CCR8 regulates contact hypersensitivity by restricting cutaneous dendritic cell migration to the draining lymph nodes

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Abstract

Allergic contact dermatitis (ACD) is a typical occupational disease in industrialized countries. Although various cytokines and chemokines are suggested to be involved in the pathogenesis of ACD, the roles of these molecules remain to be elucidated. CC chemokine receptor 8 (CCR8) is one such molecule, of which expression is up-regulated in inflammatory sites of ACD patients. In this study, we found that Ccr8⁺/− mice developed severer contact hypersensitivity (CHS) responses to 2,4-dinitrofluorobenzene, a murine model of ACD, compared with wild-type mice. T cells from Ccr8⁺/− mice showed enhanced proliferative recall responses and T h 1 and T h 17 cell populations were expanded in these mice. However, CHS responses were similar between SCID mice adoptively transferred with Ccr8⁺/− and wild-type T cells, suggesting that CCR8 in T cells is not responsible for the exacerbation of CHS. Notably, skin-resident dendritic cells (DCs), such as Langerhans cells and dermal DCs, and inflammatory DCs were highly accumulated in lymph nodes (LN) of Ccr8⁺/− mice after sensitization. Consistent with this, Ccr8⁺/− antigen-presenting cells readily migrated from the skin to the draining LNs after sensitization. These observations suggest that CCR8 negatively regulates migration of cutaneous DCs from the skin to the draining LNs in CHS by keeping these cells in the skin.

Keywords: CCR8, contact hypersensitivity, dermal dendritic cells, Langerhans cells, migration

Introduction

The prevalence of contact allergy is ~20% in the populations of North America and Western Europe, and sometimes these people develop allergic contact dermatitis (ACD), resulting in an impairment in the quality of life (1, 2). Contact hypersensitivity (CHS), a mouse model of ACD, is triggered by repetitive contact-exposure of low-molecular weight chemicals termed as hapten to the skin. CHS is constituted of two phases (3–5). (i) Sensitization phase: exposure of skin to hapten activates cutaneous dendritic cells (DCs), resulting in the migration of DCs into draining lymph nodes (dLN) in a CCR7-CCL19/CCL21-dependent manner (6). Then, these haptenized protein-bearing DCs sensitize naïve T cells, resulting in the generation of effector/memory T cells in the dLN. (ii) Elicitation phase: a second challenge of the same hapten to sensitized individuals causes the activation of memory T cells, resulting in the elicitation of local inflammation. Thus, the migration of cutaneous DCs to dLN during the sensitization phase is crucial in the development of CHS. Langerhans cells (LCs) are a subset of DCs, which are specifically localized in skin epidermis. Upon antigenic challenge to skin, LCs acquire antigens(s) and migrate to dLN, where they present the antigens to naive T cells (7, 8). Dermal dendritic cells (dDCs) are another subset of epidermis/dermis-localized DCs (9). These DC subsets are the most important...
antigen-presenting cells (APCs) for the immune responses in the skin (9, 10). In addition, inflammatory DCs (iDCs), which are likely to be differentiated from peripheral blood-circulating monocytes (11), are also suggested to be involved in this response (12). However, the pathological roles of each DC subset in the development of CHS are still controversial (13–18).

In the elicitation stage, various types of pro-inflammatory cytokines and chemokines are released from immune cells and keratinocytes [KCs (19, 20)]. Elevated levels of cytokines and chemokines, such as IFN-γ, IL-4, TNF, IL-10, CCL1 (also known as l-309 and TCA-3), are detected in the skin biopsies from ACD patients sensitized with nickel (21). Notably, CC chemokine receptor 8 (CCR8), the chemokine receptor for CCL1 and CCL8, was also up-regulated in the biopsies, suggesting possible involvement of the CCR8–CCL1/CCL8 axis in the pathogenesis of ACD.

