

# Differential Effects of Dendritic Cell Transfer on Airway Hyperresponsiveness and Inflammation

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Dendritic cells (DCs) are considered to be the most efficient antigen-presenting cells. Intratracheal administration of allergen-pulsed bone marrow–derived dendritic cells (BMDCs) before allergen challenge induces airway hyperresponsiveness (AHR) and inflammation. Ovalbumin (OVA)-pulsed BMDCs from wild-type (WT) mice were transferred into naive WT, CD4<sup>-/-</sup>, CD8<sup>-/-</sup>, or IL-13<sup>-/-</sup> mice. Two days (short protocol) or 10 days (long protocol) after BMDC transfer, mice were challenged with 1% OVA for 3 days and assayed 2 days later. Transfer of OVA-primed BMDCs into BALB/c or C57BL/6 mice elicited AHR in both protocols. Airway eosinophilia, Th2 cytokines, or goblet cell metaplasia were increased in the long but not short protocol. Lung T cells from both protocols produced Th2 cytokines in response to OVA *in vitro*. Carboxyfluorescein diacetate succinimidylester–labeled BMDCs were observed in bronchoalveolar lavage (BAL) fluid and lung parenchyma at early time points, and were detected in draining lymph nodes 48 hours after transfer. CD8<sup>-/-</sup> mice developed AHR comparable to WT mice in the short protocol, but decreased levels of AHR, airway eosinophilia, Th2 cytokines in BAL fluid, and goblet cell metaplasia compared with WT mice in the long protocol. CD4<sup>-/-</sup> or IL-13<sup>-/-</sup> mice did not develop AHR or airway inflammation in either protocol. These data suggest that allergen-pulsed BMDCs initiate development of AHR that is dependent initially on CD4<sup>+</sup> T cells, and at later time periods on CD8<sup>+</sup> T cells and IL-13. Thus, the timing of allergen challenge after transfer of allergen-pulsed BMDC affects the development of AHR and airway inflammation.

**Keywords:** dendritic cells; CD8<sup>+</sup> T cells; airway hyperresponsiveness

Eosinophilic airway inflammation, Th2 cytokine production, and airway hyperresponsiveness (AHR) are characteristic features of allergic bronchial asthma (1). The immunologic basis of this disease has been a topic of intense investigation. T cell responses to allergens are critical in the pathogenesis of asthma. In addition, a large number of cytokines, chemokines, adhesion molecules, and costimulatory molecules have been shown to be involved (2).

A critical step in the induction of a T cell immune response is the uptake, processing, and presentation of antigen by antigen-presenting cells (APCs). Among the different types of APCs, dendritic cells (DCs) are the most predominant APCs in the airways (3, 4). In rats, allergen-primed lung DCs induce allergen-specific Th2-mediated IgG1 with little Th1-directed allergen-specific IgG2b generation (3). Lung DCs are potent regulators of Th2-biased responses to inhalant allergens (4).

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Intratracheally administered allergen-pulsed DCs migrate to draining lymph nodes and initiate priming of T cells (5). After allergen challenge they elicit AHR and allergic airway inflammation (6–9). These studies showed that maturation, migration to draining lymph nodes, and expression of costimulatory molecules on DCs were all essential for the priming of T cells and for the development of allergic airway inflammation. Moreover, Fcγ receptors and Toll-like receptors, Fas ligand, Syk kinase activity, or epithelial-derived cytokines such as thymic stromal lymphopoietin (TSLP), have all been shown to regulate lung allergic responses through interactions with DCs (10–13). However, the interactions between DCs and specific T cell subsets in the development of AHR and airway allergic inflammation have not been defined.

In the present study, we investigated the role of locally administered DCs in the priming of CD4<sup>+</sup> or CD8<sup>+</sup> T cells in the development of allergen-induced AHR and airway inflammation. In this model, it appeared that the interval between lung allergen exposure and intratracheal administration of allergen-pulsed DCs dictated the outcomes and the requirements for specific T cell subsets.

## MATERIALS AND METHODS

### Animals

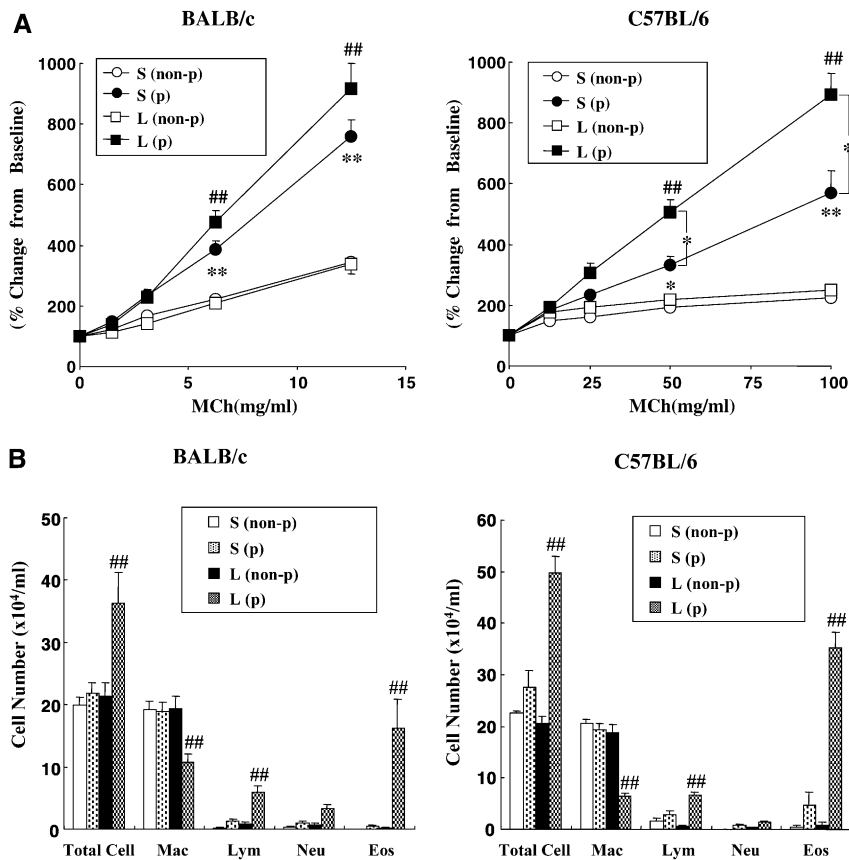
Female BALB/c and C57BL/6 (B6) mice were purchased at 8 to 12 weeks of age from Jackson Laboratories (Bar Harbor, ME). Age-matched CD4<sup>-/-</sup> mice and CD8<sup>-/-</sup> mice, generated by targeting the CD4 or CD8α chain gene in B6 mice, respectively (14, 15), were obtained from Dr. P. Marrack (National Jewish Health, Denver, CO). IL-13<sup>-/-</sup> mice (BALB/c background) initially provided by Dr. D. Umetsu (Stanford University, Stanford, CA) (16), were housed under specific pathogen–free conditions and maintained on an ovalbumin (OVA)-free diet. Experiments were conducted under a protocol approved by Institutional Animal Care and Use Committee of the National Jewish Health.

