Dendritic cells and alveolar macrophages mediate IL-13–induced airway inflammation and chemokine production

Margaret Crapster-Pregont, BS, Janice Yeo, BS, Raquel L. Sanchez, BS, and Douglas A. Kuperman, PhD *Chicago, Ill*

Background: IL-13 in the airway induces pathologies that are highly characteristic of asthma, including mucus metaplasia, airway hyperreactivity (AHR), and airway inflammation. As such, it is important to identify the IL-13–responding cell types that mediate each of the above pathologies. For example, IL-13's effects on epithelium contribute to mucus metaplasia and AHR. IL-13's effects on smooth muscle also contribute to AHR. However, it has been difficult to identify the cell types that mediate IL-13–induced airway inflammation.
Objective: We sought to determine which cell types mediate IL-13–induced airway inflammation.
Methods: We treated the airways of mice with IL-13 alone or in combination with IFN-γ. We associated the inhibitory effect of IFN-γ on IL-13–induced airway inflammation and chemokine production with cell types in the lung that coexpress IL-13 and IFN-γ receptors. We then evaluated IL-13–induced responses in CD11c promoter–directed diphtheria toxin receptor–expressing mice that were depleted of both dendritic cells and alveolar macrophages and in CD11b promoter–directed diphtheria toxin receptor–expressing mice that were depleted of dendritic cells.
Results: Dendritic cell and alveolar macrophage depletion protected mice from IL-13–induced airway inflammation and CCL11, CCL24, CCL22, and CCL17 chemokine production. Preferential depletion of dendritic cells protected mice from IL-13–induced airway inflammation and CCL22 and CCL17 chemokine production but not from IL-13–induced CCL11 and CCL24 chemokine production. In either case mice were not protected from IL-13–induced AHR and mucus metaplasia.
Conclusions: Pulmonary dendritic cells and alveolar macrophages mediate IL-13–induced airway inflammation and chemokine production. (J Allergy Clin Immunol 2012;129:1621-7.)

Key words: *Asthma, airway, macrophage, chemokines, inflammation, IL-13, IFN-γ, CD11b, dendritic cell, macrophage-derived chemokine, thymus and activation-regulated chemokine*

Abbreviations used
AHR: Airway hyperreactivity
APC: Allophycocyanin
BAL: Bronchoalveolar lavage
DC-SIGN: Dendritic cell–specific intercellular adhesion molecule–grabbing nonintegrin
DT: Diphtheria toxin
DTR: Diphtheria toxin receptor
IFN-γR: IFN-γ receptor
IL-4R: IL-4 receptor
IL-13R: IL-13 receptor
PE: Phycoerythrin

T_H2 cells contribute to the pathogenesis of asthma.[1] The T_H2 cytokine IL-13 is thought to be particularly important.[2] In asthmatic patients inhalation of an antagonist of IL-4 receptor (IL-4R) α, a shared receptor for IL-4 and IL-13, resulted in significant prevention of allergen-induced responses,[3] and anti–IL-13 treatment significantly improved pulmonary function scores, particularly in a "high-T_H2" subset of asthmatic patients.[4] In mice blockade of IL-13 in the airway prevented allergen-induced experimental asthma, including the development of airway hyperreactivity (AHR), mucus metaplasia, and airway inflammation.[5] Overexpression of IL-13 in the airway was sufficient to cause AHR and mucus metaplasia, as well as chemokine production and airway inflammation.[6-8] However, different cell types mediate different subphenotypes of IL-13–induced experimental asthma. In previous studies direct effects of IL-13 on airway epithelium were sufficient for the development of mucus metaplasia and AHR, but they were not sufficient for the development of airway inflammation.[6] Recently, it was observed that IL-13's effects exclusively on smooth muscle were sufficient for AHR, but they were not sufficient for the development of either mucus metaplasia or airway inflammation.[9] The present study is the first that we know of to identify pulmonary dendritic cells and alveolar macrophages as important mediators of IL-13–induced airway inflammation.

We previously identified a large number of IL-13–induced chemokines associated with IL-13–induced airway inflammation.[7] However, we could not attribute their production to any particular cell type. In this study we associated the known inhibitory effect of IFN-γ on IL-13–induced airway inflammation,[10] with an inhibitory effect of IFN-γ on a restricted subset of IL-13–induced chemokines. This approach helped us to form the hypothesis that the cell types that mediate IL-13–induced airway inflammation coexpress IL-13 and IFN-γ receptors. Specifically, we observed that IFN-γ inhibited IL-13–induced airway inflammation in association with an inhibitory effect of IFN-γ on IL-13–induced CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL22 (macrophage-derived chemokine), and CCL17 (thymus and activation-regulated chemokine). In previous studies alveolar macrophages were identified as major producers of CCL11[11] and CCL24[12] in the allergic lung. In other studies pulmonary dendritic cells were

From the Department of Medicine, Allergy-Immunology Division, Northwestern University Feinberg School of Medicine.
D.A.K. received grant support from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (AI076315 and AI083534).
Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.
Received for publication August 11, 2011; revised January 9, 2012; accepted for publication January 17, 2012.
Available online February 25, 2012.
Corresponding author: Douglas A. Kuperman, PhD, Division of Allergy-Immunology, 240 E Huron, Rm M-317, Chicago, IL. E-mail: d-kuperman@northwestern.edu.
0091-6749/$36.00

doi:10.1016/j.jaci.2012.01.052

identified as major producers of CCL22 and CCL17 in the allergic lung.[13-15] We confirmed that pulmonary dendritic cells and alveolar macrophages coexpressed IL-13 and IFN-γ receptors by means of flow cytometry. Therefore we hypothesized that pulmonary dendritic cells and alveolar macrophages mediate IL-13–induced airway inflammation.

