

Allergen-induced Changes in Bone Marrow and Airway Dendritic Cells in Subjects with Asthma

Amani El-Gammal^{1,2}, John-Paul Oliveria², Karen Howie², Richard Watson², Patrick Mitchell², Ruchong Chen^{1,3}, Adrian Baatjes², Steven Smith², Dhuha Al-Sajee⁴, Thomas J. Hawke⁴, Kieran J. Killian², Gail M. Gauvreau², and Paul M. O'Byrne^{1,2}

¹Firestone Institute of Respiratory Health, ²Department of Medicine, and ⁴Department of Pathology and Molecular Medicine, Michael G. DeGroote School of Medicine, McMaster University, Hamilton, Ontario, Canada; and ³Guangzhou Institute of Respiratory Disease, 1st Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Abstract

Rationale: Dendritic cells (DCs) are antigen-presenting cells essential for the initiation of T-cell responses. Allergen inhalation increases the number of airway DCs and the release of epithelial-derived cytokines, such as IL-33 and thymic stromal lymphopoietin (TSLP), that activate DCs.

Objectives: To examine the effects of inhaled allergen on bone marrow production of DCs and their trafficking into the airways in subjects with allergic asthma, and to examine IL-33 and TSLP receptor expression on DCs.

Methods: Bone marrow, peripheral blood, bronchoalveolar lavage (BAL), and bronchial biopsies were obtained before and after inhalation of diluent and allergen from subjects with asthma that develop allergen-induced dual responses. Classical DCs (cDCs) were cultured from bone marrow CD34⁺ cells. cDC1s, cDC2s, and plasmacytoid DCs were measured in bone marrow aspirates, peripheral blood, and BAL by flow cytometry, and cDCs were quantified in bronchial biopsies by immunofluorescence staining.

Measurements and Main Results: Inhaled allergen increased the number of cDCs grown from bone marrow progenitors, and cDCs and plasmacytoid DCs in bone marrow aspirates 24 hours after allergen. Allergen also increased the expression of the TSLP receptor,

but not the IL-33 receptor, on bone marrow DCs. Finally, inhaled allergen increased the percentage of cDC1s and cDC2s in BAL but only cDC2s in bronchial tissues.

Conclusions: Inhaled allergen increases DCs in bone marrow and trafficking of DCs into the airway, which is associated with the development airway inflammation in subjects with allergic asthma. Inhaled allergen challenge also increases expression of TSLP, but not IL-33, receptors on bone marrow DCs.

Keywords: asthma; inhaled allergens; myeloid dendritic cells; plasmacytoid dendritic cells

At a Glance Summary

Scientific Knowledge on the Subject: Inhaled allergen increases the bone marrow's production of dendritic cells in subjects with allergic asthma, which are available for trafficking into the airways to enhance allergen-induced airway responses.

What This Study Adds to the Field: The enhanced bone marrow production and trafficking may require up-regulation of the thymic stromal lymphopoietin, but not the IL-33, receptor on dendritic cells.

(Received in original form August 17, 2015; accepted in final form January 26, 2016)

Author Contributions: A.E.-G. designed the study, conducted the experiments, analyzed the data, and wrote the manuscript. J.-P.O., K.H., P.M., R.C., A.B., and S.S. conducted experiments and contributed to the development of the manuscript. R.W. recruited subjects and conducted the inhaled allergen challenges. D.A.-S. and T.J.H. conducted the immunostaining and quantification of the bronchial biopsies. K.J.K. provided statistical advice. G.M.G. and P.M.O'B. designed the study, assisted with data analysis, and wrote the manuscript. All authors approved the manuscript's submission.