### Local Administration of DCs for the Induction of Airway Allergic Inflammation

DCs were generated from bone marrow cells of naive BALB/c or B6 mice according to the procedure described previously (17). Briefly, bone marrow cells were obtained from femurs and iliac bones of mice and placed in DC culture medium (DC-CM; RPMI 1640 containing 10% heat-inactivated fetal calf serum [FCS], 50 μM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin) (GIBCO, Carlsbad, CA), 10 ng/ml recombinant mouse GM-CSF, and 10 ng/ml recombinant mouse IL-4 (R&D Systems, Minneapolis, MN).

On Day 8, nonadherent cells were used as DCs. These cells were more than 95% CD11c<sup>+</sup>CD11b<sup>+</sup>. The remaining cells were CD11c<sup>+</sup>B220<sup>+</sup> or CD11c<sup>+</sup>CD11b<sup>+</sup> cells, but no CD3<sup>+</sup> cells were detected. DCs were pulsed with OVA (200 μg/ml) for 24 hours and washed three times with PBS. As control, DCs were cultured without OVA. After incubation with OVA, the expression of costimulatory factors such as CD80 or CD86 on DCs were not significantly different compared with DCs not pulsed with OVA.

OVA-pulsed or nonpulsed DCs (1 × 10<sup>6</sup> cells) were instilled intratracheally into naive mice. In the short protocol, animals were



**Figure 1.** Effect of dendritic cell (DC) transfer on lung allergic responses. (A) Changes in airway resistance ( $R_i$ ) after the short and long protocols. Increasing concentrations of nebulized MCh were administered through the tracheal cannula 48 hours after the last ovalbumin (OVA) challenge in BALB/c and C57BL/6 mice. (B) Bronchoalveolar lavage (BAL) cellular composition in BALB/c and C57BL/6 mice. (C) Cytokine levels in BAL fluid. (D) Sections of lung tissues obtained 48 hours after the last challenge were stained with periodic acid-Schiff (PAS). Representative histologic sections (BALB/c [a–d] and C57BL/6 mice [e–h]) after the short protocol with OVA nonpulsed DCs (a, f), short protocol with OVA-pulsed DCs (b, g), long protocol with OVA nonpulsed DCs (c, h), and long protocol with OVA-pulsed DCs (d, i). Quantitative analysis of PAS+ cells in BALB/c mice (e) and C57BL/6 mice (j). Data represent the means  $\pm$  SEM from three independent experiments consisting of four mice/group ( $n = 12$ ). \* $P < 0.05$  or \*\* $P < 0.01$  compared with recipients of OVA nonpulsed DCs in short protocol or as indicated. ## $P < 0.01$  compared with recipients of OVA nonpulsed DCs in long protocol. S: short protocol, L: long protocol, non-p: DCs were not pulsed with OVA, p: DCs were pulsed with OVA. Mac: macrophages, Lym: lymphocytes, Neu: neutrophils, Eos: eosinophils, bm: basement membrane.

challenged with nebulized OVA (1% in saline) for 20 minutes for 3 consecutive days beginning 2 days after DC instillation. In the long protocol, animals were challenged with OVA on 3 consecutive days beginning 10 days after DC instillation. Forty-eight hours after the last OVA challenge in both protocols, assays were performed.

#### Determination of Airway Responsiveness and Airway Inflammation

Airway responsiveness was assessed as previously described by measuring changes in lung resistance ( $R_L$ ) in response to increasing doses of inhaled methacholine (MCh) (18). Many studies have demonstrated strain differences in airway responsiveness to inhaled MCh challenge (19, 20). C57BL/6 mice showing comparable changes in airway resistance as BALB/c mice but at higher MCh concentrations. Data are expressed as the percent change from baseline  $R_L$  values obtained after inhalation of saline. Baseline levels of  $R_L$  were not significantly different among the groups.

Immediately after measurement of AHR, lungs were lavaged via the tracheal tube as previously described (21). Briefly, bronchoalveolar lavage (BAL) fluid was collected with 1 ml of Hanks' balanced salt solution (HBSS) via the tracheal tube and slides were prepared with Cytospin III (Shandon Scientific, Pittsburgh, PA) and stained with May-Giemsa for differential cell counts. BAL was obtained after MCh challenge. Cell composition and cytokine levels in BAL fluid were not altered by MCh challenge.

Lungs were fixed in 10% formalin and processed into paraffin. Mucus-containing goblet cells were detected by staining of paraffin sections (5  $\mu$ m thick) with periodic acid-Schiff (PAS). Histologic analyses were done as previously described (21).

#### Cell Preparations of Lung T Cells and Cultures with OVA

Lung cells were isolated as previously described (17) using collagenase digestion. Cells were resuspended in HBSS, and mononuclear cells

(MNCs) were purified over 35% Percoll (Sigma-Aldrich, St. Louis, MO). Lung T cells were purified from lung using the Mouse T Cell Recovery Column Kit (Cederlane, Hornby, ON, Canada) (purity  $> 95\%$ ). Cells were washed, counted, and suspended at  $4 \times 10^6$  cells/ml in RPMI 1640 (Life Technologies, Gaithersburg, MD) tissue culture medium, containing heat-inactivated 10% FCS, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, 5 mM glutamine, and 50  $\mu$ M 2-mercaptoethanol. As APCs, spleen MNCs were irradiated (30 Gy). Lung T cells were cultured with irradiated spleen MNCs and OVA (10  $\mu$ g/ml) for 72 hours, at which time supernates were recovered for cytokine assays.

#### Measurement of Cytokines in Supernatants from Cell Culture or BAL Fluid

Supernatants from cell cultures or BAL fluid were stored at  $-80^\circ\text{C}$  until used for cytokine measurements. Levels of cytokines were determined using commercially available enzyme-linked immunosorbent assays (ELISAs) following the manufacturers' instructions. ELISA kits for detection of IL-4, IL-5, and IFN- $\gamma$  in supernatants were obtained from BD Pharmingen (San Diego, CA). The IL-13 ELISA kit was purchased from R&D Systems. The limits of detection for each assay were as follows: 4 pg/ml for IL-4, IL-5, and IFN- $\gamma$ ; and 1.5 pg/ml for IL-13.

#### Tracking of Transferred DCs

DCs from C57BL/6 mice were labeled with carboxyfluorescein diacetate succinimidylester (CFSE) for 12 hours before intratracheal transfer. Twenty-four hours, 48 h, 96 h, and 7 d after bone marrow-derived DC (BMDC) transfer in the absence of challenge, BAL fluid, lungs, peribronchial lymph nodes, and spleens were taken. Cells were isolated from each organ and analyzed by flow cytometry for CFSE+ CD11c+ staining.