Pulmonary dendritic cells express CD11b at a high level,[16,17] and although macrophages in most organs also express CD11b at a high level,[18] alveolar macrophages are an exception in that they express CD11b at a low level.[16,17] Both alveolar macrophages and pulmonary dendritic cells express CD11c at a high level.[16,17] Consistent with this, a previous study showed that both alveolar macrophages and pulmonary dendritic cells become depleted in the lungs of CD11c–diphtheria toxin receptor (DTR) mice.[19] Furthermore, a previous study showed that dendritic cells, but not alveolar macrophages or granulocytes, become depleted in the lungs of CD11b-DTR mice.[14] We confirmed these observations in both models, and on the basis of the responses of these mice to IL-13 in the airway, we concluded that pulmonary dendritic cells and alveolar macrophages mediate IL-13–induced airway inflammation potentially involving the production of CCL11, CCL24, CCL22, and CCL17.

METHODS

Mice

The experiments were approved by the Northwestern University Animal Care and Use Committee and complied with the "Guide for the care and use of laboratory animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press (revised 1996). BALB/c wild-type and CD11c-DTR mice[19] and FVB wild-type and CD11b-DTR mice[18] were used.

Protocols

Mice received 3 cytokine treatments after achievement of anesthesia (45 mg/kg ketamine and 8 mg/kg xylazine administered intraperitoneally), each occurring 2 days apart with 10 μg of recombinant murine IL-13 or a mixture of 10 μg of recombinant murine IL-13 and 10 μg of recombinant murine IFN-γ (PeproTech, Rocky Hill, NJ) in 50 μL of PBS through the intratracheal route. Samples were harvested 2 days after the third cytokine treatment. CD11b-DTR mice were administered diphtheria toxin (DT) twice (25 ng/g administered intraperitoneally) 1 day apart, ending 1 day before the first cytokine treatment. CD11c-DTR mice were administered DT once (25 ng administered intratracheally) 1 day before the first cytokine treatment.

Samples

Bronchoalveolar lavage (BAL) fluid was collected through a tracheotomy by washing the lumen of the lung with 0.9 mL of PBS. One microliter of acridine orange (100 μg/mL) was added to 19 μL of BAL fluid, and a Cellometer AutoX4 (Nexcelom Bioscience, Lawrence, Mass) automated cell counter was used to count the numbers of nucleated cells. The lavage fluid was centrifuged (1200 rpm × 5 minutes), and the supernatant was removed and stored at −80°C. The cell pellet was resuspended in PBS, and cytospin preparations were stained for the identification of cells by means of light microscopy. In some mice the lung was fixed in 10% formalin, dehydrated in ethanol, embedded in paraffin blocks, cut into 5-μm sections, mounted on slides, and stained with hematoxylin and periodic acid–Schiff. In other mice the lung was homogenized in Trizol (Sigma, St Louis, Mo), and the isolated RNA was converted to cDNA by means of reverse transcription.

Transcripts

The Cyber Green method of real-time PCR was used to detect a preverified panel of chemokines and inflammatory markers (mouse chemokine array; SABiosciences, Frederick, Md). Preverified Taqman assays were used to detect dendritic cell–specific intercellular adhesion molecule–grabbing nonintegrin *(DC-SIGN)*, *Ccl2*, *Ccl8*, *Ccl11*, *Ccl17*, *Ccl22*, and *Ccl24* (Applied Biosystems, Foster City, Calif). Copy numbers were normalized to those of glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)*.

ELISAs

BAL fluid was analyzed by using ELISAs (R&D Systems, Minneapolis, Minn) specific for CCL2, CCL8, CCL11, CCL17, CCL22, and CCL24.

Flow cytometry

Lungs were digested in DNase (0.25 mg/mL) and collagenase (2.5 mg/mL) for 1 hour at 37°C with gentle rotation and passed through a 200-μm filter into Dulbecco modified Eagle medium/10% FBS to identify IL-13 receptor (IL-13R)– and IFN-γ receptor (IFN-γR)–coexpressing cells. The cells were counted and resuspended in 90 μL of MACS running buffer into which 2.4G2 anti-FcεRI/III mAb (BD PharMingen, San Diego, Calif) was added to block nonspecific binding and either no beads (mock separation) or 10 μL of anti–MHC-II MicroBeads (Miltenyi Biotech, Bergisch Gladbach, Germany) per 10^7 cells was added. After 30 minutes at 20°C with gentle rotation, aliquots were incubated with no antibodies, concentration-matched isotype control antibodies, or anti–MHC-II (allophycocyanin [APC]; Miltenyi Biotech), anti–IL-4Rα (phycoerythrin [PE]; BD PharMingen), anti–IFN-γRα (Biotin; BD PharMingen) conjugated to streptavadin–Alexa Fluor 488 (Invitrogen, Carlsbad, Calif), anti-CD11b (APC-Cy7; BD PharMingen), and anti-CD11c (PE-Cy7; BD PharMingen). After 30 minutes at 20°C with gentle rotation, the cells were washed, resuspended in 500 μL of MACS buffer, and passed through a 40-μm filter. The cells were run twice through an autoMACS separator using the possel-s mode, and the flow-through and bound fractions were counted and analyzed by using flow cytometry. For evaluation of the effects of DT treatment, anti-CD11b (APC-Cy7; BD PharMingen) and anti-CD11c (PE-Cy7; BD PharMingen) were used to detect dendritic cells, and anti-F4/80 (PE-Cy7; eBioscience, San Diego, Calif) and anti-CD204 (PE; eBioscience) were used were used to detect macrophages. 4′-6-Diamidino-2-phenylindole dihydrochloride was used to gate on living cells. Fifty thousand events per sample were collected and analyzed with FACSDiva Software (BD Biosciences).

