chimeric protein (provided by Daniel Campbell) followed by biotinylated donkey anti-human IgG $F(ab')_2$ (Jackson ImmunoResearch) and phycoerythrin-conjugated streptavidin (BD Bioscience). Dead cells were stained using the LIVE/DEAD Fixable Aqua Dead Cell Stain Kit and excluded from standard analysis or used in combination with annexin V binding to quantify dead and apoptotic cells according to the manufacturer's instructions (Life Technologies). To detect intracellular cytokines, mouse Th1 cells were stimulated with antigen-pulsed BMDCs for 5 hours. Additionally, mouse and sheep T cells were stimulated with ionomycin and PMA, and stained as described (9) using monoclonal antibodies recognizing mouse IFN-γ (XMG1.2; eBioscience), IL-4 (11B11; eBioscience), and IL-10 (JES5-16E3; BD Bioscience) or ovine IFN-γ (CC302; Serotec) and IL-17A (eBio64DEC17; eBioscience). Samples were acquired on a BD LSRII or LSRFortessa using FACSDiVa software (BD Biosciences), and analyzed with FlowJo software (Tree Star).

Statistical analysis

The Mann Whitney U test (GraphPad Prism) was used for statistical analyses, and $P < 0.05$ was considered statistically significant.

Results

Antigen recognition at the effector site decreases tissue egress of effector Th1 cells

T cell-mediated inflammation involves antigenic activation of pro-inflammatory T cells at the target site. To develop a model of T cell-mediated inflammation in which the expression of T cell exit receptors is the sole variable and that is independent of cell recruitment from the blood, we modified Streilein's "local adoptive transfer assay of delayed hypersensitivity" (28). Using OVA-specific $CD4^+$ T cells from TCR-transgenic (OTII) mice, pro-inflammatory Th1 cells were generated, co-incubated for one hour with OVA-pulsed APCs and transferred into the footpads of mice. The induced local tissue

inflammation was transient and measurable as footpad swelling that depended on the number of transferred Th1 cells (Fig. 1A).

To test the influence of antigen recognition on egress from the inflamed site, CFSE-labeled CD45.1$^+$ polyclonal and CD45.2$^+$ (OVA-specific) OTII Th1 cells were mixed, and co-incubated with APCs pulsed with OVA or a control protein (BSA) before transfer into the footpads of recipient mice. As previously (4, 9, 27), we assessed T cell tissue egress by enumerating donor T cells that had left the skin and migrated into the draining popliteal lymph node (dLN) (Fig. 1B, 1C). While at 20 h after transfer many donor Th1 cells had egressed from the inflamed site and reached the dLN, transferred cells in the contralateral lymph node or the spleen were below the level of detection (Fig. 1B, 1C, and data not shown), confirming migration via afferent lymph before transferred cells enter the blood circulation. In the presence of OVA-pulsed APCs, the number of OTII Th1 cells that left the site of inflammation and reached the dLN was drastically reduced relative to that of polyclonal Th1 cells (~75% reduction on average; $P < 0.0001$; Fig. 1C). We conclude that antigen recognition leads to down-regulation of effector CD4$^+$ T cell egress from inflamed tissue.

Transgenic expression of CCR7 enhances effector T cell egress from inflamed tissue and accelerates resolution of inflammation