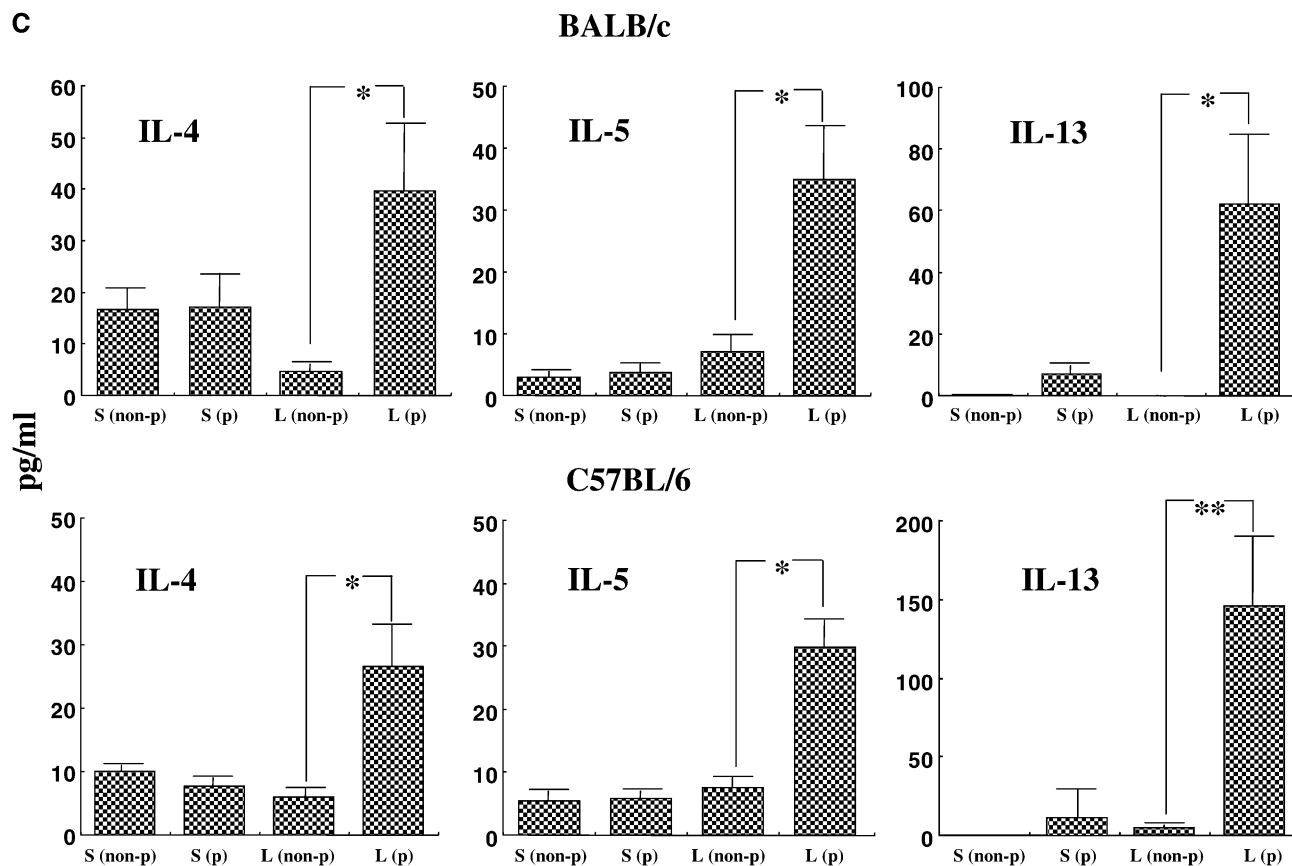


Figure 1. (continued).

### Statistical Analysis

The ANOVA *t* test was used to determine differences between two groups, and the Tukey-Kramer test was used for comparisons between multiple groups. Mann-Whitney U tests were also used to determine if the levels of difference between all groups remained significant, even if the underlying distribution was uncertain. The data were pooled from three independent experiments with four mice/group in each experiment ( $n = 12$ ). Comparisons for all pairs were performed by Kruskal-Wallis test. Significance was assumed at *P* values of  $< 0.05$  for all tests. Values for all measurements are expressed as means  $\pm$  SEM.

## RESULTS

### Effect of Locally Administered Allergen-Pulsed DCs followed by Allergen Challenge

We initially examined the effects of DC transfer in recipients that were challenged at two time-points after transfer. Naive mice received OVA-pulsed BMDCs intratracheally on Day 0, then were challenged with OVA via the airways on Days 2 to 4 (short) or from Days 10 to 12 (long). As shown in Figure 1A, AHR developed under both the short and long protocols in both BALB/c and C57BL/6 mice after transfer of OVA-pulsed DCs but not after transfer of nonpulsed DCs. In C57BL/6 mice, the degree of AHR in the long protocol was greater than in the short protocol (Figure 1A). As reported previously, the responsiveness to MCh was different between these strain of mice (19, 20); the values of RL in C57BL/6 mice were comparable to those in BALB/c mice at higher concentrations of MCh after adoptive transfer of OVA-pulsed DCs and OVA challenge. The

numbers of eosinophils and lymphocytes in BAL fluid were also significantly increased under the long but not short protocol in both strains (Figure 1B). Assays of levels of Th2 cytokines in BAL fluid showed that intratracheal transfer of OVA-pulsed DCs induced significant increases in both strains compared with transfer of nonpulsed DCs, but these increases were only seen under the long protocol (Figure 1C). PAS staining of lung sections revealed that intratracheal administration of OVA-pulsed DCs induced goblet cell metaplasia under the long protocol; PAS+ cells were also increased under the short protocol, but to a lesser degree (Figure 1D).

### Allergen-Specific Responses in Lung T Cells after DC Transfer

To investigate T cell responsiveness to allergen after DC transfer, lung T cells from C57BL/6 mice were purified, cultured *in vitro*, and supernates assayed for Th2-type cytokine production. Lung T cells from mice that received nonpulsed DC followed by allergen challenge did not show any production of cytokines, whereas lung T cells from recipients of allergen-pulsed DCs produced Th2-type cytokines in response to allergen (Figure 2). The levels of IL-5 and IL-13 from lung T cells (long protocol) were significantly higher than those obtained under the short protocol (Figure 2). The results were similar when lung T cells from BALB/c mice were assayed (data not shown).

### Trafficking of Intratracheally Transferred DCs

To investigate the fate of transferred DCs, DCs from C57BL/6 mice were labeled with CFSE and tissues were examined by