Correspondence and requests for reprints should be addressed to Paul M. O'Byrne, M.B., Room 3W10, McMaster University Medical Center, 1280 Main Street West, Hamilton, ON, L8S 4K1 Canada. E-mail: obyrynep@mcmaster.ca

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 194, Iss 2, pp 169–177, Jul 15, 2016

Copyright © 2016 by the American Thoracic Society

Originally Published in Press as DOI: 10.1164/rccm.201508-1623OC on February 4, 2016

Internet address: www.atsjournals.org

Asthma is characterized by variable airflow obstruction, airway inflammation, mucus production, and airway hyperresponsiveness. Inhaled environmental allergens cause all of these features of asthma through the activation of immune cells (1). Dendritic cells (DCs) are the most potent antigen-presenting cells in the airways and are essential for initiating adaptive immune responses (2, 3). Human CD34⁺ bone marrow progenitors cultured in the presence of granulocyte-macrophage colony-stimulating factor develop along a myeloid pathway, and the addition of exogenous tumor necrosis factor leads to the differentiation of DCs among the myeloid progeny (4, 5). DCs traffic through the bloodstream to lymphoid tissues and the epithelia of body surfaces, including the airways. In the airways, DCs undergo maturation and acquire the ability to take up antigens. After antigen uptake, DCs migrate to draining lymph nodes, where they present airway-derived antigens to naive T cells.

There are two major human DC subsets: classical DCs (cDCs) (6), generally considered immunogenic (7), and plasmacytoid DCs (pDCs), which exhibit suppressive functions on allergen-driven cell-mediated responses (8). The number of airway DCs is increased in subjects with asthma compared with normal control subjects in bronchial biopsies, and is lower in patients treated with inhaled corticosteroids (9). Several human studies have addressed DC kinetics associated with allergen-

induced responses. Circulating cDCs are reduced within 3 hours after allergen inhalation (10, 11), suggesting their recruitment into the airway mucosa. Also, the number of sputum cDCs and pDCs increase significantly 24 hours after allergen inhalation (12), as do the number of what were previously called myeloid DCs type 2 in sputum (13), but which are now classified as cDC1s (6). Also, the number of both cDCs and pDCs increases in bronchoalveolar lavage (BAL) after segmental allergen challenge (14).

The mechanism of trafficking of DCs into the airways is not fully understood. Studies in an animal model of asthma demonstrated that accumulation of airway DCs was not caused by reduced migration to the mediastinal lymph nodes. Rather, the increases in airway and lymph node DCs were associated with an almost threefold expansion of myeloid hematopoietic progenitors in the bone marrow (15, 16). This suggests that the bone marrow increases its output of myeloid precursors to meet the enhanced demand for DCs. However, it is not known whether the same mechanism exists in humans.

The airway epithelium plays a central role in the Th2-cell sensitization process, partly because of its ability to recruit DCs (17). On allergen recognition, epithelial cells release cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), which activate the DC network and other innate immune cells, such as basophils and type 2 innate lymphoid cells (18).

The purpose of the current study was to test the hypothesis that allergen inhalation causes the expansion of DCs and their precursors in the bone marrow, and to evaluate the trafficking of DCs into the airways after allergen inhalation in subjects with mild, allergic asthma. A secondary objective was to further understand the potential role of the epithelial-derived cytokines, IL-33 and TSLP, in this allergen-induced process, by examining the expression of their receptor subunits ST2R, TSLPR, and IL-7R α on DCs in the bone marrow, peripheral circulation, and the airways.

Methods

Subjects

Ten subjects with mild allergic asthma were enrolled. They were asymptomatic and not on any regular asthma treatment and using short-acting inhaled β_2 -agonists as reliever medication less than daily (Table 1). All subjects were studied when not naturally exposed to a seasonal allergen to which they were sensitized. The details of the subjects' characteristics are contained in the online supplement. Approval was granted from the Hamilton Integrated Research Ethics Board, and all subjects provided signed informed consent.

Study Design

The study was a randomized, diluent-controlled, crossover study. At screening,

Table 1. Subject Characteristics

Subject	Sex (M:F = 7:3)	Age (yr)	FEV ₁ (% Predicted)	PC ₂₀ (Baseline)	Allergen	Fall in FEV ₁ (%)	
						EAR	LAR
1	M	53	97.6	5.43	HDMDP	36.2	19.5
2	M	50	98.4	0.29	Cat	33.8	22.53
3	M	66	95.4	3.37	Cat	48.53	38.8
4	M	27	112.4	9.56	HDMDP	49.4	17.7
5	M	23	88.8	0.32	Cat	22	22.3
6	M	21	94.3	4.7	Tree	35.3	50.71
7	F	21	80.7	1.55	Ragweed	50	31
8	F	23	80.6	3.96	HDMDP	28.7	19.5
9	M	35	85.1	7.73	Grass	27.5	12.6
10	F	21	96.1	5.65	Horse	41.3	26.5
Mean $\pm$ SEM		34 $\pm$ 5.2	92.9 $\pm$ 3.0	4.2 $\pm$ 0.96*		37.3 $\pm$ 3.1	26.1 $\pm$ 3.6

Definition of abbreviations: EAR = early asthmatic responses; HDMDP = house dust mite *Dermatophagoides pteronyssinus*; LAR = late asthmatic responses; PC₂₀ = provocative concentration of methacholine causing a 20% drop in FEV₁.

