# Requirement for Chemokine Receptor 5 in the Development of Allergen-Induced Airway Hyperresponsiveness and Inflammation

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**Chemokine receptor (CCR) 5 is expressed on dendritic cells, macrophages, CD8 cells, memory CD4 T cells, and stromal cells, and is frequently used as a marker of T helper type 1 cells. Interventions that abrogate CCR5 or interfere with its ligand binding have been shown to alter T helper type 2–induced inflammatory responses. The role of CCR5 on allergic airway responses is not defined. CCR5-deficient ($CCR5^{-/-}$) and wild-type ($CCR5^{+/+}$) mice were sensitized and challenged with ovalbumin (OVA) and allergic airway responses were monitored 48 hours after the last OVA challenge. Cytokine levels in lung cell culture supernatants were also assessed. $CCR5^{-/-}$ mice showed significantly lower airway hyperresponsiveness (AHR) and lower numbers of total cells, eosinophils, and lymphocytes in bronchoalveolar lavage (BAL) fluid compared with $CCR5^{+/+}$ mice after sensitization and challenge. The levels of IL-4 and IL-13 in BAL fluid of $CCR5^{-/-}$ mice were lower than in $CCR5^{+/+}$ mice. Decreased numbers of lung T cells were also detected in $CCR5^{-/-}$ mice after sensitization and challenge. Transfer of OVA-sensitized T cells from $CCR5^{+/+}$, but not transfer of $CCR5^{-/-}$ cells, into $CCR5^{-/-}$ mice restored AHR and numbers of eosinophils in BAL fluid after OVA challenge. Accordingly, the numbers of airway-infiltrating donor T cells were significantly higher in the recipients of $CCR5^{+/+}$ T cells. Taken together, these data suggest that CCR5 plays a pivotal role in allergen-induced AHR and airway inflammation, and that CCR5 expression on T cells is essential to the accumulation of these cells in the airways.**

**Keywords:** rodent; T cells; cytokines; chemokines; lung

Bronchial asthma is characterized by chronic airway inflammation and airway hyperresponsiveness (AHR). Airway inflammation results from the influx of activated eosinophils and T cells at the site of inflammation. T cells, especially T helper (Th) type 2 cells, which release IL-4, IL-5, and IL-13, are central to the development of AHR and eosinophilic inflammation (1, 2).

Chemokines induce cell migration and activation by binding to specific G-protein–coupled cell surface receptors on target cells. Chemokine receptor (CCR) 5 is expressed on macrophages, activated T cells, immature dendritic cells (DCs), and natural killer cells (3–6). The major ligands for CCR5 are regulated upon activation normal T cell expressed and secreted (RANTES/CCL5), macrophage inflammatory protein (MIP)-1α (CCL3) and MIP-1β/CCL4 (7). CCR5 and its ligands have been reported to play a critical role in Th1 inflammation and immunity, as in tuberculosis, cryptococcal infection, toxoplasmosis, sarcoidosis, Wegener's granulomatosis, rheumatoid arthritis, periodontitis, and acute and chronic transplant rejection (8, 9). The association between CCR5 and Th1 responses is strong enough that CCR5 is frequently used as a marker of Th1 cells (10). Recently, it has been reported that CCR5 regulated AHR and allergic airway inflammation in mouse models of asthma (11–13). Down-regulation of CCR5 by a compound and a chemokine ligand suppressed AHR and airway inflammation (12, 13). However, it has also been shown that CCR5 regulates the development of AHR, but not eosinophilic inflammation in a mouse model of allergen-induced airway responses (14). Thus, CCR5 might control allergic airway responses, but its role in airway inflammation is controversial. More importantly, the exact mechanisms whereby CCR5 impacts allergic airway responses remain unclear.

In the present study, we investigated the role of CCR5 in the development of allergen-induced AHR and airway inflammation using CCR5-deficient ($CCR5^{-/-}$) mice. $CCR5^{-/-}$ mice showed significantly decreased allergen-induced AHR and airway inflammation, which was associated with lower numbers of lung T cells. Transfer of antigen-sensitized $CCR5^{+/+}$ T cells into $CCR5^{-/-}$ mice enhanced ovalbumin (OVA)-induced AHR and inflammation, and transferred $CCR5^{+/+}$, but not $CCR5^{-/-}$, T cells were detected in the lungs, indicating that CCR5 expression on T cells may play an important role in migration of these cells to the lungs and in mediating allergen-induced airway responses.

## MATERIALS AND METHODS

An expanded description of our methods can be found in the online supplement.

### Animals

Female C57BL/6 ($CCR5^{+/+}$) and C57BL/6-CD45.1 ($CD45.1^{+}CCR5^{+/+}$) mice were purchased from Charles River (Yokohama, Japan). $CCR5^{-/-}$ mice were provided by Dr. K. Matsushima (Tokyo, Japan) (15).

### Sensitization and Airway Challenge

Mice were sensitized to and challenged with OVA (OVA/OVA) as previously described (16). Control mice were nonsensitized, but challenged with OVA (PBS/OVA).

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### Determination of Airway Responsiveness

Lung resistance (RL) in response to increasing doses of inhaled methacholine was monitored (17).

### Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) cells were counted and differentiated in a blinded fashion by counting at least 300 cells under light microscopy (18).

### Lung Histology

Lungs sections were stained and examined under light microscopy as previously described (19).

### Lung Cell Isolation

Lung cells were isolated as previously described after collagenase digestion (19).

### Cell Preparation and Culture

Mononuclear cells (MNCs) from spleens, peribronchial lymph nodes (PBLNs), and lungs were isolated as previously described (18). MNCs ($4 \times 10^5$) were cultured as previously described (20).

### Flow Cytometry

After purification, lung MNCs, PBLN MNCs, and BAL cells were incubated with antibodies and then analyzed by flow cytometry (18).

### Measurement of Cytokines

Cytokine concentrations in BAL and culture supernatants were measured by ELISA (19).

### Adaptive Transfer of T Cells

Spleens from sensitized $CCR5^{+/+}$ and $CCR5^{-/-}$ mice were removed and T cells were obtained by negative selection (Miltenyi Biotec, Auburn, CA) with over 95% purity.