The chemokine–chemokine receptor system plays important roles in the trafficking of leukocytes from the circulation to specific organs or inflammatory sites (22). CCR8 induces chemotactic migration of cells by activating calcium influx in response to CCL1 (23, 24). CCR8 is expressed in monocytes and macrophages, and is involved in the development of peritoneal adhesions (25), type I diabetes (26) and hepatitis (27) through recruitment of monocytes/macrophages to the inflammatory sites. CCR8 is also expressed in T2 cells, and mediates chemotaxis and calcium mobilization of these cells (28). Consistent with this, in asthma, a T2-type disorder (29), the CCR8+ T cell population is expanded, and Ccr8−/− mice are refractory to experimental allergic airway inflammation (30), suggesting that CCR8 is involved in the pathogenesis of T2-mediated diseases. Recently, Islam et al. (31) reported that CCL8, a newly identified CCR8 agonist, promotes atopic dermatitis in mice by recruiting CCR8-expressing T2 cells. On the other hand, CCR8 suppresses septic peritonitis by suppressing activation of macrophages (32). A regulatory role of CCR8 is also suggested in ACD, because CCR8-expressing, nickel-specific skin-homing T cells produce IL-10 (21). Therefore, the role of CCR8 in the pathogenesis of ACD remains still controversial.

In this report, we have generated Ccr8−/− mice, and found that Ccr8−/− mice were more susceptible to 2,4-dinitrofluorobenzene (DNFB)-induced CHS compared with wild-type (WT) mice. This was because migration of DCs from the skin to dLNls was enhanced in Ccr8−/− mice. These results suggest that CCR8 regulates migration of DCs from skin to dLNls in contact allergic inflammation.

Methods

Mice

Ccr8−/− mice were generated by homologous recombination techniques using E14.1 ES cells as described in Supporting Materials and Methods, available at International Immunology Online, and backcrossed to the BALB/cA mice for 8 to 10 generations (Supplementary Figure 1 is available at International Immunology Online). WT and SCID mice were purchased from CLEA Japan. Mice were bred under specific pathogen-free conditions in an environmentally controlled clean room at the IMSUT, and the RIBS. Age- (8- to 12-week-old) and sex-matched mice were used for all experiments. All experiments were approved by the institutional animal use committees and were conducted according to the institutional ethical guidelines for animal experiments and safety guidelines for gene manipulation experiments.

Contact hypersensitivity

CHS was induced as described previously (33). Briefly, mice were sensitized by painting the shaved abdomen with 50 µl of 0.5% DNFB (Tokyo chemical industry, Tokyo, Japan) in acetone/olive oil (4:1). Five days after the first immunization, the mice were challenged with 25 µl of 0.2% DNFB on both surfaces of the right ear. Solvent alone was applied to the left ear as a control. Twenty-four hours after the second challenge, a small piece of the earlobe was excised using a 6-mm punch instrument and weighed. Earlobe swelling was assessed as follows: [increment of earlobe swelling (mg)] = [weight of challenged earlobe (mg)] – [weight of vehicle-treated earlobe (mg)]. Ear thickness was daily measured with a micrometer caliper. Ear swelling was shown as a percentage of thickness compared with ears at day 0.

Immunohistochemistry

Ear biopsies were fixed with 10% neutral formalin, dehydrated and paraffin-embedded. About 5 µm sections were stained with hematoxylin and eosin (HE).

Antibody titers

Sera were collected 3 days after the second challenge and levels of IgG specific for dinitrophenyl (DNP)-BSA were measured by an ELISA-based assay. An aliquot of serum (50 µl) was applied to a DNP-BSA-coated 96-well plate, and the plate was incubated at room temperature for 2 h. Then, wells were washed and incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (Zymed, San Francisco, CA, USA) at room temperature for 1 h, followed by the incubation with substrate phosphate Sigma 104 (Sigma-Aldrich, St. Louis, MO, USA). The absorbance at 415 nm was measured using a microplate reader (MTP-300, HITACHI, Japan).