To address whether tissue exit receptors can be targeted to modulate tissue egress of antigen-responding pro-inflammatory T cells, we generated Th1 cells from OTII mice and OTII mice whose T cells are *Ccr7*-transgenic (*Ccr7*^{TG}). *Ccr7*^{TG} OTII Th1 cells showed a more uniformly high CCR7 expression relative to *Ccr7*^{WT} OTII Th1 cells (Fig. 2A). These Th1 cell subsets were differentially labeled with fluorescent dyes and, after co-incubation with OVA-pulsed APCs, co-injected into the footpads of recipient mice. Strikingly, *Ccr7*^{TG} OTII Th1 cells showed a significantly enhanced capacity to egress from the inflamed skin and enter the dLN 20 h after transfer relative to *Ccr7*^{WT} OTII Th1 cells ($P = 0.0002$; Fig. 2B, 2C). At this time point after cell transfer, both *Ccr7*^{TG} and *Ccr7*^{WT} Th1 cells were below the level of detection in spleens and contralateral lymph nodes (data not shown), confirming that cells had reached the dLN through the afferent lymph. Migratory differences were independent of the cell labels, as there were no differences in the migration of differentially labeled *Ccr7*^{WT} OTII Th1 cells (Fig. 2C). At this time point, we could not detect differences in cell proliferation or survival between *Ccr7*^{WT} and *Ccr7*^{TG} OTII Th1 cells in skin or dLN (Supplemental Fig. 1A–C). The CCR7 transgene did not affect differentiation into Th1 cells as indicated by similar cytokine expression profiles after stimulation with OVA-pulsed APCs or PMA and ionomycin prior to injection (Fig. 2D, and data not shown) and after re-stimulation of transferred Th1 cells recovered from skin (Supplemental Fig. 1D). Strikingly, although both Th1 cell subsets initially induced a similar magnitude of inflammation, resolution of inflammation was accelerated in mice that had received *Ccr7*^{TG} OTII Th1 cells (Fig. 2E). We conclude that enhancing exit receptor expression is able to dislodge antigen-sequestered effector T cells from inflamed tissue, translating into faster resolution of inflammation.

Lack of T cell CCR7 enhances local tissue inflammation

Next, we analyzed *in vitro* polarized Th1 cells from $Ccr7^{-/-}$ OTII and $Ccr7^{WT}$ OTII mice (Fig. 3A), which produced cytokines at similar frequency after stimulation with OVA-pulsed APCs or PMA and ionomycin (Fig. 3B, and data not shown). Consistent with CCR7 as a major exit receptor (4, 5, 9), $Ccr7^{-/-}$ Th1 cells were drastically reduced in the dLN relative to $Ccr7^{WT}$ Th1 cells ($P = 0.0159$) and below the level of detection in the spleens of most recipients even 3 days after transfer together with OVA-loaded APCs into footpad skin (Fig. 3C, and data not shown). These results are in line with data showing that lack of T cell CCR7 promotes T cell retention at effector sites (11, 16, 17, 29). Importantly, the reduced egress of $Ccr7^{-/-}$ OTII Th1 cells from the site of inflammation translated into enhanced and prolonged inflammation in our hypersensitivity model (Fig. 3D). Together with our findings that enhancing T cell egress ameliorates the inflammatory response (Fig. 2E), we conclude that the tissue egress capacity of effector T cells is a critical determinant of the course of inflammation and that tissue exit receptors can be targeted to modulate inflammation.

The CCR7-CCL21 axis is active in tissue egressing pro-inflammatory T cells

If the CCR7-CCL21 axis is a physiological target relevant to the egress of pro-inflammatory T cells, then effector T cells that have entered the afferent lymph draining inflamed sites should be responsive to CCR7 ligand. Because analysis of afferent lymph T cells is not feasible in mice and humans, the sheep has emerged as a classical model of afferent lymph cannulation, allowing for the analysis of tissue-recirculating lymphocytes (1, 4, 8, 30). Afferent lymph vessel cannulation in sheep revealed that lymph-borne IFN-γ^+ Th1 and IL-17^+ Th17 cells egressing from uninflamed or chronically inflamed skin migrated toward CCL21 in an *ex vivo* chemotaxis assay (Fig. 4). We conclude that the CCR7-CCL21 axis is not only active in adoptively transferred effector T cells but is also a physiological target in pro-inflammatory T cells that recirculate through extralymphoid sites.

Discussion

In this paper we demonstrate that the magnitude and duration of T cell-mediated inflammation is a function of the capacity of pro-inflammatory T cells to leave the inflamed tissue via the afferent lymph. This fills an important gap in our knowledge regarding the regulation of T cell-driven inflammation and highlights the importance of studies delineating the mechanisms that regulate T cell egress versus retention.