T cells were transferred to $CCR5^{-/-}$ mice intravenously just before the first OVA challenge. Mice were challenged with OVA, then AHR was assessed and BAL and lung tissue obtained for analysis. To quantitate transferred $CCR5^{-/-}$ and $CCR5^{+/+}$ T cells in the airways, recipient $CD45.1^{+}CCR5^{+/+}$ mice were sensitized, before OVA challenge; T cells ($CD45.2^{+}$) obtained from OVA-sensitized $CCR5^{-/-}$ ($CD45.2^{+}CCR5^{-/-}$) or $CCR5^{+/+}$ ($CD45.2^{+}CCR5^{+/+}$) mice were injected intravenously. After OVA challenge, lungs were removed, and isolated lung MNCs were analyzed by flow cytometry.

### Generation of Bone Marrow–Derived DCs

DCs were generated from bone marrow of naive $CCR5^{+/+}$ or $CCR5^{-/-}$ mice, as previously described (21).

### Transfer of Bone Marrow–Derived DCs

Bone marrow–derived DCs (BMDCs) from naive $CCR5^{+/+}$ or $CCR5^{-/-}$ mice were transferred into $CCR5^{-/-}$ mice intravenously 1 day before the first sensitization. After transfer of BMDCs, the mice were sensitized and challenged with OVA, and then AHR and BAL were assessed.

In some experiments, OVA-pulsed BMDCs were instilled intratracheally into naive $CCR5^{+/+}$ mice. At 10 days after BMDC transfer, mice were exposed to OVA for 3 days; 48 hours after the last challenge, AHR was assessed and BAL fluid and lung tissues were obtained (21).

### Statistical Analysis

All results are expressed as means (±SEM). ANOVA was used to determine the levels of difference between all groups. Pairs of groups of samples distributed parametrically were compared by unpaired, two-tailed Student's *t* test, and those samples distributed nonparametrically were compared by Mann-Whitney *U* test. Significance was assumed at *P* less than 0.05.

## RESULTS

### $CCR5^{-/-}$ Mice Develop Significantly Lower AHR Compared with $CCR5^{+/+}$ Mice after Sensitization and Challenge

We first assessed airway responsiveness to increasing doses of inhaled methacholine in $CCR5^{-/-}$ and $CCR5^{+/+}$ mice. Intraperitoneal OVA sensitization and airway challenge led to the development of increased AHR in $CCR5^{+/+}$ mice, as shown by significant increases in RL compared with mice challenged (nonsensitized) alone with OVA (Figure 1A). In contrast, OVA-sensitized and -challenged $CCR5^{-/-}$ mice developed lower increases in RL compared with sensitized and challenged $CCR5^{+/+}$ mice, but, nonetheless, the changes were significantly greater than $CCR5^{-/-}$ mice that were challenged alone.

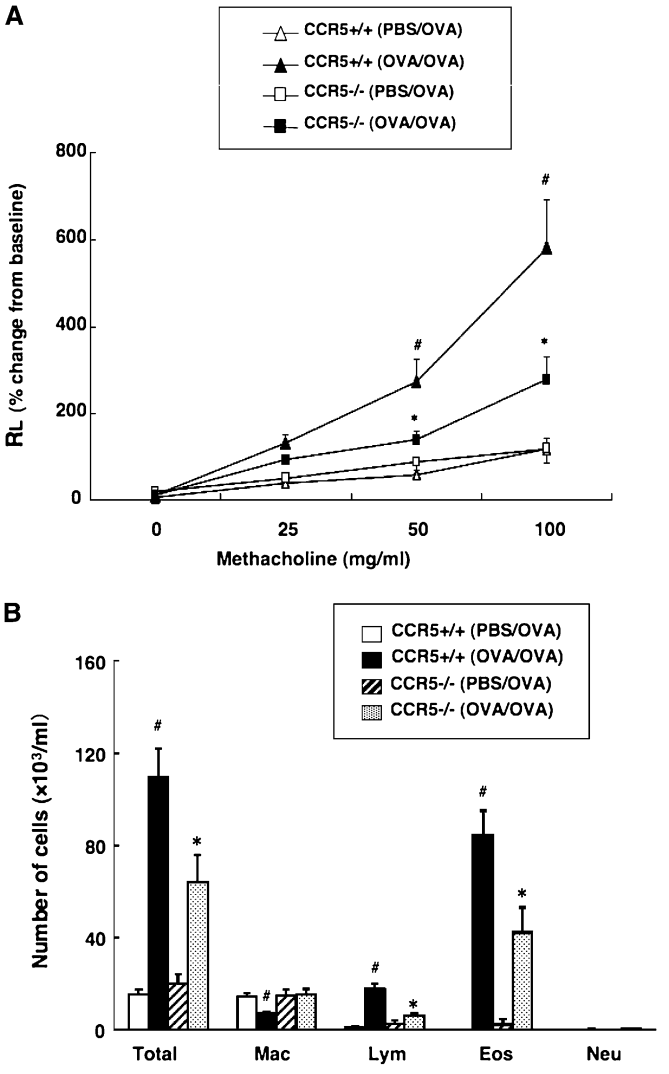


*Figure 1.* (*A*) Development of altered airway function in chemokine receptor (CCR) $5^{-/-}$ and $CCR5^{+/+}$ mice. Lung resistance (RL) was monitored in response to increasing concentrations of inhaled methacholine (MCh), as described in MATERIALS AND METHODS ($n = 10$–16 in each group). (*B*) Cellular composition in bronchoalveolar lavage (BAL) fluid. *Significant differences ($P < 0.05$) between CCR5-deficient ($CCR5^{-/-}$ ovalbumin [OVA]-sensitized and -challenged [OVA/OVA]) and CCR5-sufficient ($CCR5^{+/+}$ OVA/OVA) mice. #Significant differences ($P < 0.05$) between $CCR5^{+/+}$ OVA/OVA versus $CCR5^{-/-}$ PBS/OVA and $CCR5^{+/+}$ PBS/OVA mice.