Recall proliferation assay

Single cell suspensions were prepared from dLNls 24 h after sensitization with 0.5% DNFB, and were cultured in the presence of indicated concentrations of DNBS (Tokyo chemical industry) in 96-well plates (2 x 10^5 cells). PMA and ionomycin (50 and 500 ng/ml) treated cells were used as the controls. After incubation for 66 h, cells were labeled with [3H]-thymidine (0.25 µCi/ml, PerkinElmer, Waltham, MA, USA) for 6 h, and harvested using a Micro 96 cell harvester (Skatron, Sterling, VA, USA). [3H]-radioactivity in the acid insoluble fraction was measured using a Micro Beta Systems (Pharmacia Biotech, Piscataway, NJ, USA). IFN-γ and IL-17 concentrations in culture supernatants after 72 h stimulation with DNBS were determined using OptiEIA IFN-γ (BD Pharmingen, San Diego, CA, USA) and Duo set IL-17 (R&D systems, Minneapolis, MN, USA), respectively, according to the manufacturer’s protocols. The proportions of Th and Treg cells were determined by flow cytometry as described below.
Isolation of T and B cells by magnetic cell sorter

T and B cells were isolated from the spleen and LNs using autoMACS (Miltenyi Biotech, Bergish Gladbach, Germany) with anti-CD90.2- and anti-B220-conjugated microbeads (Miltenyi Biotech), respectively, in accordance with the manufacturer’s instructions.

T cell proliferation

T cells (2×10^5 cells) were cultured in anti-CD3 (clone 145-2C11; eBioscience, San Diego, CA, USA)-coated-96-well plates. T-cell proliferation was determined as described above.

Adoptive T cell transfer

T cells were purified from both spleens and LNs using autoMACS with anti-CD90.2-microbeads (89% pure; 1×10^5 or 1×10^6), and they were intravenously injected into recipient SCID mice. After 1 day, these mice were subjected to DNFB-induced CHS.

In the case of DNFB-sensitized T cell transfer into WT mice, mice were sensitized by painting with 0.5% DNFB. Five days after sensitization, T cells were isolated from dLNs by MACS with anti-CD90.2-microbeads. The resulting cells (2×10^7 cells/mouse; 98% pure) were intravenously injected into WT mice. Six hours after injection, mice were challenged with DNFB.

Flow cytometry

Antibodies used for flow cytometric analysis are listed in Supplementary Table 1, available at International Immunology Online. Flow cytometry was performed as described previously (34). Briefly, cells were stained with primary antibodies for 30 min at 4°C after blocking with 2.4G2, followed by secondary antibodies. Cells were analysed by flow cytometers, FACSCalibur (Becton Dickinson, Sparks, MD, USA) or FACSCantoII (Becton Dickinson) with FlowJo (Tree Star). Dead cells were stained with either 7AAD (Sigma–Aldrich) or LIVE/DEAD Fixable Aqua (Invitrogen). For staining of DC subsets, LNs were prepared by digesting LNs with 200 U/ml type VIII collagenase (Sigma–Aldrich). For intracellular cytokine staining, cells were cultured in the presence of 50 ng/ml PMA, 500 ng/ml ionomycin and 1 μM monensin for 5 h. Cells were washed, fixed with 4% paraformaldehyde (PFA), and permeabilized with 0.1% saponin, followed by staining with anti-IFN-γ, anti-IL-17 or anti-Foxp3 antibodies. For preparation of highly purified cells, cells were sorted by FACSAria II (Becton Dickinson) or MoFlo XDP (Beckman Coulter, Miami, FL, USA).

Quantitative reverse-transcription (RT)-PCR

RNAs from tissues and cells were purified using the Sepasol-RNA I Super (Nacalai Tesque, Kyoto, Japan) and GenElute mammalian total RNA miniprep kit (Sigma–Aldrich), respectively. Resulting RNAs were reverse-transcribed using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). For semi-quantitative analysis, synthesized cDNA was amplified by PCR with Ex Taq DNA polymerase (TaKaRa, Kyoto, Japan) and a primer set (Supplementary Table 2 is available at International Immunology Online). For quantitative analysis, SYBR Green qPCR kit (TaKaRa) was used with specific primer sets (Supplementary Table 3 is available at International Immunology Online).