*Geometric mean.

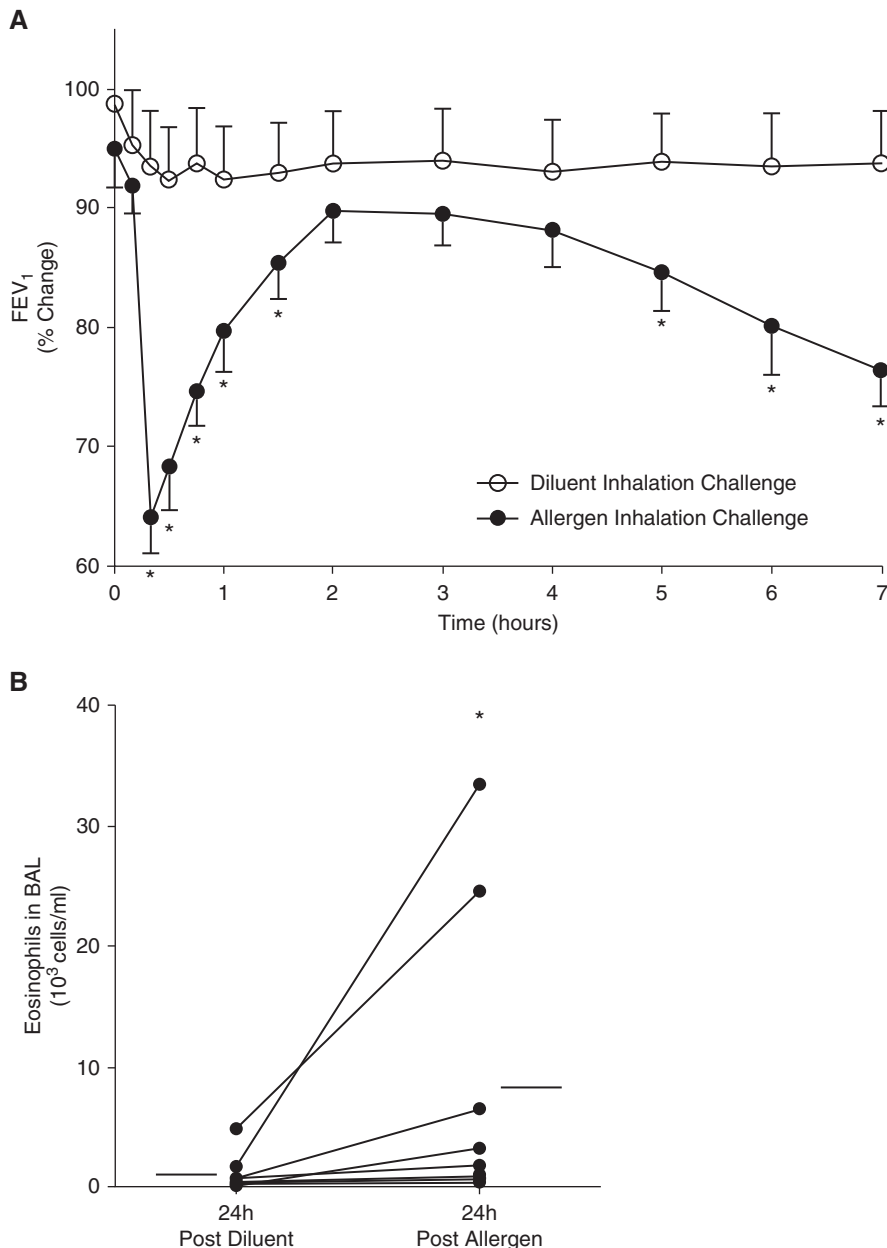


Figure 1. Allergen inhalation induces airway responses in patients with mild allergic asthma. (A) Percentage change in FEV₁ induced by allergen inhalation versus diluent. (B) Bronchoalveolar lavage (BAL) eosinophil count after allergen and diluent. Data are presented as mean $\pm$ SEM. * $P < 0.05$.