### Lung Inflammation in CCR5$^{-/-}$ and CCR5$^{+/+}$ Mice

The numbers of inflammatory cells in BAL fluid were determined 48 hours after the last allergen challenge. In PBS/OVA mice, total cells were very few and macrophages comprised more than 80% of the cells. In OVA/OVA mice, total cells, eosinophils, and lymphocytes were increased compared with PBS/OVA mice. However, numbers of total cells, eosinophils, and lymphocytes in BAL fluid of CCR5$^{-/-}$ mice were significantly lower (Figure 1B).

Inflammatory cell infiltration was further investigated by histological examination of Hematoxylin and eosin–stained slides. In PBS/OVA mice, very few cells were detected in the peribronchial and perivascular areas. CCR5$^{+/+}$ mice showed accumulation of inflammatory cells after sensitization and challenge with OVA. In contrast, CCR5$^{-/-}$ mice showed lower numbers of inflammatory cells compared with CCR5$^{-/-}$ mice after sensitization and challenge. (Figure 2A). To quantify the numbers of leukocytes in the lung, lung tissue was first digested and cells recovered. The numbers of lymphocytes and eosinophils in the lung of CCR5$^{-/-}$ mice were lower compared with CCR5$^{+/+}$ mice after sensitization and challenge (*see* Figure E1 in the online supplement).

Goblet cell metaplasia was evaluated by periodic acid Schiff staining and quantification of positive cells (Figures 2A and 2B). The numbers of periodic acid Schiff–positive goblet cells were significantly lower in CCR5$^{-/-}$ mice compared with CCR5$^{+/+}$ mice after sensitization and challenge with OVA (Figure 2B).

### Cytokine Levels in BAL Fluid in CCR5$^{+/+}$ and CCR5$^{-/-}$ Mice

Cytokine levels in BAL fluid were measured by ELISA. OVA sensitization and challenge resulted in significant increases in IL-4, IL-5, and IL-13 levels in CCR5$^{+/+}$ mice (Figure 3A). In contrast, CCR5$^{-/-}$ mice showed significantly lower levels of IL-4, IL-5, and IL-13 after sensitization and challenge with OVA.

### Decreased Numbers of CD3, CD4, and CD8 T Cells in the Lungs of CCR5$^{-/-}$ Mice

To determine if the accumulation of T cells in the airways of sensitized and challenged mice was affected by absent expression of CCR5, BAL and lung cells were isolated and numbers of CD3$^+$, CD4$^+$, and CD8$^+$ T cells were determined by flow cytometry. The numbers of CD3$^+$, CD4$^+$, and CD8$^+$ T cells in the lungs of sensitized and challenged CCR5$^{-/-}$ mice were significantly lower than in CCR5$^{+/+}$ mice (Figure 3B). The numbers of CD3$^+$, CD4$^+$, and CD8$^+$ T cells in the BAL fluid of CCR5$^{-/-}$ mice were also lower (Figure 3C). In contrast, numbers of CD11c$^+$ cells and CD11c$^+$CD11b$^+$Gr1$^-$ cells in the lungs and PBLNs were not different in CCR5$^{+/+}$ and CCR5$^{-/-}$ mice after sensitization and challenge (Figure E2). These data indicate that accumulation of CD4$^+$ and CD8$^+$ T cells into the airways of sensitized mice after challenge was reduced in the absence of CCR5 expression on T cells, whereas the accumulation of DCs or myeloid DCs was not affected.

### *In Vitro* Cytokine Production from Spleen, PBLNs, and Lung MNCs

To determine if the attenuated Th2 cytokine secretion observed *in vivo* in CCR5$^{-/-}$ mice was due to impaired Th2 cytokine production from T cells, we assessed cytokine production from spleen, PBLNs, and lung MNCs *in vitro*. MNCs were isolated from sensitized and challenged mice and were restimulated with OVA *in vitro*. Levels of IL-4, IL-5, IL-13, and IFN-γ were measured in the culture supernatants from spleen (Figure 4A), PBLNs (Figure 4B), and lung (Figure 4C) MNCs by ELISA. There were no significant differences between CCR5$^{-/-}$ and CCR5$^{+/+}$ mice in terms of IL-4, IL-5, IL-13, and IFN-γ production after culture with medium alone or with OVA (data not shown).