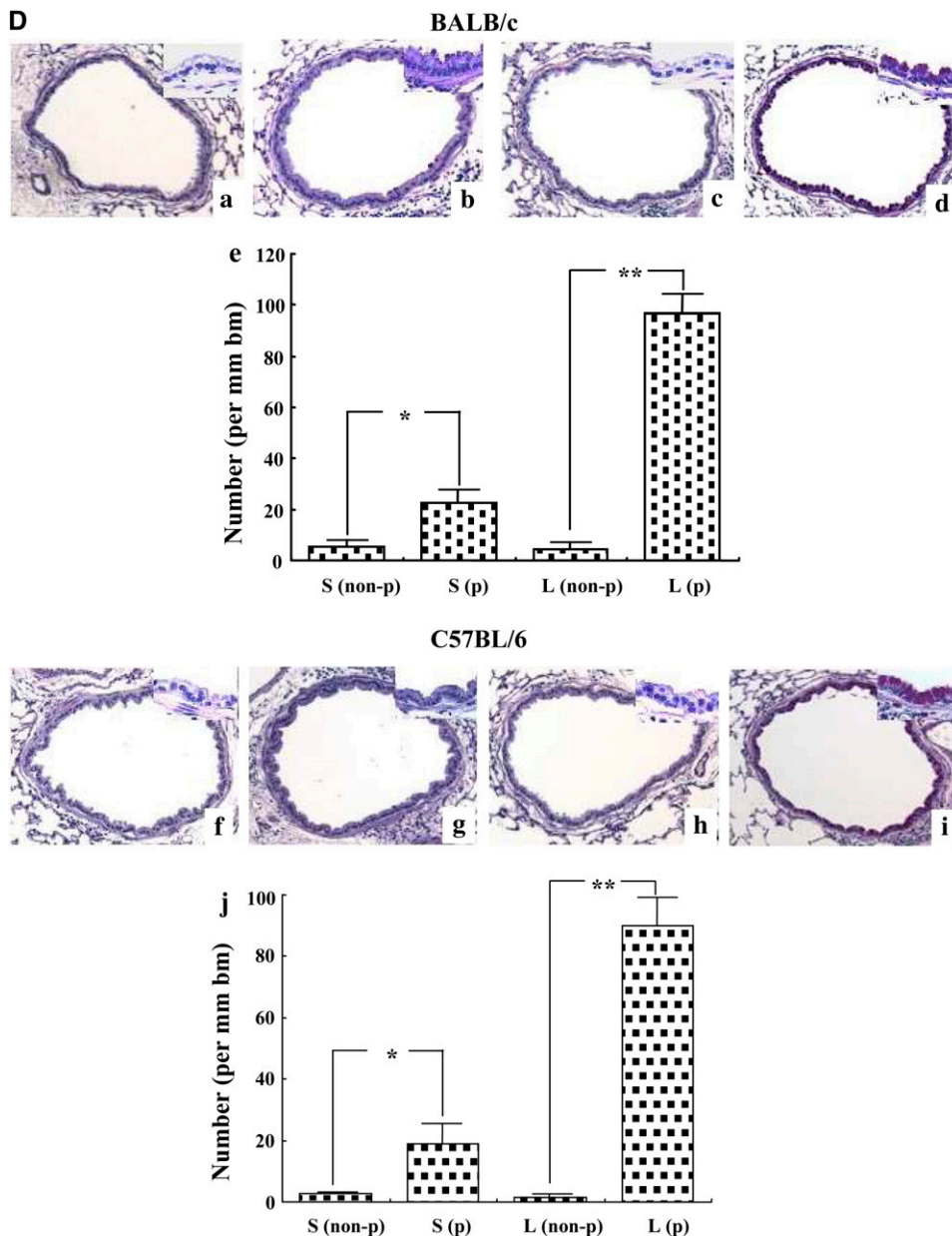


Figure 1. (continued).

flow cytometry gating on CFSE+CD11c+ cells. Transferred DCs were observed by 24 hours in BAL fluid and lung parenchyma. After 48 hours, transferred DCs were detected in draining lymph nodes. Few transferred DCs were found in the spleen (Figure 3). Experiments performed in the same manner in BALB/c mice revealed similar results (data not shown).

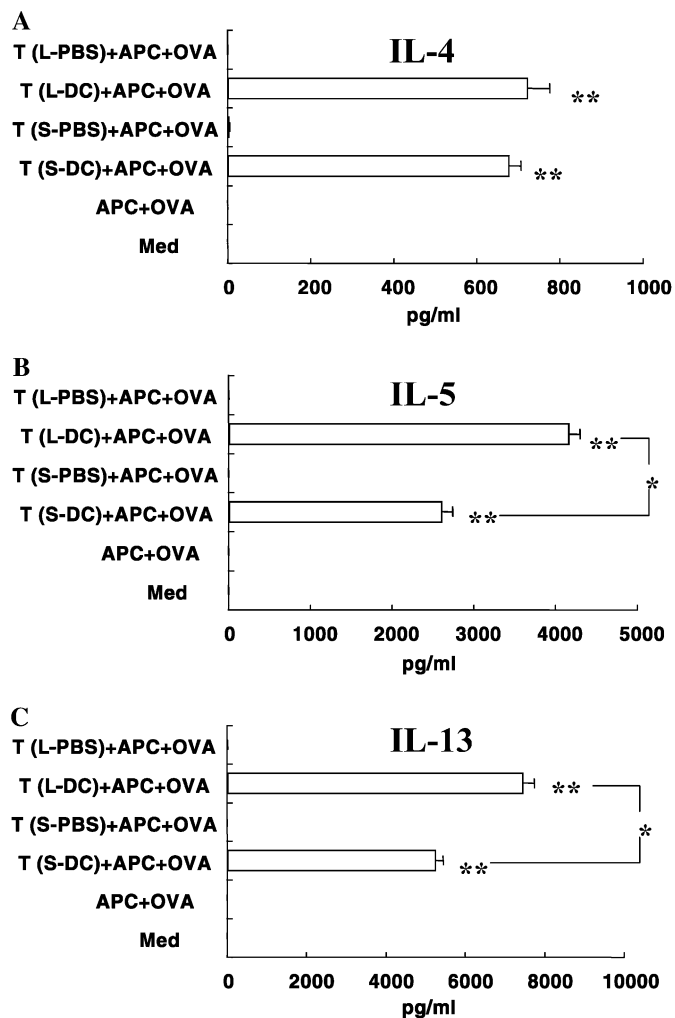
#### The Role of IL-13 in DC Transfer-Induced AHR and Allergic Airway Inflammation

To determine the role of IL-13 in the development of lung allergic responses after DC transfer, IL-13-deficient or WT recipients were studied after transfer of WT DCs. After intratracheal administration of OVA-pulsed DCs and allergen challenge, IL-13-deficient mice failed to develop AHR or airway eosinophilia under both the short and long protocols (Figures 4A and 4B). Levels of IL-4, IL-5, and IL-13 in BAL fluid in the IL-13-deficient mice that received WT DCs were significantly lower compared with WT recipient mice (Figure

4C), whereas the levels of IFN- $\gamma$  were not significantly different (data not shown). IL-13-deficient mice also failed to develop goblet cell metaplasia under both protocols (Figure 4D).

#### The Role of CD4<sup>+</sup> and CD8<sup>+</sup> T Cells

To determine the role of CD4<sup>+</sup> or CD8<sup>+</sup> T cells in the development of lung allergic responses after DC transfer and allergen challenge, CD4-deficient and CD8-deficient recipient mice were compared with WT recipients. Intratracheal administration of OVA-pulsed DCs into CD4-deficient mice induced little AHR or airway eosinophilia in both protocols (Figures 5A and 5B). Transfer of DCs to CD8-deficient mice did lead to some AHR, comparable to that in WT recipients, under the short protocol. However, under the long protocol, CD8-deficient mice showed significantly lower AHR and airway eosinophilia compared with WT mice (Figures 5A and 5B). Levels of IL-4, IL-5, and IL-13 in BAL fluid from CD4- or CD8-deficient mice receiving OVA-pulsed WT DCs were also significantly lower