AHR

Airway reactivity to intravenous acetylcholine challenge was measured as previously described.[20] Mice were anesthetized with sodium pentobarbital (90 mg/kg), intubated, and mechanically ventilated. A laparotomy was performed to expose the abdominal vena cava, and decamethonium bromide (25 mg/kg administered intravenously) was administered for paralysis followed by acetylcholine (50 μg/kg administered intravenously) after which dynamic airway pressure was integrated for 5 minutes.

Statistics

ANOVA and the Tukey-Kramer post test were used. Means and SEs are shown. *P* values of .05 or less were considered statistically significant.

RESULTS

The numbers and types of cells in BAL fluid were compared between groups of mice that received PBS, IL-13, or an IL-13/IFN-γ mixture to the airway (Fig 1, *A*). Compared with PBS, there was no effect of IL-13 or IL-13/IFN-γ on the numbers of alveolar macrophages. However, IL-13 induced a significant increase in eosinophil and lymphocyte numbers and caused neutrophil

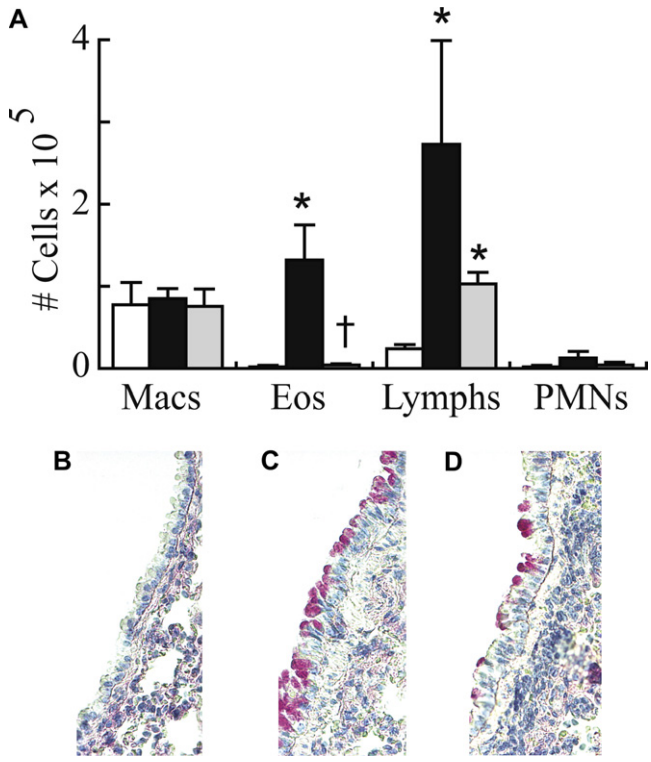

FIG 1. IFN-γ inhibits IL-13–induced airway inflammation and mucus metaplasia. **A,** Cell types and numbers retrieved in BAL fluid from PBS-treated *(open bars)*, IL-13–treated *(black bars)*, and IL-13/IFN-γ–treated mice *(shaded bars)*. *Eos*, Eosinophils; *Lymphs*, lymphocytes; *Macs*, macrophages. **B-D,** Representative images of mucus metaplasia in PBS-treated (Fig 1, *B*), IL-13–treated (Fig 1, *C*), and IL-13/IFN-γ–treated (Fig 1, *D*) wild-type mice (n = 6 mice per group). *$P \leq .05$ versus PBS. †$P \leq .05$ versus IL-13.

numbers to trend higher. Mice treated with IL-13/IFN-γ had completely normalized eosinophil numbers. Both lymphocyte and neutrophil numbers trended lower but were not significantly lower in IL-13/IFN-γ–treated mice. No mucus metaplasia was observed in PBS-treated control animals (Fig 1, *B*). However, IL-13 caused severe mucus metaplasia (Fig 1, *C*), which was diminished in mice treated with IL-13/IFN-γ (Fig 1, *D*). These results confirm an inhibitory effect of IFN-γ on IL-13–induced airway inflammation[10] and extend this observation to IL-13–induced mucus metaplasia.

To identify chemokines associated with the inhibitory effect of IFN-γ on IL-13–induced airway inflammation, we used a chemokine-themed real-time PCR miniarray. Compared with PBS, IL-13 increased the expression of *Ccl8*, *Ccr5*, *Inhba*, *Ccl2*, *FceR2a*, and *Itgb2*, and IFN-γ did not inhibit the expression of these genes. However, IL-13 also increased the expression of *Ccl17* and *Ccl11*, and the IL-13–induced expression of these genes was inhibited by IFN-γ (Table I). We extended this study by using ELISA of chemokines in BAL fluid. Because of its relatedness to CCL11 (eotaxin-1), we included CCL24 (eotaxin-2). As a consequence of detection of CCL17 (thymus and activation-regulated chemokine), we included CCL22 (macrophage-derived chemokine) because they are genetically linked,[21] are both dendritic cell derived,[13-15] and both contribute to allergen-induced airway inflammation through their common receptor, CCR4.[15] In agreement with their gene expression, IL-13 increased the production of CCL11, CCL24, CCL22, and CCL17 in the lung, and the IL-13–induced production of these chemokines was inhibited by IFN-γ (Fig 2, *A-D*). Also in agreement with their gene expression, IL-13 increased the levels of CCL8 and CCL2 in the lung, and the IL-13–induced production of these chemokines was not inhibited by IFN-γ (Fig 2, *E* and *F*). Thus IL-13– and IFN-γ–counterregulated airway inflammation was associated with IL-13 and IFN-γ counterregulated CCL11, CCL24, CCL22, and CCL17 chemokine production.