We found that $CD4^+$ effector T cells that recognize antigen at the inflamed site greatly reduce their egress from the tissue (Fig. 1). This is in agreement with data demonstrating that upon antigenic stimulation *in situ*, virus-specific $CD8^+$ T cells decrease their egress from the lung during influenza virus infection (10) and/or develop into long-term T_{RM} cells in the vesicular stomatitis virus-infected brain (31). As T cell entry into tissues is independent of antigen specificity (32), enhancing the retention of antigen-specific T cells by decreased egress from sites of inflammation and infection ensures continued T cell effector functions, while allowing bystander T cells to egress and redistribute. Notably, also long-term retention of $CD4^+$ T cells as T_{RM} cells at effector sites was proposed to involve antigenic stimulation (33). In contrast, formation of $CD8^+$ T_{RM} cells requires additional or

alternative antigen-independent cytokine-dependent transcriptional reprograming (reviewed in (34, 35)).

Importantly, we show that tissue exit receptors can be targeted to modulate inflammation: transgenic expression of T cell CCR7 enhanced egress and accelerated resolution of inflammation, while lack of T cell CCR7 exacerbated inflammation. These data are consistent with reduced delayed hypersensitivity responses in *Ccr7*^{TG} mice, a phenotype that was attributed to effector T cell redistribution to the splenic white pulp (19). We demonstrate that an additional or alternative explanation is that *Ccr7*^{TG} effector T cells may enter inflamed tissues but egress prematurely via afferent lymph, thereby dampening local inflammation. This is likely accomplished by enhanced infiltrate clearance and/or limited access to antigen bearing APCs at the effector site. Thus, enhancing T cell egress or preventing reduced egress from the effector site represents a novel potential target of anti-inflammatory therapy.

The finding that the CCR7-CCL21 axis is active in physiologically recirculating effector T cells that have entered the inflammation-draining afferent lymph (Fig. 4) highlights the potential importance of this receptor-ligand pair as a therapeutic target. While many inflammatory signals upregulate lymphatic CCL21 at effector sites, its expression levels as well as that of other lymphatically expressed chemokines and adhesion molecules are strongly stimulus-dependent during inflammation (reviewed in (36)). Since we show that diminished T cell egress exacerbates inflammation, future studies will be critical to reveal the regulation of CCL21 on lymphatic endothelial cells and CCR7 on tissue-infiltrating T cells in addition to identifying alternative tissue exit receptors active in different types of inflammation.

Dampening inflammation by guiding pro-inflammatory T cells out of the inflamed site adds to the various anti-inflammatory functions of lymphatic endothelial cells (reviewed in (21)). One example of counter-regulation of inflammation by lymphatic endothelial cells is the expression of atypical chemokine receptor 2 (ACKR2, D6), which scavenges inflammatory chemokines while preserving CCL21 (37, 38). Other examples demonstrate that lymphatic vessel function and activation (*e.g.* by VEGF-C and VEGF-D) decrease inflammation by expanding the lymphatic network and enhancing the drainage of fluid and inflammatory mediators from the tissue (21, 36). Thus, afferent lymphatics fulfill a key anti-inflammatory role by counteracting the function of blood vascular endothelial cells, which allow for the entry of pro-inflammatory cells, fluid and mediators from the blood into the tissue.