**Figure 2.** *In vitro* cytokine levels. Cytokine levels were determined in supernates from co-cultures of lung T cells together with irradiated spleen MNCs and OVA. Lung T cells from mice that received DCs were isolated as described in MATERIALS AND METHODS. The lung T cells ( $2 \times 10^5$ /well) were cultured with irradiated spleen MNCs ( $2 \times 10^5$ /well) and OVA (10  $\mu$ g/ml) in 96-well culture plates for 72 hours. T (L-PBS): Lung T cells from mice that received PBS after the long protocol. T (L-DC): Lung T cells from mice that received OVA-pulsed DC after the long protocol. T (S-PBS): Lung T cells from mice that received PBS after the short protocol. T (S-DC): Lung T cells from mice that received OVA-pulsed DC transfer after the short protocol. Data represent means  $\pm$  SEM from three independent experiments performed in triplicate. \* $P < 0.05$  or \*\* $P < 0.01$  comparing PBS groups to DC groups, or between the groups indicated.

compared with WT recipients under the long protocol (Figure 5C), whereas the levels of IFN- $\gamma$  were not significantly different (data not shown). Similarly, goblet cell metaplasia in CD4- or CD8-deficient mice was also significantly lower than in WT mice recipients (long protocol), while CD8-deficient mice but not CD4-deficient mice did develop an increase in goblet cell numbers in the short protocol, similar to WT recipients (Figure 5D).

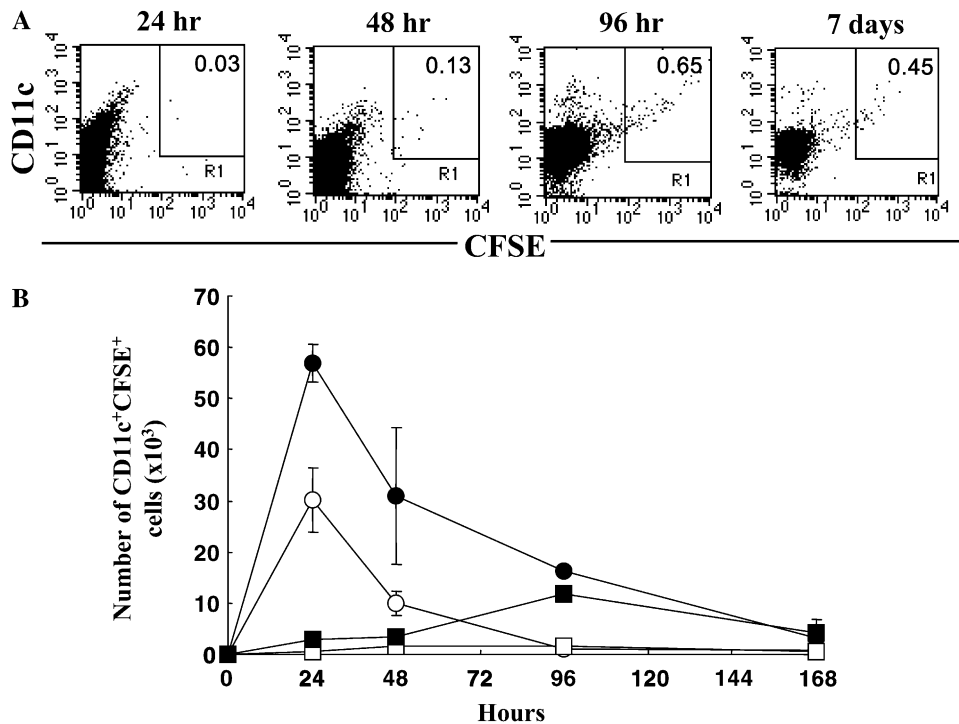
## DISCUSSION

DCs play a critical role in the innate immune response as well as in the initiation of adaptive immune responses through the

priming of naive T cells. To function efficiently as APCs, DCs need to have matured after receiving a signal from a pathogen, often referred as a danger signal. BMDCs are differentiated, mature myeloid type DCs (22). In contrast, resident lung DCs are a mixed population of myeloid, plasmacytoid, and a recently identified CD103<sup>+</sup> subset of DCs (23). Interestingly, among these DCs, only BMDCs have been shown to be potent inducers of AHR and allergic airway inflammation. This may explain some of the controversy of the failure of resident lung DCs to induce airway allergic inflammation in some reports (11, 24). Stampfli and coworkers showed that the overexpression of GM-CSF in bronchial mucosa induced the maturation of lung resident DCs and, after allergen challenge, resulted in airway allergic inflammation (25). In addition, diesel exhaust particles were shown to lead to the maturation of lung DCs, which primarily induced Th2 airway inflammation (26). Based on these findings, it appears that the maturation level of airway DCs is a pivotal component in the development of Th2-type airway inflammation in conjunction with AHR.

In the present study, intratracheally administered OVA-pulsed DCs were initially detected in BAL fluid and lung tissue, and later in draining lymph nodes before allergen challenge. After the two protocols, which varied the interval between DC transfer and allergen challenge, these DCs were capable of priming the recipients to allergen, as detected by the development of AHR *in vivo* and cytokine production in response to *in vitro* culture of lung T cells with OVA. However, after allergen challenge at the two different time-points after DC transfer, the responses could be distinguished when airway allergic inflammation, including eosinophilia, levels of Th2-type cytokines in BAL fluid, and goblet cell metaplasia were monitored. The differences observed after the two protocols were likely attributable to differences in the localization of transferred DCs before allergen challenge. In the short protocol, transferred DCs were not detected in the draining lymph nodes before allergen challenge, whereas allergen challenge was only begun after DCs had trafficked to the draining lymph nodes in the long protocol. Previous work has suggested that DCs migrate to draining lymph nodes to prime T cells after uptake of antigen (27). The results from this study indicated that transferred DCs also remained in the lung and were capable of priming lung T cells to allergen.

In the lungs, there are small populations of many other cell types besides  $\alpha\beta$ <sup>+</sup> T cells, which may play a role in the development of AHR. For example,  $\gamma\delta$  T cells in the lung, especially in the peribronchial regions, play an important role in the induction of mucosal immune responses (28, 29). Thus, locally transferred DCs could interact with resident  $\gamma\delta$  T cells, resulting in AHR. However, the present results identify another pathway, since CD4-deficient mice were unable to develop AHR and most of the  $\gamma\delta$  T cells express CD8<sup>+</sup> or are double-negative T cells (30). Based on the report that  $\gamma\delta$  T cells have the potential to present antigen to conventional  $\alpha\beta$  T cells (31), resident  $\gamma\delta$  T cells might enhance the transfer of information from DCs to resident CD4<sup>+</sup> T cells. Further, locally administered DCs may interact with other resident cell types in the airways, such as B cells, mast cells, airway epithelial cells, or smooth muscle cells. A recent study showed that airway epithelial cells are a potent source of cytokines and chemokines, such as GM-CSF, IL-1 $\beta$ , or TNF- $\alpha$  (32), which are all known to activate DCs. Thymic stromal lymphopoietin (TSLP), which was recently demonstrated to induce Th2-type inflammation by modulating the activity of DCs (33), is also produced from epithelial cells and activates mast cells to produce Th2 cytokines (34). Therefore, transferred DCs might interact with airway epithelial cells to produce TSLP, which stimulate mast cells to