We hypothesized that cell types that coexpress IL-13 and IFN-γ receptors and that were known to produce CCL11, CCL24, CCL22, and CCL17 might mediate the counterregulatory effects of IL-13 and IFN-γ on airway inflammation. In previously published studies alveolar macrophages were identified as a major source of CCL11[11] and CCL24[12] in the allergic lung. In other studies dendritic cells were identified as a major source of CCL22 and CCL17 in the allergic lung.[13-15] Specifically, for MHC-II$^+$ cells in the murine lung, dendritic cells have been defined as CD11b$^+$/CD11c$^+$ cells, and alveolar macrophages have been defined as CD11b$^-$/CD11c$^+$ cells.[16,17] Therefore to determine whether alveolar macrophages and pulmonary dendritic cells coexpressed IL-13 and IFN-γ receptors, we evaluated the expression of CD11b, CD11c, IL-4Rα and IFN-γRα on MHC-II$^+$ cell–depleted and MHC-II$^+$ cell–enriched lung cells from PBS- and IL-13–treated mice.

We used flow cytometry to determine the percentages of MHC-II$^+$ cells in a single-cell suspension made from whole lungs after a mock and an anti–MHC-II magnetic bead–based separation procedure. After the mock separation procedure, the percentages of MHC-II$^+$ cells in the flow-through and bound fractions were essentially the same, regardless of whether they were from the lungs of PBS- or IL-13–treated mice, and they comprised, on average, 2.9% of the total lung cell population. After MHC-II–targeted separation, the percentage of MHC-II$^+$ cells in the flow-through fraction decreased to an average of 0.1%, and the percentage of MHC-II$^+$ cells in the bound fraction increased to an average of 11.5% (see Table E1 in this article's Online Repository at www.jacionline.org).

We next evaluated the expression of IL-4Rα, IFN-γRα, CD11b, and CD11c on the MHC-II$^+$ cell–depleted flow-through fraction and the MHC-II$^+$ cell–enriched bound fraction of lung cells from PBS- and IL-13–treated mice. There were much greater numbers of IL-4Rα$^+$/IFN-γRα$^+$ cells detected among the MHC-II$^+$ cell–enriched bound fraction compared with the MHC-II$^+$ cell–depleted flow-through fraction (Fig 3, *A*). There were no differences detected between PBS- and IL-13–treated mice. However, we detected 2 major populations of IL-4Rα$^+$/IFN-γRα$^+$ cells in the MHC-II$^+$ cell–enriched bound fraction that were only detected at low levels in the MHC-II$^+$ cell–depleted flow-through fraction. One of these populations was comprised of CD11b$^+$/CD11c$^+$ cells, and the other population was comprised of CD11b$^-$/CD11c$^+$ cells (Fig 3, *B*). These cells fit the definition of dendritic cells and alveolar macrophages, respectively.[16,17] On the basis of their coexpression of IL-13 and IFN-γ receptor subunits, we concluded that pulmonary dendritic cells, alveolar macrophages, or both could be responsible for the counterregulatory effects of IL-13 and IFN-γ on airway inflammation and chemokine production.

In previously published studies pulmonary dendritic cells and alveolar macrophages became depleted in CD11c-DTR mice,[19] and dendritic cells, but not alveolar macrophages, became depleted in the lungs of CD11b-DTR mice.[14] In this study we

TABLE I. Effect of IFN-γ on IL-13–induced chemokine gene expression

Gene	PBS vs IL-13	PBS vs IL-13/IFN-γ	IL-13 vs IL-13/IFN-γ	Fold induction, PBS vs IL-13
P values for genes induced by IL-13 and not inhibited by IFN-γ				
Ccl8	**.003**	**.015**	.209	4.9
Ccr5	**.007**	**.002**	.292	3.2
Inhba	**.015**	**.010**	.532	2.6
Ccl2	**.044**	**.050**	.605	2.5
FceR2a	**.022**	**.028**	.557	2.4
Itgb2	**.002**	**.009**	.263	2.0
P values for genes induced by IL-13 and inhibited by IFN-γ				
Ccl17	**.002**	.117	**.021**	9.6
Ccl11	**.053**	.694	**.025**	1.5

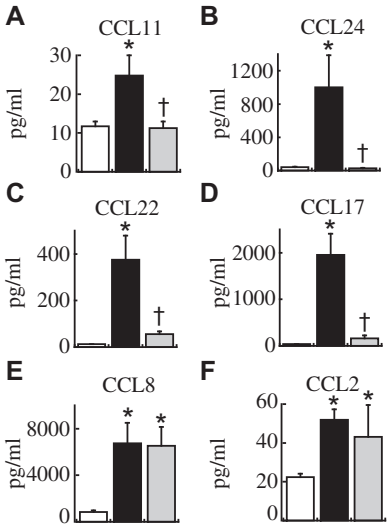

FIG 2. IFN-γ inhibits a subset of IL-13–induced chemokines. Levels of CCL11 **(A)**, CCL24 **(B)**, CCL22 **(C)**, CCL17 **(D)**, CCL8 **(E)**, and CCL2 **(F)** in BAL fluid from PBS-treated *(open bars)*, IL-13–treated *(black bars)*, and IL-13/IFN-γ–treated wild-type mice *(shaded bars)* are shown (n = 6 mice per group). **P* ≤ .05 versus PBS. †*P* ≤ .05 versus IL-13.