Staining of epidermal sheets

Epidermal sheets were obtained from earlobes according to a previous report (55). Earlobes were cut off at the base, and split into dorsal and ventral halves. The dorsal sheets were incubated with 20 mM EDTA/PBS for 2 h at 37°C. The epidermis was fixed in cold acetone for 20 min, permeabilized in 0.1% saponin/PBS, and blocked with 5% FBS/PBS. After washing, the sheets were stained with 5 μg/ml biotin-conjugated anti-CD207 monoclonal antibody (clone 92F93.01, Dendritic, Dardilly, France), followed by DyLight549-conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA, USA). Sheets were mounted with Mount-Quick (Daido Sangyo, Saitama, Japan). Images were obtained using fluorescence microscopy, BZ-9000 (Keyence, Osaka, Japan). Cell numbers were counted in three independent fields per section of a mouse epidermal sheet.

FITC migration

Mice were sensitized by painting with 50 μl of 0.5% FITC isomer I (Sigma–Aldrich) in acetone/dibutylphthalate (1:1, vol:vol) on the shaved back skin. After 24 h, dLNs were collected and a single cell suspension was prepared by digesting with collagenase. Cells were blocked, and were stained with antibodies. The contents of FITC+ DC subsets were analysed by flow cytometry.

Immunostaining

LNs were embedded in OCT compound (Sakura Finetek Japan, Tokyo, Japan) and frozen with liquid nitrogen. About 7 μm serial cryosections were fixed with 4% PFA, blocked with 5% goat serum in PBS/0.3% Triton X-100 and stained with hamster anti-mouse CD11c antibody (clone, N418; Biolegend, San Diego, CA, USA), followed by DyLight594-conjugated goat anti-hamster IgG (Biolegend). The coverslips were mounted with ProLong Gold Antifade mountant with DAPI (Molecular Probes, Eugene, OR, USA). Images were acquired using fluorescence microscopy, BZ-9000.

Isolation of epidermal cells from the skin

Shaved skin was cut out, and subcutaneous adipose tissues were removed by gently rubbing. After washing, the skin was floated on 0.25 mg/ml thermolysin (Sigma–Aldrich) for 1 h at 37°C. Epidermis was peeled off, minced by scissors and disaggregated by vigorous pipetting. Epidermal cells were isolated by a cell sorter.

Generation of bone marrow-derived DCs and macrophages

For generation of bone marrow (BM)-derived DCs (BMDCs), BM cells were cultured at 2×10^6 cells/10 ml RPMI1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% 2-mercaptoethanol and 20 ng/ml recombinant mouse GM-CSF (Peprotech, Rocky Hill, NJ, USA) in
100-mm non-treated dishes. On day 3, 10 ml of the same fresh medium was added to the dishes. On day 6 and 8, 5 ml of the medium was replaced with fresh medium, which contained 10 ng/ml GM-CSF. On day 10, non-adherent cells were collected as BMDCs. For generation of BM-derived macrophages (BMMPs), BM cells were cultured at 5 × 10^7 cells/10 ml RPMI1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 1% 2-mercaptoethanol and 20 ng/ml recombinant mouse M-CSF (R&D systems) in 100-mm non-treated dishes. On day 3, 5 ml of the same fresh medium was added to the dishes, and on day 7, adherent cells were collected by a cell scraper with 2.5 mM EDTA.

Statistics

Two-tailed unpaired Student’s t-test was used for statistical evaluation of all experiments. P value < 0.05 was considered as significant; * < 0.05; ** < 0.01; *** < 0.001. Data are expressed as a mean ± SD.