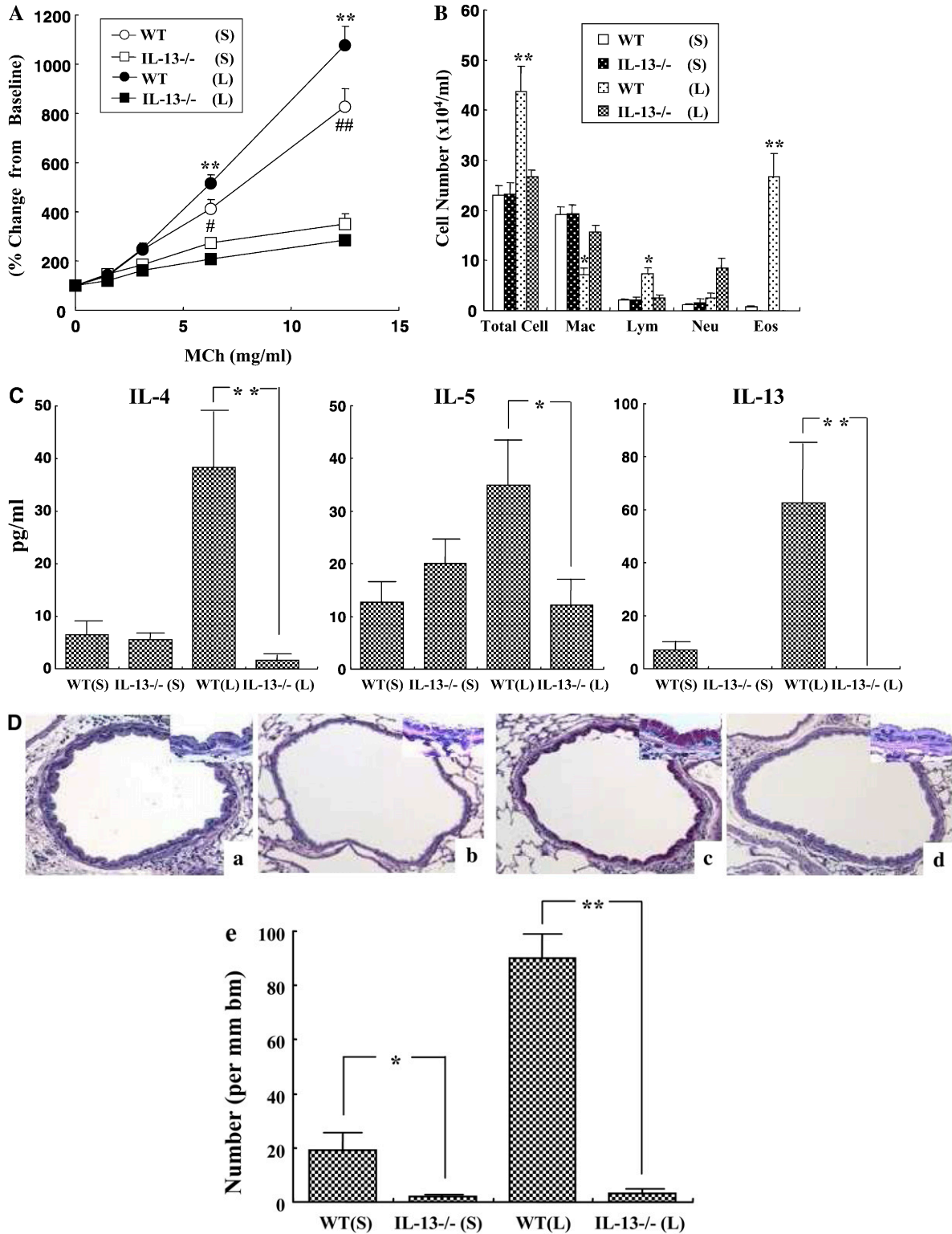
**Figure 3.** Localization of carboxyfluorescein diacetate succinimidylester (CFSE)-labeled DCs after intratracheal administration. CFSE-labeled DCs from B6 mice ( $1 \times 10^6$ ) were transferred via the trachea, then were detected in BAL fluid, lungs, peribronchial lymph nodes, and spleen by flow cytometry. Transferred DCs could be discriminated from endogenous DCs by the green fluorescence of CFSE. (A) Representative scattergram of CFSE-labeled DCs detected in the peribronchial lymph node cells at different time-points. (B) Kinetics of CFSE-labeled DCs in BAL fluid (open circles), lungs (solid circles), peribronchial lymph nodes (solid squares), and spleen (open squares). Each time point represents four mice/group in two independent experiments ( $n = 8$ ).

secrete a variety of chemical mediators or cytokines, such as histamine or IL-13, contributing to the development of AHR. DCs also express receptors for neuropeptides and they are capable of releasing substance P and neurokinin A (NKA), and these neuropeptides modulate smooth muscle cells through neurokinin receptor interactions (35). Potential autocrine or paracrine mechanisms may modulate airway function, but in the absence of inflammatory cell accumulation in the airways, as shown in the short protocol (36). Thus, the local instillation of allergen-pulsed DCs may activate distinct pathways that are associated with the development of AHR in the presence or absence of allergic airway inflammation.