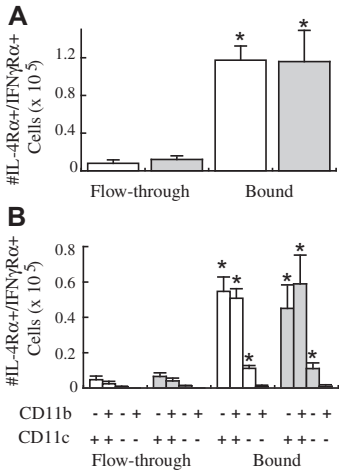

FIG 3. Dendritic cells and alveolar macrophages coexpress IL-13R and IFN-γR subunits. Numbers of IL-4Rα$^+$/IFN-γRα$^+$ cells **(A)** and their proportions that express CD11b and CD11c **(B)** from the lungs of PBS-treated *(open bars)* and IL-13–treated *(shaded bars)* wild-type mice in MHC-II$^+$ cell–depleted flow-through and MHC-II$^+$ cell–enriched bound fractions are shown (n = 6 mice per group). **P* ≤ .05 versus flow-through.

used flow cytometry of whole lung cells to evaluate the percentages of CD11b$^+$/CD11c$^+$ cells as a marker of dendritic cells and the percentages of (F4/80)$^+$/CD204$^+$ cells as a marker of macrophages. The analysis occurred 1 day after the last DT treatment, corresponding to the time point of the first IL-13 treatment in subsequent studies. Although the depletions were not complete, in CD11c-DTR mice a single intratracheal treatment of DT caused an average 3-fold reduction in the percentages of dendritic cells, and an average 3-fold reduction in the percentages of macrophages was detected. In CD11b-DTR mice 2 intraperitoneal treatments of DT caused an average 2-fold reduction in the percentages of dendritic cells, and the percentages of macrophages were unaltered (see Fig E1 in this article's Online Repository at www.jacionline.org).

In CD11c-DTR mice IL-13 increased the gene expression of the dendritic cell–specific marker *DC-SIGN*.[22,23] This result is indicative of IL-13–induced dendritic cell activation. However, DT treatment caused CD11c-DTR mice to have near ablation of baseline and IL-13–induced gene expression of *DC-SIGN* (Fig 4, *A*) and significantly lower numbers of alveolar macrophages in BAL fluid (Fig 4, *B*). These results are consistent with pulmonary dendritic cell and alveolar macrophage depletion in these mice.[19] Furthermore, DT treatment protected these mice from IL-13–induced eosinophilic and lymphocytic airway inflammation but had no effect on neutrophil numbers (Fig 4, *C*). This result indicated that either dendritic cells, alveolar macrophages, or both mediated IL-13–induced eosinophilic and lymphocytic airway inflammation.

To confirm the observed protective effect of dendritic cell and alveolar macrophage depletion on IL-13–induced airway inflammation, we measured the levels of chemokines in BAL fluid. In CD11c-DTR mice IL-13 increased the production of CCL11, CCL24, CCL22, and CCL17 chemokines. DT treatment protected these mice from the IL-13–induced chemokine response (Fig 4, *D*). We extended this study to the effects of IL-13 on mucus metaplasia and AHR. In previous studies these pathologies were attributed to IL-13's effects on airway epithelial and smooth muscle cells.[6,9] As expected, we observed that the development of IL-13–induced AHR and mucus metaplasia was not prevented by DT treatment of CD11c-DTR mice (see Fig E2 in this article's Online Repository at www.jacionline.org).

In CD11b-DTR mice IL-13 increased the gene expression of *DC-SIGN*, and DT treatment almost abolished the baseline and IL-13–induced gene expression of *DC-SIGN* in the lung (Fig 5,

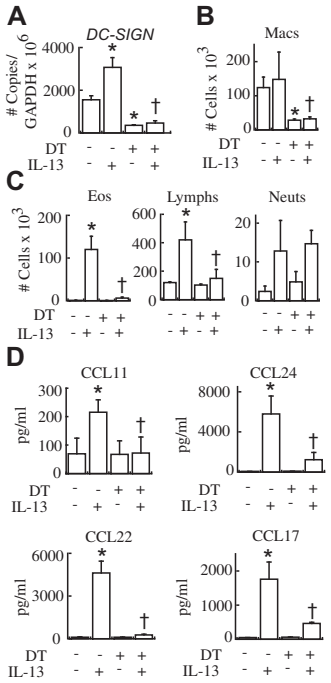

FIG 4. Dendritic cell and alveolar macrophage targeting protects mice from IL-13-induced airway inflammation and chemokine production. **A,** Gene expression levels of *DC-SIGN* in whole lung. *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase. **B-D,** Numbers of alveolar macrophages (*Macs*; Fig 4, *B*); numbers of eosinophils *(Eos)*, lymphocytes *(Lymphs)*, and neutrophils (*Neuts*; Fig 4, *C*); and levels of chemokines (Fig 4, *D*) in BAL fluid from CD11c-DTR mice (n = 6 mice per group). *$P \leq .05$ versus PBS. †$P \leq .05$ versus IL-13.