In conclusion, our data show a proof-of-principle that the capacity of effector T cells to egress from the inflamed site via the afferent lymph modulates the local inflammatory response, revealing a novel checkpoint of tissue inflammation with relevance to autoimmune and inflammatory diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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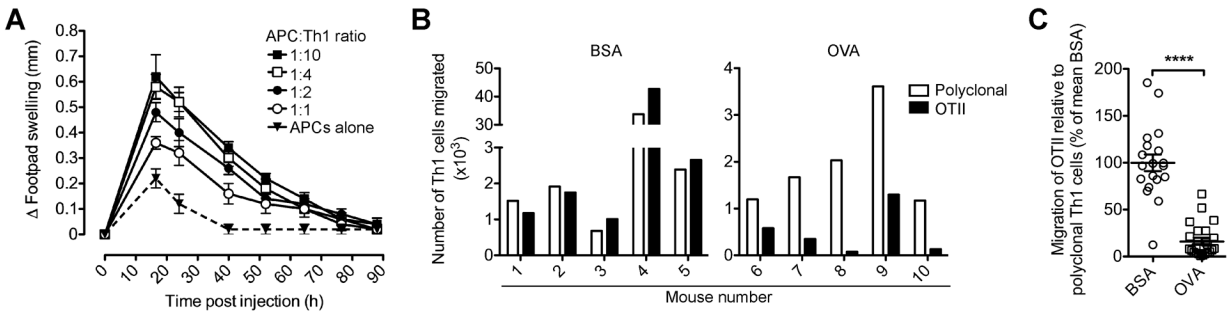

FIGURE 1. Antigen recognition reduces the tissue egress of CD4$^+$ effector T cells

(**A**) Different ratios of OVA-specific OTII Th1 cells and OVA-pulsed APCs (constant per recipient) were co-incubated and subcutaneously injected into the footpads of WT recipient mice. Footpad thickness was measured and corrected for pre-injection values (Δ swelling). Data points show mean ± SEM (5 mice per group) in one representative of 3 performed experiments. (**B–C**) A mixture of CFSE-labeled polyclonal CD45.1$^+$ and OTII CD45.2$^+$ Th1 cells were co-incubated with BSA- or OVA-pulsed APCs before injection into the footpads of recipient mice. 20 h later, donor cells that had egressed from the effector site and reached the draining lymph node (dLN) were enumerated by flow cytometry. Migration of OTII Th1 cells relative to polyclonal Th1 cells (**B**) in one representative experiment (5 mice per group) or (**C**) in all (n=4) independent experiments combined and expressed relative to the mean ratio of migrated OTII Th1 cells to polyclonal Th1 cells in the presence of BSA (set as 100%). Data points indicate individual recipients and the mean ± SEM of each group. ****$P < 0.0001$.

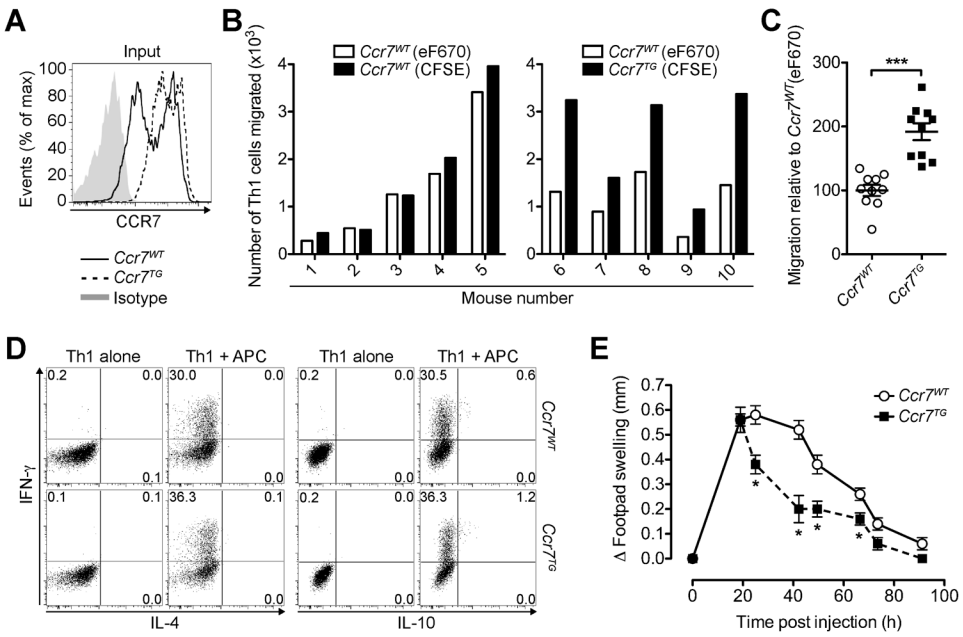

FIGURE 2. Transgenic expression of CCR7 enhances effector T cell egress from inflamed tissue and accelerates resolution of inflammation