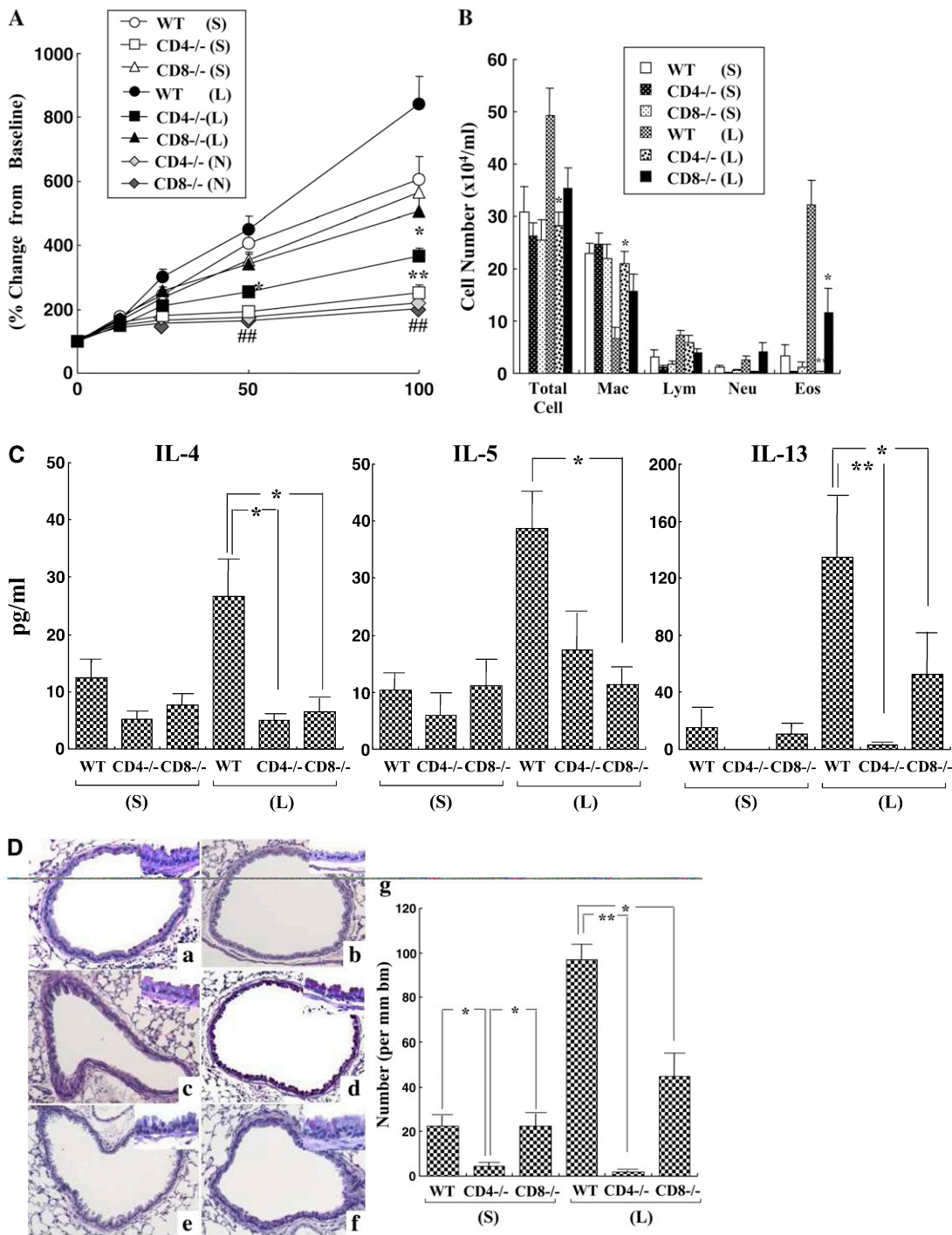
In the present study, IL-13 was shown to play a role in the development of AHR and airway inflammation in both protocols using IL-13-deficient mice. Recent studies also suggest that IL-13 is produced not only by T cells, including Th2-polarized CD4 or CD8 T cells, but also non-T cell populations such as mast cells, basophils, and eosinophils (37, 38). Bellinghausen and colleagues reported that BMDCs can produce IL-13 *in vitro* (39). Nevertheless, IL-13 production from DCs was not likely essential, as there was no significant effect of administration of IL-13-sufficient DCs into IL-13-deficient recipients when AHR, airway inflammation, and induction of Th2-cytokine responses were examined, even in the long protocol. It is possible that IL-13 was released from another cell type. Temann and coworkers reported that IL-9 can induce the production of IL-13 from airway epithelium in the absence of inflammatory cells such as T cells or eosinophils (40). Padilla and colleagues demonstrated that endogenous IL-13 in the sensitization phase induces DC maturation in the lung and elicits strong immune responses to inhaled antigen (41), suggesting that IL-13 could play some role in the initial development of responsiveness in this DC transfer model. However, the failure to restore AHR after adoptive transfer of allergen-pulsed, IL-13-sufficient DCs into IL-13-deficient recipients suggested a critical role for IL-13 in the allergen challenge phase (effector phase) rather than in the sensitization phase (initial phase). Other studies have also

indicated that IL-13 is an essential cytokine in the effector phase response, whereas IL-4 may be more critical for the initial development and expansion of antigen-specific Th2 polarized cells (41–43).

To determine the role of T cells in the response to transfer of allergen-pulsed DCs, CD4- or CD8-deficient mice were investigated. CD4<sup>+</sup> T cells, similar to IL-13, were shown to be essential for the development of AHR and airway inflammation under both protocols (Figure 5). In the long protocol, the deficiency of CD8<sup>+</sup> T cells was seen to result in a marked decrease in AHR and allergic inflammation compared with WT recipients, whereas AHR still developed in these mice under the short protocol. We recently reported that the functional activation of CD8<sup>+</sup> T cells in a similar allergic airway model required CD4<sup>+</sup> T cells from IL-4-sufficient mice in the sensitization phase but not in the challenge phase (44). Therefore, after longer periods as in the long protocol, the functional activation of CD8<sup>+</sup> T cells induced after initial DC-CD4<sup>+</sup> T cell interactions may play a more critical role in the development of AHR and airway inflammation. This is supported by other studies, which showed that the development of memory CD8<sup>+</sup> T cells to bacterial or viral challenges are impaired in CD4<sup>+</sup> T cell-deficient animals (45, 46). APC-CD4<sup>+</sup> T cell interactions are also critical for induction of effective CD8<sup>+</sup> CTL responses (47). Taken together, and as illustrated in Figure 6, transferred DCs may initiate contact with CD4<sup>+</sup> T cells in the local environment and prime and activate them, as observed under the short protocol. With additional time and after DC migration into the draining lymph nodes, in cooperation with primed CD4<sup>+</sup> T cells, priming and activation of CD8<sup>+</sup> T cells occurs as suggested in the long protocol, resulting in the full and enhanced development of altered airway function and airway inflammation. Allergen-specific immunoglobulin, IgE and IgG1, have all been demonstrated to play a role in the development of allergic inflammation under different conditions (48). However, the serum levels of OVA-specific IgE and IgG1 were very low in the DC transfer model, far lower than seen after systemic



**Figure 4.** Comparison of the response of wild-type (WT) and IL-13-deficient mice to DC transfer. (A) Changes in R<sub>L</sub> in WT or IL-13-deficient (IL-13<sup>-/-</sup>) mice that received OVA-pulsed DCs after the short or long protocol. (B) BAL cellular composition. (C) Cytokine levels in BAL fluid. (D) Representative PAS-stained histologic sections of lung tissues obtained 48 hours after the last challenge in (a) WT recipients of OVA-pulsed DCs after the short protocol, (b) IL-13-deficient recipients of OVA-pulsed DCs after the short protocol, (c) WT recipients of OVA-pulsed DCs after the long protocol, (d) IL-13-deficient recipients of OVA-pulsed DCs after the long protocol, (e) Quantitative analysis of PAS<sup>+</sup> cells. WT (S): WT mice following the short protocol. IL-13<sup>-/-</sup> (S): IL-13-deficient mice after the short protocol. WT (L): WT mice in long protocol. IL-13<sup>-/-</sup> (L): IL-13-deficient mice after the long protocol. Data represent means ± SEM from three independent experiments (n = 12). \*P < 0.05 or \*\*P < 0.01 compared with IL-13-deficient recipients of OVA-pulsed DCs after the long protocol. #P < 0.05 or ##P < 0.01 compared with IL-13-deficient recipients of OVA-pulsed DCs in short protocol. Mac: macrophages, Lym: lymphocytes, Neu: neutrophils, Eos: eosinophils, bm: basement membrane.