subjects provided consent, completed a skin prick test, and a medical history was obtained. On Day 1, baseline measurements of provocative concentration of methacholine (PC₂₀), peripheral blood, and a bone marrow aspirate were conducted. On Day 2, subjects were randomized to inhale diluent or allergen and blood collections

(3 and 6 h after the inhalation challenge) were performed. On Day 3, at 24 hours after the inhalation challenge, spirometry, bone marrow aspiration, blood collection, and bronchoscopy (to obtain BAL fluid and endobronchial biopsies) were performed. This triad was repeated after a 4-week recovery period.

Airway Challenges

Methacholine inhalation challenges were performed using Wright nebulizer and the tidal breathing method, as previously described (19). PC₂₀ was calculated as the provocative concentration of methacholine inducing a 20% decrease in FEV₁. Allergen inhalation challenge was performed as previously described (1).

Bone Marrow Aspirate, CD34⁺ Cell Isolation, and Culture

Bone marrow aspirates were obtained from the posterior iliac crest using a bone marrow aspiration needle. Ten milliliters of bone marrow were aspirated into a syringe containing 1 ml of sterile heparin (1000 U/ml). The details of the cell isolation and culture are provided in the online supplement.

Fiberoptic Bronchoscopy

Fiberoptic bronchoscopy was performed as previously described (20). BAL was collected from the right middle lobe and at least six endobronchial biopsy samples were obtained using a sterile cup and spike forceps from the second generation of the right lower lobe bronchus.

Histology and Tissue Processing

Airway biopsies were fixed in 10% neutral-buffered formalin. After overnight fixation, the blocks were processed and embedded in paraffin. Sections 10- μ m thick from the paraffin blocks were stained with hematoxylin and eosin to assess the quality of the biopsies and the integrity of the epithelium.

BAL and Cell Isolation

The details of the handling of BAL samples are contained in the online supplement.

Immunofluorescence Staining and Flow Cytometric Gating Strategy

Details of immunofluorescence staining and flow gating strategy are contained in the online supplement.

Multicolor Immunofluorescence Staining of Mucosal Biopsies

Formalin-fixed bronchial biopsies were used to determine the density of two subsets of