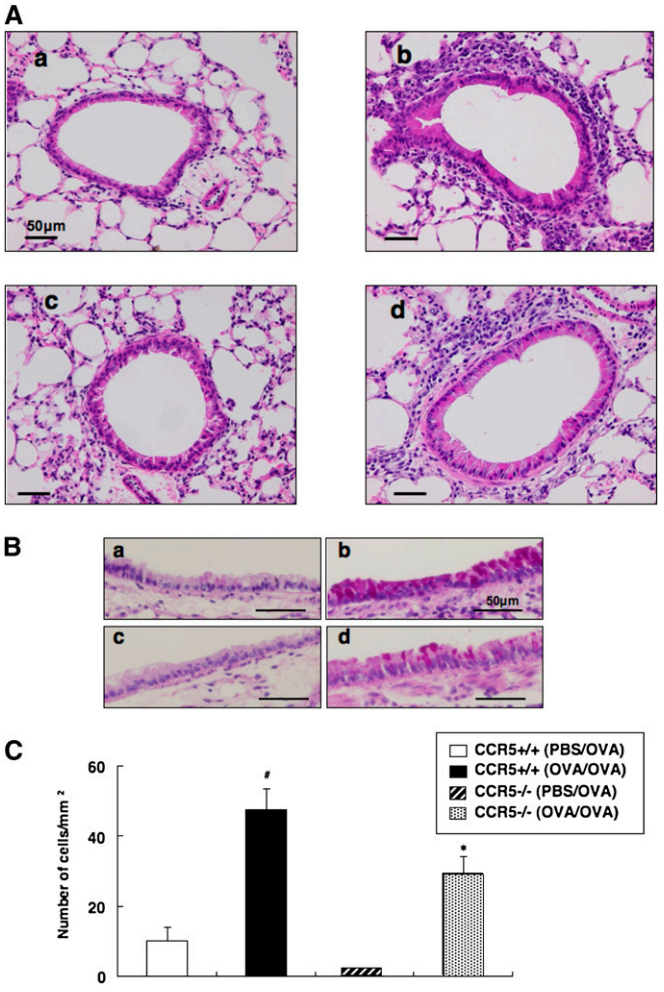


*Figure 2.* (*A*) Hematoxylin and eosin–stained sections. (*B*) Development of goblet cell metaplasia in CCR5$^{-/-}$ and CCR5$^{+/+}$ mice. Goblet cell metaplasia was quantified in periodic acid Schiff (PAS)–stained sections as described in Materials and Methods. (*a*) Nonsensitized CCR5$^{+/+}$ mice, (*b*) OVA/OVA CCR5$^{+/+}$ mice, (*c*) nonsensitized CCR5$^{-/-}$ mice, and (*d*) OVA/OVA CCR5$^{-/-}$ mice. (*C*) Quantitative analysis of PAS$^+$ cells in lung tissues was performed as described in Materials and Methods. *Significant differences ($P < 0.05$) between CCR5-deficient mice (CCR5$^{-/-}$ OVA/OVA) versus CCR5$^{-/-}$ PBS/OVA and CCR5$^{+/+}$ PBS/OVA. #Significant differences ($P < 0.05$) between CCR5$^{+/+}$ OVA/OVA versus CCR5$^{-/-}$ PBS/OVA and CCR5$^{+/+}$ PBS/OVA mice.

### Transfer of CCR5$^{+/+}$ T Cells Enhances AHR and Eosinophilic Inflammation in CCR5$^{-/-}$ Recipients

CCR5 is expressed on lung T cells (12). To address whether the absence of CCR5 expression on T cells in the CCR5$^{-/-}$ mice was responsible for the decreases in AHR and inflammation, we reconstituted CCR5$^{-/-}$ mice with CCR5$^{+/+}$ T cells. CCR5$^{-/-}$ recipients were sensitized with OVA on Days 1 and 14. On Day 28, 2 hours before OVA challenge, $1 \times 10^6$ or $5 \times 10^6$ CCR5$^{+/+}$

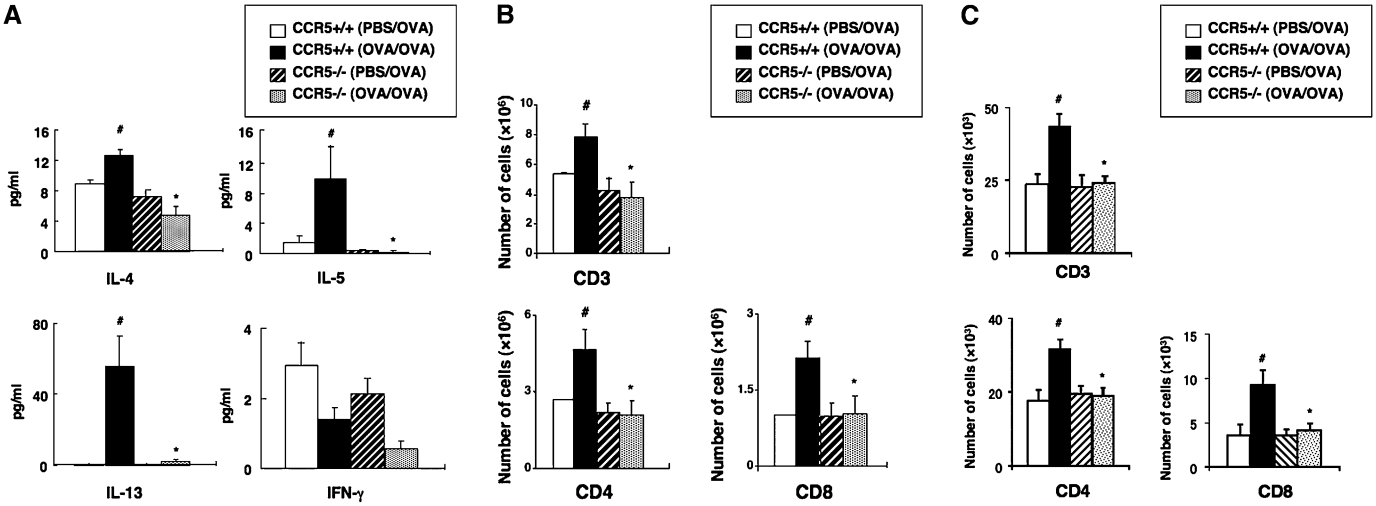


*Figure 3.* (*A*) Cytokine levels in BAL fluid. IL-4, IL-5, IL-13, and IFN-γ levels in BAL fluid from the same groups of mice as in Figure 1 were measured by ELISA. Numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T cells in the lung (*B*) and BAL fluid (*C*) after sensitization and challenge. Numbers of cells in the lung were determined as described in MATERIALS AND METHODS. #Significant differences ($P < 0.05$) between PBS/OVA and OVA/OVA. *Significant differences ($P < 0.05$) between $CCR5^{-/-}$ OVA/OVA and $CCR5^{+/+}$ OVA/OVA.

T cells were injected intravenously via the tail vein. As a control, some $CCR5^{-/-}$ recipients received $CCR5^{-/-}$ T cells. As assessed by flow cytometry, the proportion of the transferred cells that expressed CD3 from OVA-sensitized $CCR5^{+/+}$ or $CCR5^{-/-}$ mice exceeded 95%.

After transfer of cells, recipient mice were challenged with OVA on 3 consecutive days; 48 hours after last challenge, AHR was measured. Figure 5A shows the results of reconstitution with $CCR5^{+/+}$ T cells on airway responsiveness. Transfer of antigen-primed $5 \times 10^6$ $CCR5^{+/+}$ T cells into the sensitized $CCR5^{-/-}$ mice significantly enhanced the development of AHR after challenge. In contrast, transfer of $CCR5^{-/-}$ T cells failed to enhance AHR.

Analysis of the inflammatory cell populations in the BAL fluid of recipient mice showed that transfer of OVA-sensitized $5 \times 10^6$ $CCR5^{+/+}$ T cells increased the numbers of eosinophils and lymphocytes (Figure 5B), in parallel to the development of AHR. However, $CCR5^{-/-}$ T cell transfer failed to increase the numbers of eosinophils. In addition, transfer of OVA-sensitized $CCR5^{+/+}$ T cells, but not $CCR5^{-/-}$ T cells, enhanced the levels of IL-4, IL-5, and IL-13 in the BAL fluid (Figure E3).