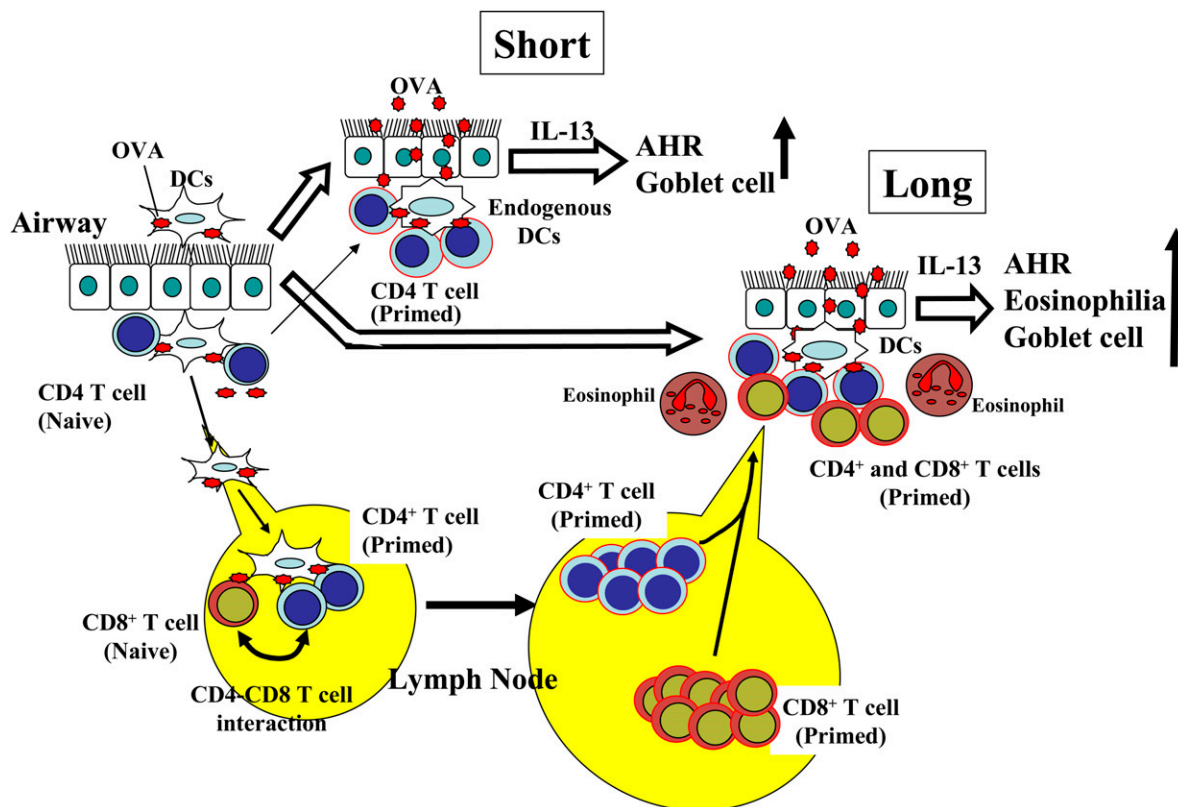
**Figure 5.** Effects of CD4 and CD8 T cell depletion on the response to DC transfer. (A) Changes in RL in WT, CD4-deficient, or CD8-deficient recipients of OVA-pulsed DCs after the short or long protocols. CD4<sup>-/-</sup> (N); CD4-deficient mice in the absence of DC transfer but after OVA challenge. CD8<sup>-/-</sup> (N); CD8-deficient mice in the absence of DC transfer but after OVA challenge. (B) BAL cell composition. (C) Cytokine levels in BAL fluid (left, IL-4; middle, IL-5; right, IL-13). (D) Representative PAS-stained histologic sections of lung tissues obtained 48 hours after the last challenge: (a) WT mice that received OVA-pulsed DCs after the short protocol, (b) CD4-deficient mice that received OVA-pulsed DCs after the short protocol, (c) CD8-deficient mice that received OVA-pulsed DCs after the short protocol, (d) WT mice that received OVA-pulsed DCs after the long protocol, (e) CD4-deficient mice that received OVA-pulsed DCs after the long protocol, (f) CD8-deficient mice that received OVA-pulsed DCs after the long protocol, and (g) quantitative analysis of PAS<sup>+</sup> cells. Data represent means ± SEM from three independent experiments (n = 12). \*P < 0.05 or \*\*P < 0.01 compared with WT mice that received OVA-pulsed DCs in long protocol. ##P < 0.01 compared with WT mice that received OVA-pulsed DCs in short protocol. Mac: macrophages, Lym: lymphocytes, Neu: neutrophils, Eos: eosinophils, bm: basement membrane.

sensitization and challenge (data not shown). Thus, these results are similar to those seen when B cell-deficient mice were sensitized (systemically) and challenged, but nonetheless developed comparable levels of AHR to WT mice (49).

A significant feature of DC transfer under the short protocol was the ability to elicit AHR after allergen challenge in the apparent absence of an accompanying and robust BAL (and tissue) eosinophilia or elevation of Th2 cytokine levels; only a modest increase in goblet cell numbers was detected. This dissociation of AHR and airway eosinophilia has been previously reported under varying experimental conditions (50–53) and was obviously different from the long protocol, in which

AHR was associated with a marked airway eosinophilia, Th2 cytokine elevations, and goblet cell metaplasia. Further, the dissociation of AHR and eosinophilia was recently attributed to strain-specific differences (54), but here, after the short protocol, both BALB/c and C57BL/6 mice developed AHR (albeit to a lower degree in the latter). The development of AHR after the short protocol was dependent on antigen pulsing of DCs before transfer as well as CD4<sup>+</sup> but not CD8<sup>+</sup> T cells. IL-13 was also required as IL-13-deficient mice failed to develop AHR. Whether this induction of AHR represents a different mechanism than that observed in association with airway eosinophilia is unclear at present. At a minimum, the dissociation may only





**Figure 6.** Schematic illustration of the potential mechanisms underlying responses after the short and long DC transfer protocol. Transferred DCs initially make contact and prime CD4<sup>+</sup> T cells in the airways and later migrate to draining lymph nodes, where they further prime naive T cells. Subsequent antigen challenge induces AHR and goblet cell metaplasia mediated by CD4<sup>+</sup> T cells in the short protocol. After the long protocol, there is further interaction, activation, and priming of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the draining lymph nodes, and allergen exposure then induces a marked eosinophilia and Th2 cytokine production as well as AHR and goblet cell metaplasia.

indicate that the pathways activated under both conditions differ quantitatively and not qualitatively, and that the requirements for AHR development are less than required for airway eosinophilia. The requirement for development of increases in goblet cell numbers may lie between, and all three responses are absolutely IL-13 dependent.

In summary, we have investigated the interactions between DCs and CD4<sup>+</sup> and CD8<sup>+</sup> T cells. When allergen-pulsed DCs were given intratracheally followed by allergen challenge after a short interval, in both BALB/c and C57BL/6 mice AHR and a modest but significant goblet metaplasia developed. These findings were not observed in CD4- or IL-13-deficient mice, but were observed in CD8-deficient animals. When the interval between DC transfer and allergen challenge was extended, the mice fully developed AHR and airway allergic inflammation, but these responses were restricted to WT mice and not seen to the same degree in any of the deficient mice; these responses were significantly lower in CD8-deficient mice, and almost completely eliminated in the CD4- or IL-13-deficient animals. These results identify a complex series of interactions between DCs on one hand, and T cell subsets on the other hand, governed to some extent by the location where these interactions take place.

**Conflict of Interest Statement:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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