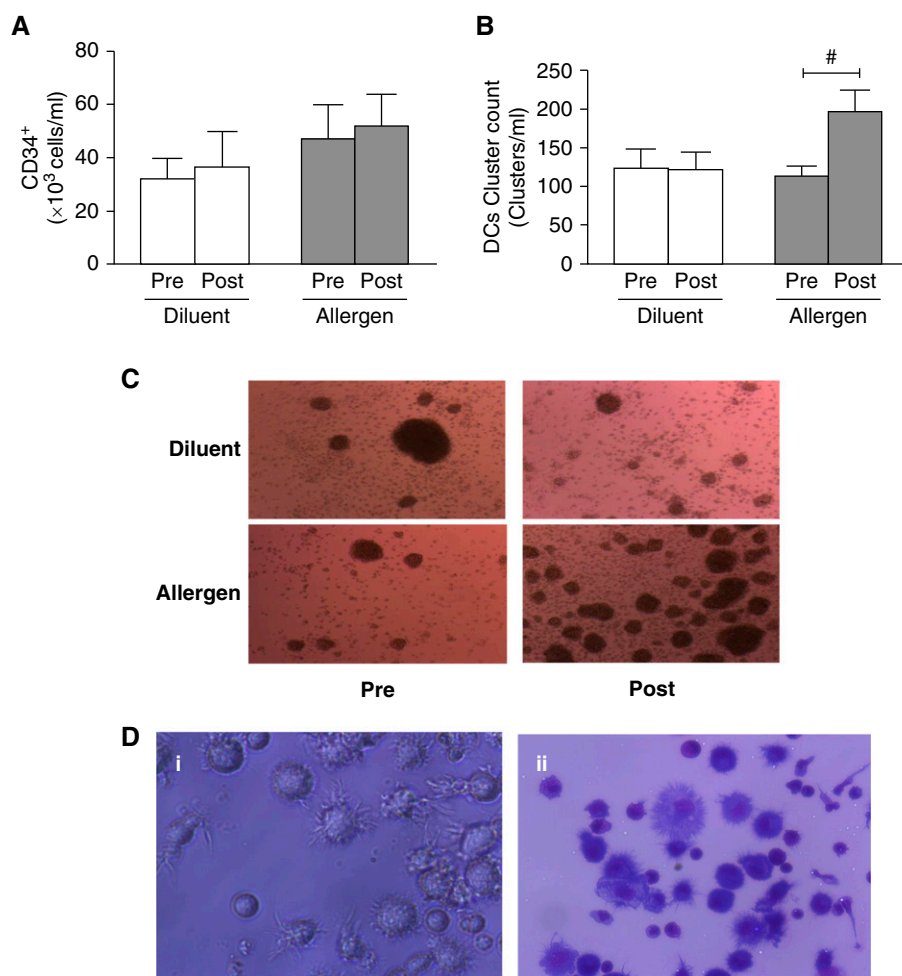


Figure 2. Effect of allergen inhalation on dendritic cell (DC) progenitors (CD34⁺) and clusters. (A) CD34⁺ cells in bone marrow before inhalation and 24 hours after inhalation of allergen or diluent. There was no significant change in CD34⁺ cells after allergen inhalation. (B) DC clusters measured after 14-day culture from bone marrow before inhalation and 24 hours after inhalation of allergen or diluent. Allergen inhalation significantly increased the numbers of clusters. Data are presented as mean $\pm$ SEM. [#] $P < 0.05$. (C) Photomicrographs of bone marrow–derived clusters after 14-day culture from bone marrow samples taken before allergen and after allergen or diluent challenge. Magnification, $\times 4$. (D) Morphology of DCs. (i) Photomicrograph of cells dispersed from bone marrow–derived clusters after 14-day culture. The cells have the morphology of developmentally mature DCs showing the elongated spiny cytoplasmic processes and extending veils. Magnification, $\times 40$. (ii) The cells stained with Diff-Quick stain.

DCs (cDC1 and cDC2) using specific markers for both in bronchial tissue. Details of the staining of mucosal biopsies are contained in the online supplement.

Microscopic Image Analysis

Images were captured using Nikon 90i eclipse inverted microscope and Nikon elements software (Nikon Inc., Mississauga, Canada) was used to analyze images. The details of the quantification of DCs are contained in the online supplement.

Statistical Analysis

All data are presented as mean $\pm$ SEM. The allergen-induced changes from baseline were analyzed by using paired Student's *t* tests with GraphPad Prism 5 software (GraphPad Software, La Jolla, CA). Methacholine PC₂₀ values were log-transformed before analyses, and expressed as the geometric mean and range. Analysis for inhalation challenges, bone marrow, and peripheral blood was performed using a two-way repeated measures analysis of variance and *post*

hoc Bonferroni test. For BAL and bone marrow culture results, where two data points available, paired Student's *t* tests were used. Significance was accepted at *P* less than 0.05. Mean case substitution was used to replace missing data points.

Results

All subjects demonstrated allergen-induced dual responses, with a mean maximum baseline fall in FEV₁ from baseline of $30.9 \pm 18.9\%$ during the early response ($P < 0.05$), and $18.6 \pm 15.2\%$ fall during the late response ($P < 0.05$). No significant change in FEV₁ from baseline occurred during the diluent challenge (Figure 1A). In BAL, there was a significant allergen-induced increase in eosinophil counts 24 hours after allergen compared with 24 hours after diluent (Figure 1B). There were no significant differences in BAL total cell numbers, or neutrophil and macrophage cell counts 24 hours after the challenges.