Results

Ccr8−/− mice are highly susceptible to DNFB-induced CHS

We first investigated Ccr8 mRNA expression in DNFB-induced CHS. Consistent with the observations in ACD patients (21), an increase of Ccr8 transcript was observed in sensitized skin compared with untreated skin (Fig. 1A). To investigate the pathological role of CCR8 in contact dermatitis, we generated Ccr8−/− mice on the BALB/cA background (Supplementary Figure 1 is available at International Immunology Online), and examined the development of CHS. When CHS was induced in the earlobe by challenging with DNFB, Ccr8−/− mice developed severer inflammation, as indicated by significantly increased punched earlobe weight at 24 h (Fig. 1B). The extent of ear thickness in Ccr8−/− mice was significantly increased at 24 h after immunization (Fig. 1C). Histological analysis showed notable swelling of the earlobe and marked accumulation of granulocytes (Fig. 1D). Analysis of cytokine gene expression revealed that the expression of the Il1b gene was significantly enhanced in Ccr8−/− mouse ears compared with WT mouse ears (Fig. 1E), while Tnf and Il10 gene expression was not. Although IgG antibody levels specific to DNP tended to increase in Ccr8−/− mice compared with WT mice, the difference was not statistically significant, consistent with our previous report that antibody levels don’t affect the development of CHS [Fig. 1F (36)].

The T cell proliferating response is enhanced in Ccr8−/− mice

Because the Ccr8 gene is highly expressed in T cells (37, 38), we examined the proliferating response of Ccr8−/− LN cells. After sensitization of mice with DNFB, dLN cells were isolated, and the proliferative response to DNBS was evaluated by [3H]-thymidine incorporation. The proliferative response of Ccr8−/− LN cells was significantly higher than that of WT LN cells, while PMA/ionomycin-induced or anti-CD3-induced proliferation was comparable (Fig. 2A and B). Under the experimental conditions, T helper 1, T helper 17 and T helper 17 cell populations were significantly expanded in Ccr8−/− mouse LNs after sensitization with DNFB compared with WT mouse LNs (Fig. 2C). Higher concentrations of IFN-γ and IL-17 were also detected in the culture supernatants from Ccr8−/− LN cell culture (Fig. 2D). The results suggest that T cell priming against DNFB is enhanced in Ccr8−/− mice.

T cells from DNFB-primed Ccr8−/− mice induce enhanced CHS responses in WT mice

We examined T cell function of Ccr8−/− mice by employing adoptive T cell transfer techniques. T cells were purified from spleens and LNs, and transferred into recipient SCID mice (Fig. 3A, left). After 24 h, CHS responses were induced in these mice. We found that earlobe swelling was comparable between WT T cell- and Ccr8−/− T cell-transferred mice (Fig. 3A, right), indicating that CCR8 deficiency in T cells does not affect the development of contact dermatitis.

Next, T cells were purified from dLNs of Ccr8−/− mice 5 days after sensitization, and were intravenously transferred into WT mice. Those mice were then challenged with DNFB (Fig. 3B, left). CHS development was aggravated in Ccr8−/− T cell-transferred mice compared with WT T cell-transferred mice (Fig. 3B, right), indicating that T cell priming is promoted in Ccr8−/− mice.

The migration of cutaneous DCs to dLNs is enhanced in Ccr8−/− mice

Because adoptive T cell-transfer experiments suggested that cutaneous DCs, but not T cells, affect priming responses, we analysed the DC function in Ccr8−/− mice in CHS. We examined the expression of CCR8 in highly purified DC subsets, which were separated according to Ruedl et al. (39). (purity > 99 %; Supplementary Figure 2 is available at International Immunology Online for the gating protocol). These DC subsets corresponded to well documented LN DC populations (35, 39, 40). We found that Ccr8 mRNA was significantly expressed in DC subsets, including cDCs (CD11c+CD40int), dDCs (CD11c+CD40hi), LCs (CD11c+CD40hi) and iDCs (CD11c+CD40hi), as well as in T and B cells, but not in BMLCs and BMDCs (Fig. 4A and Supplementary Figure S3 is available at International Immunology Online).