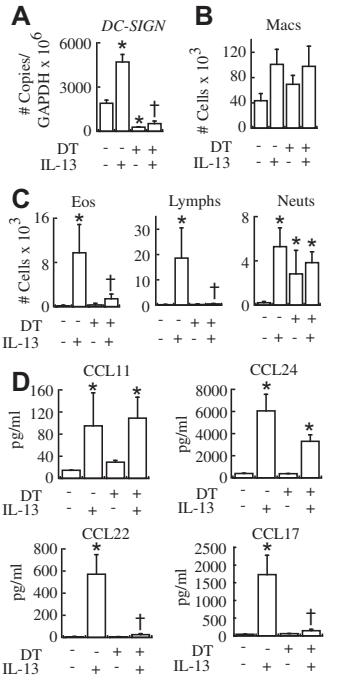

FIG 5. Dendritic cell targeting protects mice from IL-13–induced airway inflammation and a subset of IL-13–induced chemokines. **A,** Gene expression levels of *DC-SIGN* in whole lung. *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase. **B-D,** Numbers of alveolar macrophages (*Macs*; Fig 5, *B*); numbers of eosinophils *(Eos)*, lymphocytes *(Lymphs)*, and neutrophils (*Neuts*; Fig 5, *C*); and levels of chemokines (Fig 5, *D*) in BAL fluid from CD11b-DTR mice (n = 6 mice per group). *$P \leq .05$ versus PBS/PBS. †$P \leq .05$ versus PBS/IL-13.

A). However, the numbers of alveolar macrophages in BAL fluid were unchanged (Fig 5, *B*). In combination, these results are consistent with dendritic cell but not alveolar macrophage depletion in CD11b-DTR mice.[14] IL-13 induced significantly increased numbers of eosinophils, lymphocytes, and neutrophils. DT treatment protected CD11b-DTR mice from the development of eosinophilic and lymphocytic airway inflammation but had no effect on neutrophil numbers (Fig 5, *C*).

To confirm the protective effect of dendritic cell depletion on IL-13–induced airway inflammation, we evaluated the levels of IL-13–induced chemokines, including CCL22, CCL17, CCL11, and CCL24, in BAL fluid. IL-13 increased the levels of all of the chemokines assayed. DT treatment protected CD11b-DTR mice from IL-13–induced CCL22 and CCL17 but not from IL-13–induced CCL11, and there was a not quite significant inhibition of IL-13–induced CCL24 (Fig 5, *D*), which is potentially indicative of a partial role for dendritic cells in CCL24 production and/or some depletion of alveolar macrophages in the CD11b-DTR stain. IL-13–induced AHR and mucus metaplasia were not prevented by DT treatment of CD11b-DTR mice (data not shown). We conclude that pulmonary dendritic cells and alveolar macrophages cooperatively mediate the development of IL-13–induced airway inflammation and chemokine production.

DISCUSSION

In summary, IFN-γ inhibited IL-13–induced airway inflammation in association with an equivalent effect on CCL11, CCL24, CCL22, and CCL17 chemokine production. On the basis of their coexpression of IL-13 and IFN-γ receptors, pulmonary dendritic cells and alveolar macrophages were considered potential mediators. CD11c-DTR mice were depleted of both dendritic cells and alveolar macrophages, and these mice were protected from IL-13–induced airway inflammation in association with protection from CCL11, CCL24, CCL22, and CCL17 production. In comparison, CD11b-DTR mice were depleted of pulmonary dendritic cells, and these mice were also protected from IL-13–induced airway inflammation. In the case of CD11b-DTR mice, the protection from IL-13–induced airway inflammation occurred in association with protection from IL-13–induced CCL22 and CCL17 chemokine production, but these mice were not protected from IL-13–induced CCL11 production, and the majority of IL-13–induced CCL24 chemokine production was preserved. These results are consistent with previous observations that pulmonary dendritic cells are a major source of allergen-induced CCL22 and CCL17[13-15] and that alveolar macrophages are a major source of allergen-induced CCL11 and CCL24.[11,12]

On the basis of the experiments reported here, it is not possible to determine whether alveolar macrophages also mediate IL-13–induced airway inflammation through CCL11, CCL24, or both because no protocol was available for selectively depleting alveolar macrophages. However, compared with CD11c-DTR mice, we observed that in CD11b-DTR mice CCL11 and CCL24 production remained increased in the context of protection from IL-13–induced airway inflammation. This could indicate that CCL11 and CCL24 chemokine production by alveolar macrophages is not sufficient for the development of IL-13–induced airway inflammation. However, this conclusion should be made

with caution because the baseline levels of dendritic cells and alveolar macrophages and the IL-13–induced inflammatory responses were quantitatively different in the 2 strains. This might be due to genetic background differences or the effects of genetic engineering. Furthermore, the idea that CCL11 and CCL24 are dispensable for IL-13–induced airway inflammation is in contrast to a previous finding that CCR3, the receptor for CCL11 and CCL24, is necessary for IL-13–induced airway inflammation.[24] Another consideration is that there appears to be a complex mechanistic interaction between CCL11 and CCL24 chemokines and CCL22 and CCL17 chemokines. This is evidenced by a murine study in which CCR3, the receptor for CCL11 and CCL24, was observed to be important for early allergen-induced T_H2 cell recruitment into the airway, and then subsequently CCR4, the receptor for CCL22 and CCL17, became important.[25]

The present study confirmed a previous report that IL-13–induced airway inflammation was inhibited by IFN-γ.[10] IL-13 in the airway is sufficient to induce eosinophilic and lymphocytic airway inflammation[10,26] and to augment allergen-induced airway inflammation.[27] Conversely, IL-13 gene deletion protects mice from allergic airway inflammation.[28] The inhibitory effects of IFN-γ on the IL-13–induced inflammatory responses that we observed are supported by previous findings that IFN-γ in the airway inhibits allergen-induced airway inflammation[10,27,29] and that IFN-γR deletion augments allergic airway inflammation.[30] There are diverse mechanisms by which IL-13 can augment (as previously reviewed by Kuperman and Schleimer[2]), and IFN-γ can suppress allergic airway inflammation (as reviewed by Teixeira et al[31]). However, the present study, which identified pulmonary dendritic cells and alveolar macrophages as IL-13R– and IFN-γR–coexpressing cells, highlights a novel possibility that the local balance of IL-13 and IFN-γ in the airway counterregulates allergic airway inflammation by counterregulating the levels of chemokines produced by pulmonary dendritic cells and alveolar macrophages.