The number of bone marrow CD34⁺ cells did not significantly increase after either diluent or allergen challenge (Figure 2A). Also, the percentage of CD34⁺ cells expressing IL4R α did not change after diluent or allergen (data not shown). Culture of bone marrow CD34⁺ cells with granulocyte-macrophage colony-stimulating factor, stem cell factor, tumor necrosis factor- α , and IL-4 for 14 days produced large cells with polygonal, fusiform, or dendritic shape (Figure 2D). DCs formed cell clusters of more than 50 cells by Day 7. The number and size of the clusters increased with time. There was no increase in cluster numbers before and after diluent challenge after 14 days, being 123.4 ± 24.8 and 121.2 ± 23 clusters/ml, respectively (Figures 2B and 2C). By contrast, there was a significant increase in cluster numbers after allergen challenge after 14 days, being 113.7 ± 12.8 clusters/ml before challenge and 196.7 ± 28.5 clusters/ml after challenge (Figures 2B and 2C) ($P < 0.05$).

Bone marrow–derived DCs generated from CD34⁺ progenitor cells, after 14 days culture, were also analyzed by flow cytometry. When compared with diluent, the number of CD11c⁺ cells were significantly increased after

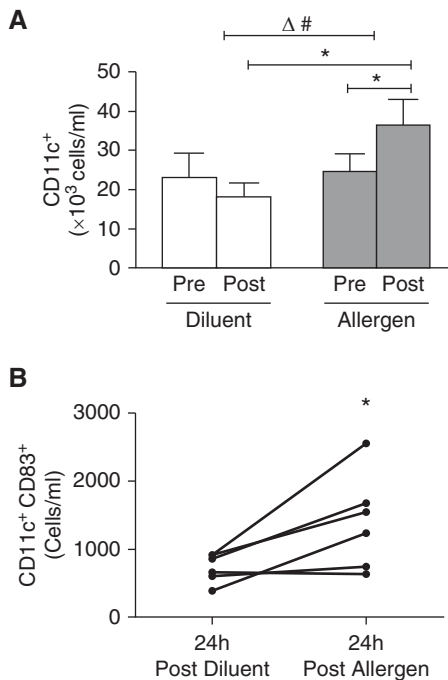


Figure 3. Allergen inhalation induced changes in bone marrow cultures. (A) Bone marrow–derived CD11c⁺ dendritic cells (classical dendritic cells) after 14-day culture analyzed by flow cytometry. The number of cells was significantly increased after allergen when compared with diluent inhalation. (B) Bone marrow–derived CD11c⁺ and CD83⁺ dendritic cells after 14-day culture. Allergen challenge significantly increased the coexpression of CD11c⁺ and CD83⁺ suggesting more developmentally mature classical dendritic cells. Data are presented as mean $\pm$ SEM. * $P < 0.05$ compared with baseline and 24 hours after diluent; $\Delta^{\#}P < 0.05$ compared with diluent challenge.

allergen to $36.4 \pm 6.6 \times 10^3$ cells/ml after allergen compared with $18.0 \pm 3.7 \times 10^3$ cells/ml after diluent ($P < 0.05$) (Figure 3A). Allergen challenge also significantly increased the coexpression of CD11c⁺ and CD83⁺ (Figure 3B) suggesting more developmentally mature cDCs.

DCs were also measured in bone marrow aspirates and peripheral blood before and after diluent and allergen challenge. There was a significant increase in the absolute number of both cDCs and pDCs in bone marrow aspirates 24 hours after allergen compared with both prechallenge and 24-hour postdiluent values ($P < 0.05$) (Figure 4A). In addition, there was a significant increase in the numbers of cDCs expressing CD83 and CCR5 after allergen compared with

preallergen and postdiluent values ($P < 0.05$), whereas there were more pDCs expressing CD83 after allergen, and CCR5 compared with preallergen and postdiluent values ($P < 0.05$) (Figure 4B). Also, there were allergen-induced increases in bone marrow–derived cDCs and pDCs expressing both components of the TSLP receptor (TSLPR and IL7Ra) ($P < 0.05$) (Figure 4C); although this was not significantly different when compared with diluent challenge for CD11c⁺/TSLPR⁺ DCs. There were no significant changes in specific median fluorescence intensity for peripheral blood, bone marrow, and BAL-derived cDCs and pDCs expressing the TSLPR (see Figure E5 in the online supplement). Lastly, there were no significant changes in the expression of IL-33 receptor (CD11c⁺/ST2⁺ or CD123⁺/ST2⁺ cells) after diluent or allergen.