Because migration of cutaneous DCs into dLNs is a prerequisite to sensitize T cells to induce allergic responses, we examined the migration of these DC subpopulations after CHS induction. Significantly enhanced accumulation of dDCs, LCs and iDCs was observed in the dLNs of Ccr8−/− mice compared with WT mice (Fig. 4B and C), whereas the proportions of plasmacytoid DCs (pDCs, CD11c+B220+) as well as T and B lymphocytes were similar between WT and Ccr8−/− mice (Supplementary Figure S4 is available at International Immunology Online). In contrast, no significant accumulation of these DC subsets was observed under physiological conditions without allergen challenge (Supplementary Figure S5 is available at International Immunology Online). High expression of MHC-II and CD86, activation markers for DCs, was similarly observed among WT and Ccr8−/− DC subsets (Fig. 4D and E). The expression of CCR7, which is required for the migration of DCs to dLNs through lymphatic vessels (6, 35), was also similar in these DCs from WT and Ccr8−/− mice. These results suggest that the migration of cutaneous DCs into dLNs is increased at the sensitization phase of CHS in Ccr8−/− mice.
Next, we measured the number of CD207+ DCs in the epidermis using fluorescence microscopy. The number of epidermal DCs was reduced after sensitization with DNFB (Fig. 5A, left). Notably, this reduction was more significantly observed in Ccr8−/− mice (Fig. 5A, right). These observations support the notion that Ccr8−/− DCs migrate
**Fig. 2.** T cell sensitization against DNFB is enhanced in Ccr8−/− mice. (A) Five days after sensitization with DNFB, dLN cells were re-stimulated with DNBS. Recall proliferation of LN cells was determined by [3H]-thymidine incorporation (n = 3 each). PMA/ionomycin stimulation was used as a control (n = 3 each). The data are representative of three independent experiments. *P < 0.05 (two-tailed unpaired Student’s t-test) (B) T cells were isolated from spleen and LNs, and were stimulated with plate-bound anti-CD3 antibody for 3 days. The proliferative response was determined by [3H]-thymidine incorporation. The data are presented as mean ± SD of triplicate wells, and representative of two independent experiments. (C) The proportion of T, T1, and T17 cells in dLNs after antigen re-stimulation was analysed by flow cytometry. CD4+ and CD8+ T cells were stained with anti-IFN-γ or anti-IL-17. Mean ± SD of three independent experiments (n = 9 each). *P < 0.05; **P < 0.01 (two-tailed unpaired Student’s t-test) (D) The concentrations of IFN-γ and IL-17 in LN cell culture supernatants were determined by ELISA. Mean ± SD of three independent experiments (n = 9 each). **P < 0.01 (two-tailed unpaired Student’s t-test).
more easily from the epidermal compartment to dLNs after sensitization.

Then, we directly examined migration of cutaneous Ccr8<sup>−/−</sup> DCs after sensitization with FITC. The proportions of FITC-labeled dDCs, LCs and iDCs were significantly increased in dLNs of Ccr8<sup>−/−</sup> mice (Fig. 5B and C). Immunofluorescent staining analysis revealed that FITC+CD11c+ cells were localized at the cortical area in dLNs, but not at the subcapsular sinus (SCS) (Fig. 6), consistent with previous reports (41, 42). We also observed that the proportion of Ccr8<sup>−/−</sup> FITC+CD11c+ cells tended to be higher in that region. These results indicate that CCR8 deficiency promotes migration of DCs from skin to dLNs.

**CCL8 expression is upregulated in the skin during the sensitization phase**

Both CCL8 and CCL1 are the ligands for CCR8. CCL8 is expressed in the skin (31), whereas CCL1 is not (43). Thus, we hypothesized that CCL8 is important for the recapture and tethering of CCR8<sup>+</sup> DCs in the skin. Then, we examined the expression of CCL8 in cutaneous and immune cells. Epidermal LCs (CD45<sup>−</sup>CD11b<sup>+</sup>) and KCs (CD45<sup>−</sup>CD11b<sup>−</sup>TC R<sub>γδ</sub>-CD117<sup>−</sup>) were highly purified by flow cytometry (>95% pure), and the expression of Ccl8 was analysed by quantitative PCR. Ccl8 was expressed in both epidermal LCs and KCs (Fig. 7A). We further compared Ccl8 expression in the skin between steady and inflammatory states. Ccl8 transcript was elevated in DNF8-challenged skin (Fig. 7B), suggesting that CCL8 expression is enhanced during inflammation in the skin.