### Migration of Transferred $CCR5^{-/-}$ and $CCR5^{+/+}$ T Cells into the Lung

To determine directly whether expression of CCR5 on antigen-sensitized T cells was critical to the recruitment of these cells to the airway, we monitored numbers of transferred $CCR5^{-/-}$ and $CCR5^{+/+}$ T cells in the airways. Recipient $CD45.1^+$ $CCR5^{+/+}$ mice were sensitized with OVA on Days 1 and 14. On Day 28, 2 hours before OVA challenge, $5 \times 10^6$ $CD45.2^+CCR5^{+/+}$ T cells or $CD45.2^+CCR5^{-/-}$ T cells were injected intravenously via the tail vein. Transferred $CCR5^{+/+}$ T cells (identified as $CD3^+CD45.2^+$) amounted to 4.0 (±0.5)% (mean [±SEM] from three independent experiments) of total MNCs in the lungs after OVA sensitization and challenge, whereas transferred $CCR5^{-/-}$ T cells were found at much lower percentages (0.3 ± 0.1% of total MNCs). Figure 6 shows the numbers of transferred T cells in the lungs. Thus, fewer $CCR5^{-/-}$ T cells migrated into the lung compared with $CCR5^{+/+}$ T cells. These results suggest that CCR5 expression on T cells plays an important role in migration of antigen-sensitized T cells into the airways.

### $CCR5^+$ T Cells Produce IL-13 in the Lung

To confirm that $CCR5^+$ T cells produce IL-13 in the airways, $CCR5^{+/+}$ mice were sensitized and challenged with OVA, then the lung MNCs were isolated, and intracellular staining for IFN-γ and IL-13 was performed. $CCR5^+$ T cells stained positively for IFN-γ and IL-13; however, the percentages of IL-13–producing cells were higher than IFN-γ–producing cells (Figure E4).

### Transfer of $CCR5^{+/+}$ DCs does not Enhance AHR or Eosinophilic Inflammation in $CCR5^{-/-}$ Recipients

In animal models of allergic airway responses, DCs have been shown to play important roles in sensitization of antigen-specific T cells and in the development of lung allergic responses. Previous studies have shown that DCs express CCR5 (3–6). Therefore, we hypothesized that DCs expressing CCR5 might play an important role in the initiation of immune responses in the airways. To evaluate $CCR5^{+/+}$ DC function, we first investigated the ability of DCs to induce AHR after intravenous injection. DCs from $CCR5^{+/+}$ or $CCR5^{-/-}$ mice were transferred intravenously to $CCR5^{-/-}$ mice, followed by sensitization and challenge with OVA, and airway responses were monitored. Figures 7A and 7B show the results of reconstitution with $CCR5^{+/+}$ DCs on allergic airway responses. Transfer of $CCR5^{+/+}$ BMDCs into $CCR5^{-/-}$ mice did not enhance the development of AHR or eosinophilic inflammation after sensitization and challenge. These data suggest that reconstitution of $CCR5^{-/-}$ mice with $CCR5^{+/+}$ DCs alone does not affect allergen-induced airway responses.

### $CCR5^{-/-}$ DCs Induce AHR

To evaluate $CCR5^{-/-}$ DC function directly, we investigated the ability of antigen-pulsed BMDCs to induce AHR after intratracheal instillation. BMDCs from $CCR5^{+/+}$ or $CCR5^{-/-}$ mice were pulsed with OVA, washed, and transferred intratracheally to $CCR5^{+/+}$ mice. At 10 days after sensitization, recipient mice were then challenged on 3 consecutive days with aerosolized OVA and evaluated for AHR. These experiments showed that OVA-pulsed DCs from $CCR5^{+/+}$ and $CCR5^{-/-}$ mice were comparable in inducing increases in AHR and eosinophilic