Previous studies using CD11b-DTR and CD11c-DTR mice with adoptive transfer of *in vitro* polarized T_H2 cells supported the conclusion that antigen presentation by pulmonary dendritic cells to effector/memory T_H2 cells led to production of T_H2 cytokines and the development of allergic airway inflammation.[14,19] Furthermore, it was observed that dendritic cell–derived CCL17 and CCL22 was critical for allergen-induced airway inflammation.[15] However, in allergen-driven models of airway inflammation such as these, it is difficult to dissect the contribution by dendritic cells that occurs as a consequence of their ability to present antigen and thus trigger cytokine production from the responsiveness of dendritic cells to the cytokines that are consequently produced. In this study allergen was not used to drive the inflammatory response, making the data easier to interpret. In combination with the above studies, our findings support a model in which dendritic cell–mediated antigen presentation to memory/effector T_H2 cells induces their production of IL-13 and then the IL-13 helps to establish allergic inflammation by causing pulmonary dendritic cells and alveolar macrophages to produce chemokines.

We thank the staff of the Northwestern University Allergy-Immunology Division for their support.

Clinical implications: This study might lead to the development of novel cell-targeted asthma therapies.

REFERENCES

1. Kay AB. Allergy and allergic diseases. First of two parts. N Engl J Med 2001;344:30-7.
2. Kuperman DA, Schleimer RP. Interleukin-4, interleukin-13, signal transducer and activator of transcription factor 6, and allergic asthma. Curr Mol Med 2008;8:384-92.
3. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. Lancet 2007;370:1422-31.
4. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365:1088-98.
5. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258-61.
6. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, et al. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nat Med 2002;8:885-9.
7. Kuperman DA, Lewis CC, Woodruff PG, Rodriguez MW, Yang YH, Dolganov GM, et al. Dissecting asthma using focused transgenic modeling and functional genomics. J Allergy Clin Immunol 2005;116:305-11.
8. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999;103:779-88.
9. Perkins C, Yanase N, Smulian G, Gildea L, Orekov T, Potter C, et al. Selective stimulation of IL-4 receptor on smooth muscle induces airway hyperresponsiveness in mice. J Exp Med 2011;208:853-67.
10. Ford JG, Rennick D, Donaldson DD, Venkayya R, McArthur C, Hansell E, et al. Il-13 and IFN-gamma: interactions in lung inflammation. J Immunol 2001;167:1769-77.
11. Ge XN, Bahaie NS, Kang BN, Hosseinkhani MR, Ha SG, Frenzel EM, et al. Allergen-induced airway remodeling is impaired in galectin-3-deficient mice. J Immunol 2010;185:1205-14.
12. Pope SM, Zimmermann N, Stringer KF, Karow ML, Rothenberg ME. The eotaxin chemokines and CCR3 are fundamental regulators of allergen-induced pulmonary eosinophilia. J Immunol 2005;175:5341-50.
13. Alferink J, Lieberam I, Reindl W, Behrens A, Weiss S, Huser N, et al. Compartmentalized production of CCL17 in vivo: strong inducibility in peripheral dendritic cells contrasts selective absence from the spleen. J Exp Med 2003;197:585-99.
14. Medoff BD, Seung E, Hong S, Thomas SY, Sandall BP, Duffield JS, et al. CD11b+ myeloid cells are the key mediators of Th2 cell homing into the airway in allergic inflammation. J Immunol 2009;182:623-35.
15. Perros F, Hoogsteden HC, Coyle AJ, Lambrecht BN, Hammad H. Blockade of CCR4 in a humanized model of asthma reveals a critical role for DC-derived CCL17 and CCL22 in attracting Th2 cells and inducing airway inflammation. Allergy 2009;64:995-1002.
16. Jakubzick C, Tacke F, Llodra J, van Rooijen N, Randolph GJ. Modulation of dendritic cell trafficking to and from the airways. J Immunol 2006;176:3578-84.
17. Vermaelen K, Pauwels R. Accurate and simple discrimination of mouse pulmonary dendritic cell and macrophage populations by flow cytometry: methodology and new insights. Cytometry A 2004;61:170-7.
18. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56-65.
19. van Rijt LS, Jung S, Kleinjan A, Vos N, Willart M, Duez C, et al. In vivo depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. J Exp Med 2005;201:981-91.
20. Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. J Exp Med 1998;187:939-48.
21. Nomiyama H, Imai T, Kusuda J, Miura R, Callen DF, Yoshie O. Human chemokines fractalkine (SCYD1), MDC (SCYA22) and TARC (SCYA17) are clustered on chromosome 16q13. Cytogenet Cell Genet 1998;81:10-1.
22. Geijtenbeek TB, Kwon DS, Torensma R, van Vliet SJ, van Duijnhoven GC, Middel J, et al. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 2000;100:587-97.
23. Geijtenbeek TB, Torensma R, van Vliet SJ, van Duijnhoven GC, Adema GJ, van Kooyk Y, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 2000;100:575-85.
24. Fulkerson PC, Fischetti CA, Rothenberg ME. Eosinophils and CCR3 regulate interleukin-13 transgene-induced pulmonary remodeling. Am J Pathol 2006;169:2117-26.
25. Lloyd CM, Delaney T, Nguyen T, Tian J, Martinez AC, Coyle AJ, et al. CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo. J Exp Med 2000;191:265-74.