**Discussion**

Chemokines mediate the trafficking of leukocytes to inflammatory sites and specific lymphatic organs (44), while adhesion molecules are important for tethering cells to a tissue (45). In the skin, LCs are anchored to the epidermis through E-cadherin bonds to KCs under steady state conditions (46, 47). Upon antigen stimulation, LCs are activated to express CCR7 and migrate into local dLNs where CCL19 and CCL21 are expressed. CCL8 is also highly expressed in LCs and KCs in the skin. We showed in this report that the expression of CCR8 on LCs and CCL8 in KCs of the skin was upregulated when the mice were cutaneously sensitized with DNF8, suggesting that the CCR8–CCL8 interaction is important for the migration and recruitment of inflammatory cells to the antigen stimulated skin. Nonetheless, we showed that Ccr8<sup>−/−</sup> mice are more susceptible to DNF8-induced CHS, suggesting that CCR8 rather negatively regulates CHS responses. Accumulation of DCs including LCs, dDCs and iDCs in the dLNs was significantly enhanced in Ccr8<sup>−/−</sup> mice compared to WT mice. This suggests that CCR8 rather negatively regulates CHS responses.
with WT mice after sensitization. Furthermore, the number of DCs in the skin significantly reduced in DNFB-sensitized Ccr8⁻/⁻ mice, suggesting that cutaneous Ccr8⁻/⁻ DCs easily migrated from the sensitized skin to dLNs compared with WT DCs. Consistent with this, we showed that migration of antigen-captured dDCs, LCs and iDCs from the skin to dLN was significantly enhanced in Ccr8⁻/⁻ mice upon sensitization with FITC. Although CCR8 signaling induces Ca²⁺ influx
Fig. 5. CCR8 regulates migration of cutaneous DCs during the sensitization phase. (A) Epidermal cell sheets were prepared from the earlobe skin after 24 h-sensitization with DNFB. The sheets were stained with a biotin-conjugated anti-CD207 monoclonal antibody, followed by DyLight549-streptavidin. Microscopic photographs were taken using fluorescence microscopy (left; original magnification: ×40), and DC numbers are shown in the right (n = 5 each). (B) Mice were sensitized by painting with FITC on the shaved back skin. Twenty-four hours later, cells were isolated from dLNs, and the contents of FITC+ DCs were analysed by flow cytometry (n = 13 each). The gating protocol is described in Supplementary Figure S2, available at International Immunology Online. The data are shown in (C) (upper panel, percentage; lower panel, number; n = 5 each). The results are reproduced in the two independent experiments. Mean ± SD (A and B). *P < 0.05; ***P < 0.001 (two-tailed unpaired Student’s t-test).
Fig. 6. Localization of migratory DCs in dLNs after treatment with FITC. Mice were sensitized with FITC. Frozen sections were prepared from dLNs of these mice, and stained with hamster anti-CD11c monoclonal antibody followed by DyLight594-anti-hamster antibody (Red). FITC appears green. Nuclei were stained with DAPI (blue). Images were acquired by fluorescence microscopy. Areas surrounded by dotted lines show the SCS. Original magnification, ×20. Arrows indicate FITC-CD11c+ cells (yellow).
showed that the migration of latex-beads+ (53) et al (MDDCs) from skin to LNs. In an earlier study, Qu exacerbation of CHS in Ccr8−/− mice. These results suggest that T cells are not responsible for the international immunology figure S6 is available at online.

Ccr8 was comparable between WT T cell-transferred and these results suggest that T cells are not responsible for the migration of these cells to the dLN. In contrast, when CHS is induced, the migration of cutaneous DCs is strongly restricted by the excess expression of CCL8 in the skin.

In conclusion, we have shown here that CCR8 is a negative regulator of APC migration from the skin to dLNs in CHS. This regulation of APC migration may be beneficial for the hosts by keeping APCs in epidermis and preventing excess hapten-induced hypersensitivity (Supplementary Figure S7 is available at International Immunology Online).

Supplementary data
Supplementary data are available at International Immunology Online.

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