In peripheral blood, in contrast to bone marrow aspirates, allergen challenge did not affect the number of circulating cDCs and pDCs because no differences were observed between allergen and diluent at any time point (see Figure E2). This was also true for the expression of surface markers CCR5, CD83, IL-33R, TSLPR, and IL7Ra on circulating cDCs and pDCs (data not shown).

In BAL, the absolute numbers cDC2s increased from 185.4 ± 37.4 cells/ml after diluent to 743.1 ± 348.8 cell/ml after allergen ($P < 0.05$) (Figure 5A). There were no significant increases in the absolute numbers of cDC1s or pDCs after allergen (Figure 5A); however, allergen inhalation significantly increased the percentage of cDC1s and cDC2s when compared with diluent inhalation. The cDC2s increased from $14.9 \pm 4.2\%$ after diluent to $28.5 \pm 5.7\%$ after allergen ($P < 0.05$), whereas cDC1s increased from $47.9 \pm 6.2\%$ after diluent to $56.9 \pm 4.5\%$ after allergen ($P < 0.05$). The percentage of cDC1s in BAL was significantly higher than cDC2s ($P < 0.05$). In addition, allergen significantly increased both the absolute numbers and percentage of BAL cDCs, but not pDCs expressing the CD83 surface marker (Figure 5B). Finally, the expression of the TSLP receptor subunits was not significantly increased on cDCs, whereas IL7R α expression was increased on pDCs (Figure 5C).

In bronchial biopsies, HLA-DR⁺ cells were found in both the epithelium

and lamina propria in all specimens examined. A proportion of the HLA-DR⁺ cells contained positive with monoclonal antibodies specific for cDC1s and cDC2s (Figures 6A and 6B). Allergen inhalation increased the numbers of cDC2s in both the epithelium and lamina propria, when compared with diluent challenge from 14.0 ± 2.9 to 24.0 ± 3.1 cells/mm² ($P < 0.05$) (Figure 6C). No allergen-induced increase in cDC1s was demonstrated (Figure 6D). The number of cDC2s was significantly higher in the bronchial mucosa after allergen challenge compared with the number of cDC1s ($P < 0.05$).

Discussion

This study has demonstrated that inhaled allergen increased the numbers of DCs cultured from bone marrow progenitors, and developmentally mature and immature cDCs and pDCs in bone marrow aspirates, 24 hours after challenge in patients with mild asthma. Inhaled allergen also increased the expression of the TSLP receptor, but not the IL-33 receptor (ST2), on bone marrow cDCs and pDCs. Finally, inhaled allergen increased the percentage of cDC1s and cDC2s in BAL, but only cDC2s in bronchial tissues.

The allergen-induced increase in DCs cultured from bone-marrow progenitors is, to our knowledge, the first description of an effect of inflammatory stimulus on these progenitors in human subjects. These findings are in agreement with observations previously made in mouse models of allergen responses (15, 16). The mechanisms of this could include an increase in the total number of bone marrow CD34⁺ cells, which was not demonstrated in this study. Another possibility is an increased number of CD34⁺ cells primed for the growth factors needed for dendropoiesis. This was evaluated by examining the effect of allergen challenge on the number of bone marrow CD34⁺ cells expressing IL-4R α , given the importance of IL-4 for the development and survival of DCs in bone marrow culture (21). However, no change in IL-4R α expression on CD34⁺ cells before and after allergen challenge was demonstrated. Another mediator important in the recruitment and