(**A–C**) CFSE-labeled *Ccr7*^{WT} or *Ccr7*^{TG} OTII Th1 cells were mixed with eF670-labeled *Ccr7*^{WT} OTII Th1 cells and incubated with OVA-pulsed APCs, before injection into the footpads of WT recipients. 20 h later, dLN were analyzed for donor cells. (**A**) Representative CCR7 staining of Th1 cells prior to injection. (**B**) Quantification of donor Th1 cells that migrated to the dLN. (**C**) Tissue egress of CFSE-labeled *Ccr7*^{WT} and *Ccr7*^{TG} OTII Th1 cells relative to eF670-labeled *Ccr7*^{WT} OTII Th1 cells. Data are expressed as a percentage of the mean ratio of CFSE^{+} to eF670^{+} *Ccr7*^{WT} Th1 cells that migrated to the dLN (set as 100%) combined from 2 experiments. (**D**) Intracellular cytokine profile of *in vitro* generated *Ccr7*^{WT} OTII and *Ccr7*^{TG} OTII Th1 cells prior to injection with and without stimulation with OVA-pulsed APCs. (**E**) *Ccr7*^{WT} OTII or *Ccr7*^{TG} OTII Th1 cells were co-incubated with OVA-pulsed APCs, injected into the footpads of separate groups of mice and inflammation was measured over time. Data points depict individual recipients (B and C) and/or the mean ± SEM of each group (C and E). One experiment of ≥ 2 with similar results (A, B, D, E) or the combined analysis of all (N=2) experiments (C) using 5 mice per group is shown. *$P < 0.05$; ***$P < 0.005$.

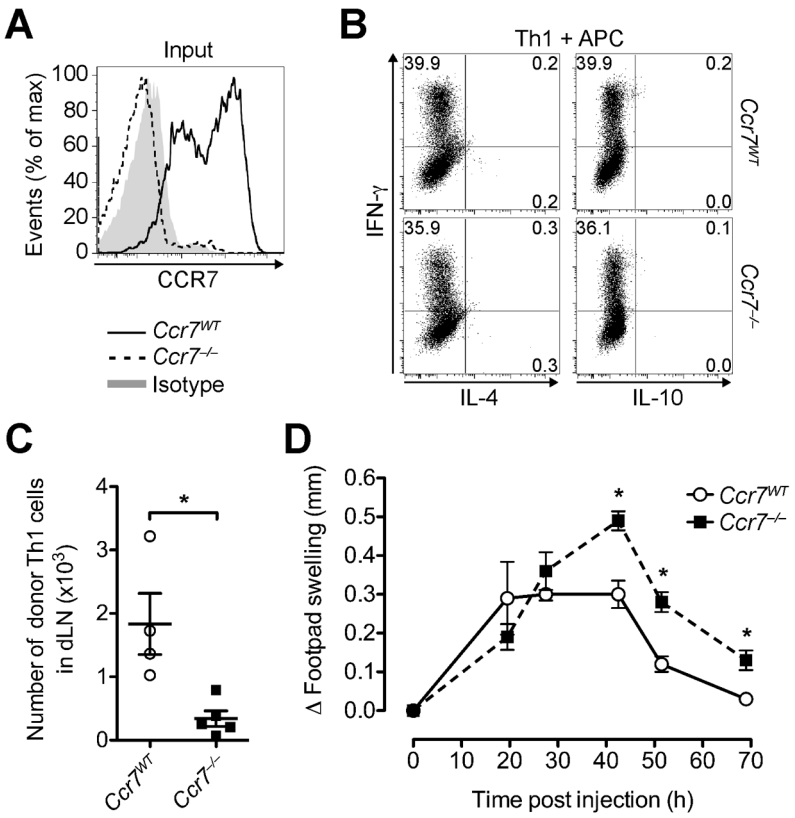

FIGURE 3. Lack of CCR7 impairs effector T cell egress and enhances local inflammation