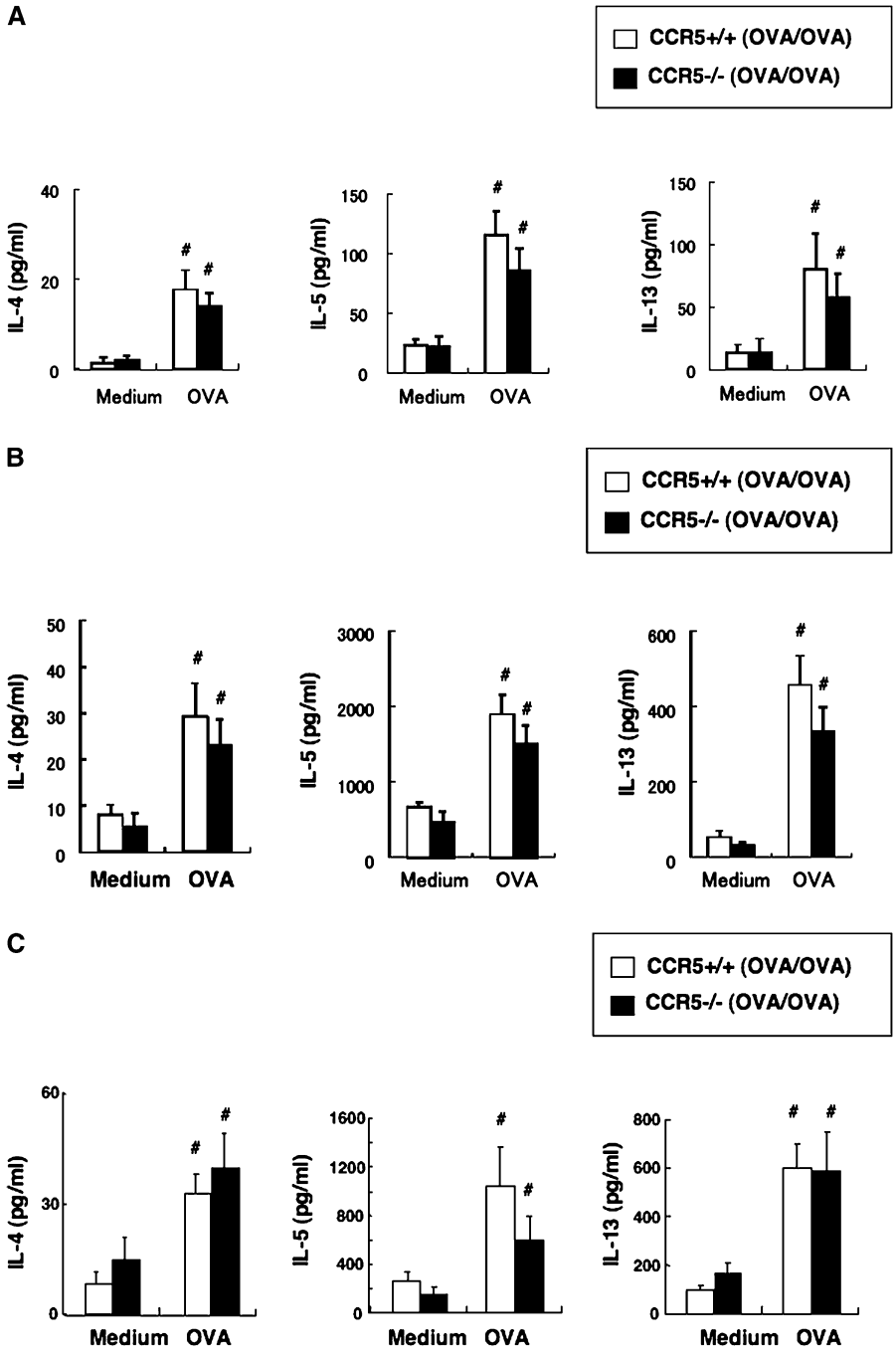


*Figure 4.* IL-4, IL-5, and IL-13 levels in supernatants from spleen (*A*), peribronchial lymph nodes (PBLNs) (*B*), and lung (*C*) mononuclear cells (MNCs), cultured in the presence or absence of OVA (10 μg/ml), were determined by ELISA. There were no significant differences between $CCR5^{-/-}$ and $CCR5^{+/+}$ mice. #Significant differences ($P < 0.05$) between medium and OVA.

inflammation, confirming the functional competence of $CCR5^{-/-}$ DCs in facilitating the development of AHR and associated inflammation in the presence of $CCR5^{+/+}$ T cells (Figures 7C and 7D).

## DISCUSSION

Previous studies have shown that CCR5 contributes to Th1-type immune responses (8–10). However, the role of CCR5 in the development of allergen-induced AHR and airway inflammation has not been well defined. In the present study, we show that CCR5 plays a critical role in the development of OVA-induced AHR and inflammation. Using $CCR5^{-/-}$ mice, OVA-induced AHR was significantly reduced compared with wild-type mice in the absence of CCR5. Analysis of BAL fluid and histology revealed that eosinophilic inflammation and goblet cell metaplasia were also attenuated in $CCR5^{-/-}$ mice. Th2 cytokine levels in BAL fluid of $CCR5^{-/-}$ mice were significantly reduced after sensitization and challenge, suggesting that the contribution of CCR5 to the development of AHR and inflammation may be linked, at least in part, to Th2 cytokine production *in vivo*.

The results of this study are consistent with those of a previous study showing that CCR5 contributes to AHR and inflammation where a different model of allergen-induced airway responses was assessed using fungal antigen (11). Gupta and colleagues (13) have also reported the effect of CCR5 blockade in suppression of allergic airway responses. However, the mechanisms through which CCR5 affects allergic airway responses have not been well defined. Here, we show that transfer of antigen-