26. Chen W, Ericksen MB, Levin LS, Khurana Hershey GK. Functional effect of the R110Q IL13 genetic variant alone and in combination with IL4RA genetic variants. J Allergy Clin Immunol 2004;114:553-60.
27. Lindley AR, Crapster-Pregont M, Liu Y, Kuperman DA. 12/15-lipoxygenase is an interleukin-13 and interferon-gamma counterregulated-mediator of allergic airway inflammation. Mediators Inflamm 2010;2010:pii:727305.
28. Kumar RK, Herbert C, Yang M, Koskinen AM, McKenzie AN, Foster PS. Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma. Clin Exp Allergy 2002;32:1104-11.
29. Lack G, Bradley KL, Hamelmann E, Renz H, Loader J, Leung DY, et al. Nebulized IFN-gamma inhibits the development of secondary allergic responses in mice. J Immunol 1996;157:1432-9.
30. Coyle AJ, Tsuyuki S, Bertrand C, Huang S, Aguet M, Alkan SS, et al. Mice lacking the IFN-gamma receptor have impaired ability to resolve a lung eosinophilic inflammatory response associated with a prolonged capacity of T cells to exhibit a Th2 cytokine profile. J Immunol 1996;156:2680-5.
31. Teixeira LK, Fonseca BP, Barboza BA, Viola JP. The role of interferon-gamma on immune and allergic responses. Mem Inst Oswaldo Cruz 2005;100(suppl 1):137-44.

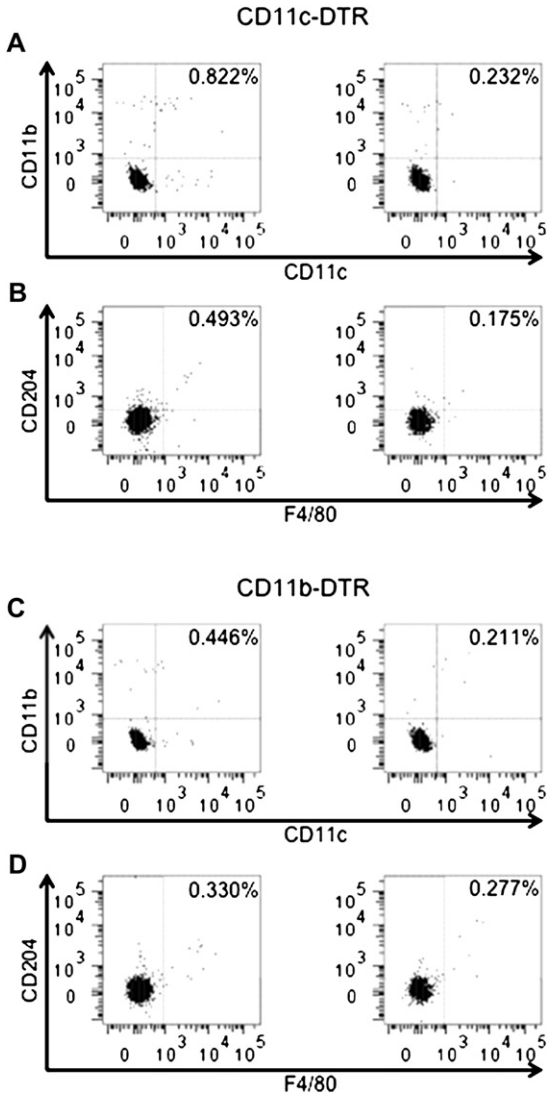

FIG E1. Effects of DT treatment on baseline levels of dendritic cells and macrophages in whole lung. **A** and **B,** Percentages of $CD11b^+/CD11c^+$ cells (Fig E1, *A*) and $CD204^+/(F4/80)^+$ cells (Fig E1, *B*) detected in whole lungs of CD11c-DTR mice. **C** and **D,** Percentages of $CD11b^+/CD11c^+$ cells (Fig E1, *C*) and $CD204^+/(F4/80)^+$ cells (Fig E1, *D*) detected in whole lungs of CD11b-DTR mice. Representative plots are shown from 4 mice per group.

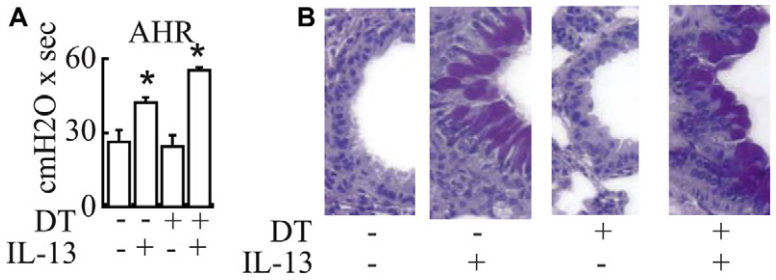

FIG E2. Dendritic cell and alveolar macrophage targeting does not protect mice from IL-13–induced AHR and mucus metaplasia. AHR to acetylcholine injection **(A)** and representative images showing mucus metaplasia **(B)** in the lungs of CD11c-DTR mice (n = 6 mice per group.) $*P \leq .05$ versus PBS.

TABLE E1. Efficiency of MHC-II$^+$ cell separation

	Mock separated		Separated	
Group	**MHC-II$^+$ cells (%)**	**SEM**	**MHC-II$^+$ cells (%)**	**SEM**
PBS flow-through	1.79	0.39	0.16	0.06
IL-13 flow-through	2.68	0.33	0.10	0.03
PBS bound	3.10	0.51	11.31	2.14
IL-13 bound	3.91	0.48	11.74	2.24