Ccr7^{WT} and *Ccr7*$^{-/-}$ (CD45.2^{+}) OTII Th1 cells were separately co-incubated with OVA-pulsed DCs, before subcutaneous injection into the footpads of two groups of CD45.1^{+} congenic WT recipients. (**A**) Representative CCR7 staining of Th1 cells prior to injection. (**B**) Intracellular cytokine profile of *Ccr7*^{WT} OTII and *Ccr7*$^{-/-}$ OTII Th1 cells prior to injection after stimulation with OVA-pulsed APCs. (**C**) 70 h after transfer, recovered donor Th1 cells were quantified in the dLN. (**D**) Footpad swelling induced by *Ccr7*^{WT} and *Ccr7*$^{-/-}$ OTII Th1 cells was measured over time. Data points depict individual recipients (C) and/or the mean ± SEM of each group (C and D). One experiment of ≥ 2 with similar results using 5 recipients per group. *$P < 0.05$.

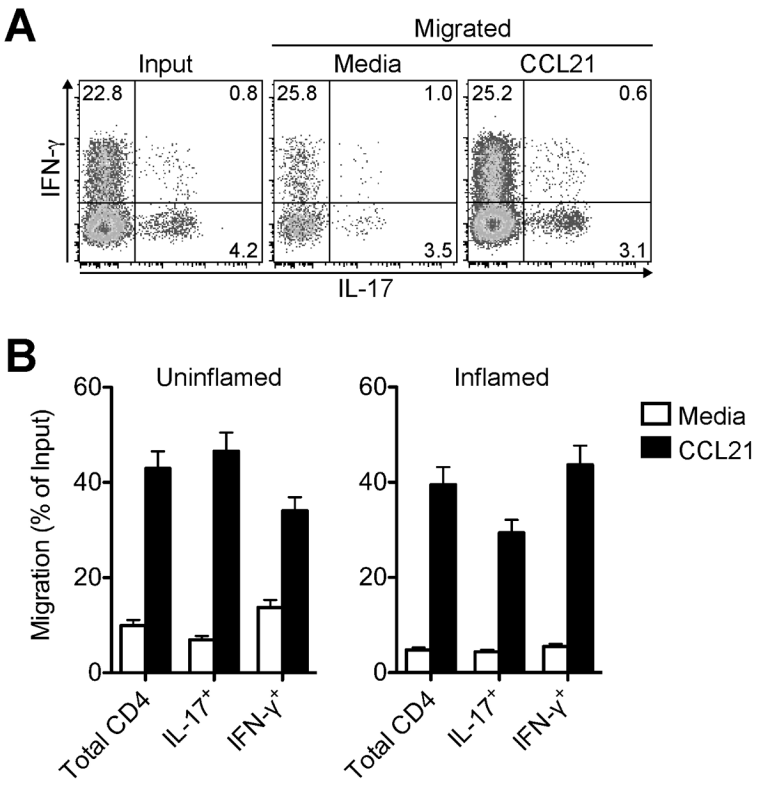

FIGURE 4. Inflammation exiting Th1 and Th17 cells chemotax to CCR7 ligand

Chronic skin inflammation was induced in sheep by subcutaneous injection of CFA. ≥ 3 weeks later afferent lymph vessels draining the inflamed and uninflamed control skin were cannulated and skin-egressing T cells collected. Chemotaxis of lymph-borne $CD4^+$ T cells toward CCL21 was tested *ex vivo* in a Transwell chemotaxis assay. (**A**) Representative intracellular cytokine staining of inflammation-draining $CD4^+$ T cells in input and migrated populations after polyclonal stimulation. (**B**) Chemotaxis of $CD4^+$ T cell subsets. Bars indicate the mean ± SD for triplicate wells for one representative animal of ≥ 3 analyzed for each condition.