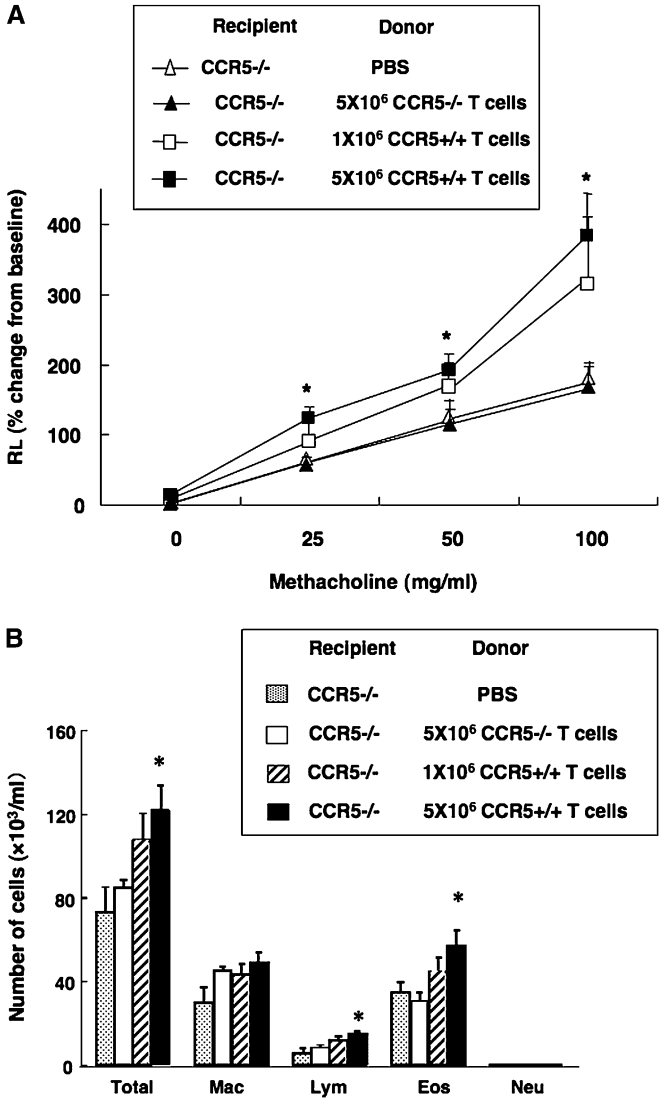


*Figure 5.* Reconstitution of CCR5$^{-/-}$ mice with antigen-primed CCR5$^{+/+}$ T cells enhances lung allergic responses. (*A*) Airway hyperresponsiveness (AHR) and (*B*) cellular composition in BAL fluid. OVA-sensitized CCR5$^{-/-}$ mice (recipient mice) received $1 \times 10^6$ or $5 \times 10^6$ T cells from spleens of OVA-sensitized CCR5$^{+/+}$ mice ($1 \times 10^6$ CCR5$^{+/+}$ donor T group or $5 \times 10^6$ CCR5$^{+/+}$ donor T group; $n = 8$ in each group) intravenously via the tail vein 2 hours before the first airway challenge with aerosolized OVA. Some recipients received $5 \times 10^6$ T cells from OVA-sensitized CCR5$^{-/-}$mice (CCR5$^{-/-}$mice donor T group; $n = 8$). Mice that received PBS only are also shown (CCR5$^{-/-}$ PBS group, $n = 8$). *Significant differences ($P < 0.05$) are indicated between $5 \times 10^6$ CCR5$^{+/+}$ donor T group versus CCR5$^{-/-}$ mouse donor T group and CCR5$^{-/-}$ mice that only received PBS.

sensitized T cells obtained from CCR5$^{+/+}$ mice into CCR5$^{-/-}$ mice enhanced AHR and eosinophilic airway inflammation, suggesting that CCR5 expression on antigen-sensitized T cells plays an important role in the induction of allergen-induced airway responses. Moreover, larger numbers of transferred CCR5$^{+/+}$ T cells accumulated in the lungs of recipient CCR5$^{-/-}$ mice compared with transfer of CCR5$^{-/-}$ T cells, suggesting that CCR5 expression on antigen-sensitized T cells is a limiting factor for migration of these cells into the lungs. Not surprisingly, *in vitro* culture of lung cells from both strains of mice showed comparable production of the Th2 cytokines IL-5 and IL-13. These findings confirm the role of CCR5 in the chemoattractant response, but

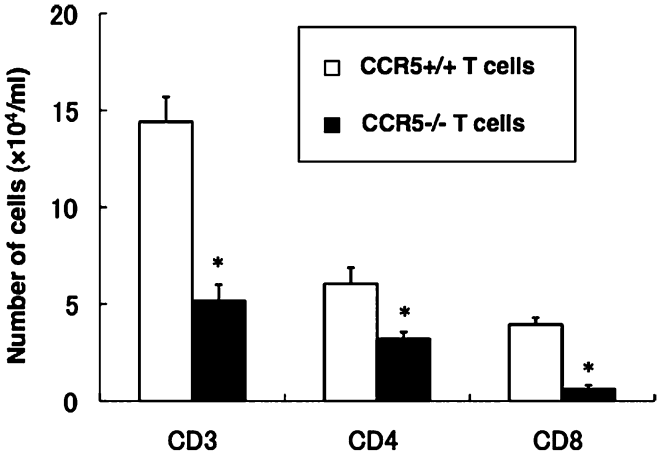


*Figure 6.* Fewer transferred CCR5$^{-/-}$ T cells can be detected in the lung. Recipient CD45.1$^+$ CCR5$^{+/+}$ mice were sensitized with OVA on Days 1 and 14. On Day 28, 2 hours before OVA challenge, $5 \times 10^6$ CD45.2$^+$CCR5$^{+/+}$ or CD45.2$^+$ CCR5$^{-/-}$ T cells were injected intravenously into recipient CD45.1$^+$CCR5$^{+/+}$ mice via the tail vein. Numbers of transferred CCR5$^{+/+}$ or CCR5$^{-/-}$ T cells in the lungs (identified as CD3$^+$CD45.2$^+$) were assessed as described in MATERIALS AND METHODS. *Significant differences ($P < 0.05$) are indicated between numbers of CCR5$^{+/+}$ versus CCR5$^{-/-}$ T cells.

without effects on function. The results also define the importance of antigen-sensitized CCR5$^{+/+}$ T cell accumulation in the lung for AHR and eosinophilic inflammation.

Walker and colleagues (14) reported that CCR5$^{-/-}$ mice showed lower AHR, but the numbers of eosinophils in CCR5$^{-/-}$ mice were similar to those in CCR5$^{+/+}$ mice. In contrast, in the current study, CCR5$^{-/-}$ mice showed lower numbers of eosinophils compared with CCR5$^{+/+}$ mice using the same background of mice, but generated differently. The basis for this discrepancy is not clear, but may reflect the use of a different protocol as well as unknown factors related to the generation of the mice. They challenged mice with OVA for 1 day, whereas, in the current study, mice were challenged for 3 days, which likely enhanced the extent of eosinophilic inflammation.

Ligands for CCR5 are thought to be RANTES, MIP-1α, and MIP-1β (7). These ligands act promiscuously and activate other chemokine receptors. RANTES acts on not only CCR5, but also CCR1 and CCR3. MIP-1α acts on CCR5 and CCR1, but MIP-1β only acts on CCR5. Blockade of RANTES may suppress allergen-induced airway responses, but not AHR (22, 23). In animals infected with respiratory syncytial virus, anti-RANTES suppressed the development of AHR (24). However, after repeated allergen challenge, anti-RANTES enhanced AHR (25). Therefore, the role of RANTES appears to be complex, and might depend on the phase of allergic responses when interference is introduced. Additional studies are needed to determine directly the role of CCR5 ligands in the development of allergic airway responses.

Migration of Th2 cells to the lung is regulated, in large part, by chemokine receptors, members of the seven-membrane-spanning receptor families. Lipid mediators and their receptors have also been reported to play important roles for migration of T cells to the lungs (26). We recently showed that the lipid mediator, LTB4, and interaction with its high affinity receptor BTL1, plays an important role in the migration of antigen-sensitized, IL-13-producing effector T cells to the lungs (20, 27). Specifically, the LTB4–BLT1 pathway resulted in the recruitment of antigen-specific effector (memory) CD8$^+$ T cells to the lungs (28–30). In the present study, absence of CCR5 also

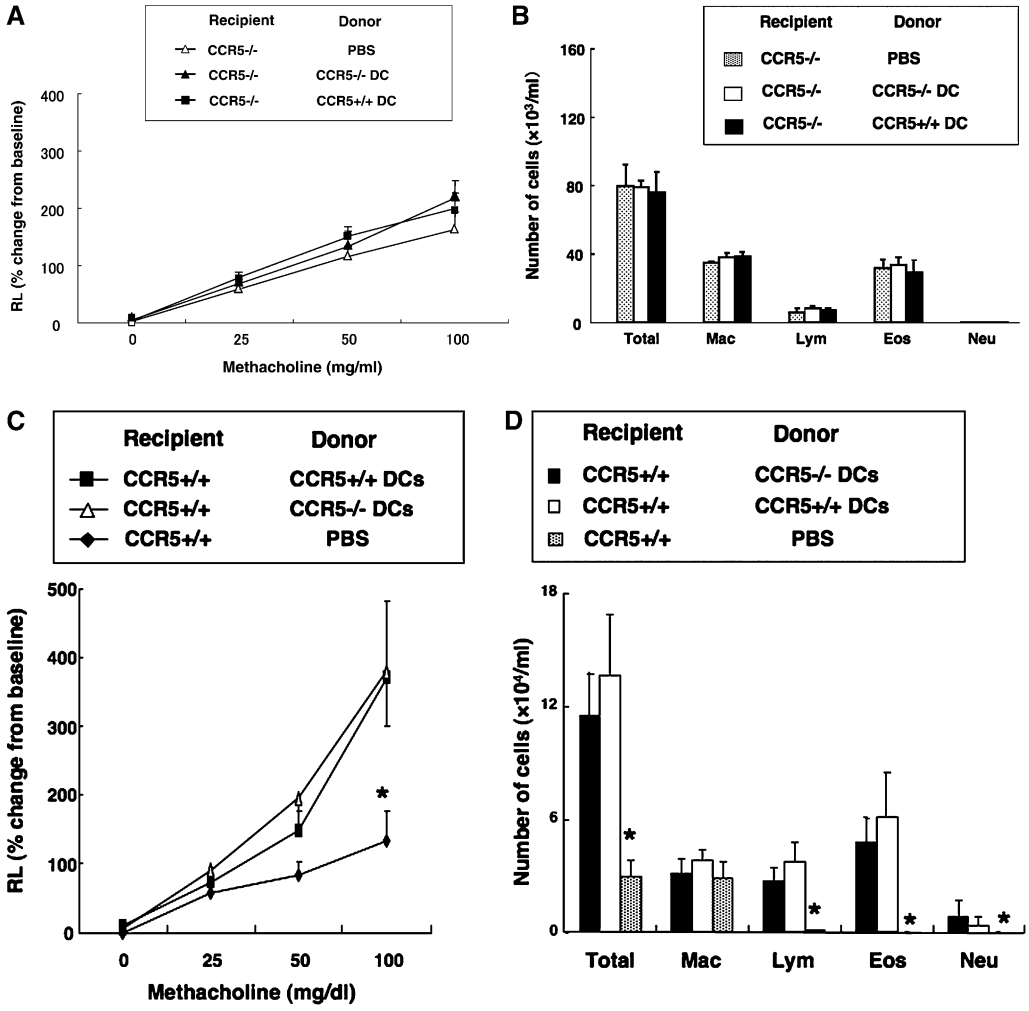


*Figure 7.* Reconstitution of $CCR5^{-/-}$ mice with dendritic cells (DCs) does not enhance lung allergic responses. (*A*) AHR or (*B*) cellular composition in BAL fluid. OVA-sensitized $CCR5^{-/-}$ mice received $1 \times 10^6$ $CCR5^{+/+}$ DCs ($CCR5^{+/+}$ donor DC group; $n = 8$) or $CCR5^{-/-}$ DC ($CCR5^{-/-}$ donor DC group; $n = 8$) intravenously via the tail vein, followed by OVA challenges. Mice that received PBS only are also shown ($CCR5^{-/-}$ PBS group; $n = 8$). Development of (*C*) altered airway function and (*D*) airway inflammation in BAL fluid in mice that received $CCR5^{+/+}$ or $CCR5^{-/-}$ DCs. OVA-pulsed $CCR5^{+/+}$ bone marrow–derived DCs (BMDCs; $1 \times 10^6$), OVA-pulsed $CCR5^{-/-}$ BMDCs ($1 \times 10^6$), or PBS were administered intratracheally to naive $CCR5^{+/+}$ mice before challenge. *Significant differences ($P < 0.05$) are indicated between PBS and $CCR5^{+/+}$ DCs or $CCR5^{-/-}$ DCs.

affected migration of T cells, especially $CD8^+$ T cells to the lungs. Interestingly, both CCR5 and BLT1 are seven-membrane-spanning receptor family members that use β-arrestin-2 for activation of the receptors (31–33). Together, the results of this and previous studies are consistent with a report showing that β-arrestin-2 regulates the development of allergic inflammation at a proximal step in the inflammatory cascade (34).

A critical stage in the induction of T cell activation is the uptake, processing, and presentation of antigen by antigen-presenting cells. Among lung antigen-presenting cells, DCs play a key role in the initiation of immune responses in the airways (35, 36). CCR5 is reported to be expressed on DCs (37, 38). To determine if impaired *in vivo*, Th2 responses observed in $CCR5^{-/-}$ mice were due to impaired $CCR5^{-/-}$ DC function, we used a BMDC-dependent model of allergic airway disease. After transfer of $CCR5^{-/-}$ BMDCs, we observed a similar development of AHR and eosinophilic inflammation as that seen after transfer of $CCR5^{+/+}$ BMDCs, indicating their competence in priming of antigen-specific T cells. Previous studies have demonstrated a need for CCR2 and CCR6 on DCs in allergic airway responses (39).

In summary, we have identified an important role for CCR5 on antigen-primed T cells in the full development of allergen-induced allergic airway responses. In the absence of CCR5, the reduction in AHR was associated with a significant reduction in numbers of lung $CD4^+$ and $CD8^+$ T cells, as well as reduced numbers of eosinophils and mucus-containing goblet cells in the airways. The cumulative data indicate that control of CCR5 expression and function would provide a novel interventional strategy for the treatment of asthma.

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