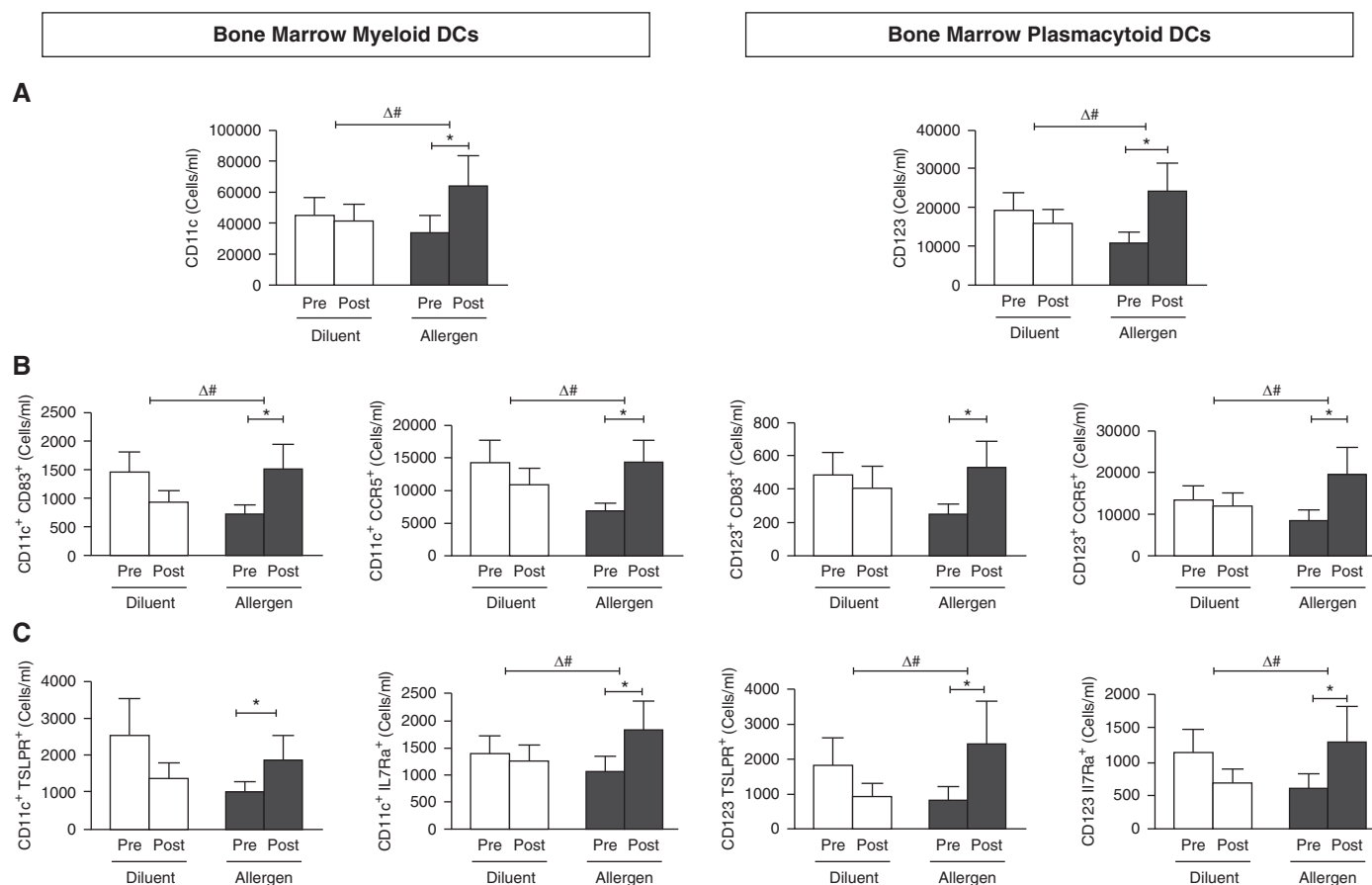


Figure 4. Allergen inhalation induced changes in bone marrow aspirates. Enumeration, phenotypic characterization, and thymic stromal lymphopoietin receptor (TSLPR) expression of CD11c⁺ (classical dendritic cell [cDCs]) and CD123⁺ (plasmacytoid DCs [pDCs]) cells 24 hours after allergen compared with both prechallenge and 24-hour postdiluent values. (A) Allergen inhalation significantly increases in the absolute number of both cDCs and pDCs. (B) CD11c⁺ CD83⁺ and CD11c⁺ CCR5⁺ (cDCs), and CD123⁺ CD83⁺ and CD123⁺ CCR5⁺ (pDCs) cells in bone marrow aspirates 24 hours after allergen compared with both prechallenge and 24-hour postdiluent values. The numbers of both cDCs and pDCs expressing these maturity markers was significantly increased after allergen compared with before allergen. Data are presented as mean $\pm$ SEM. * $P < 0.05$ compared with before allergen; $\Delta^{\#}P < 0.05$ compared with diluent challenge. (C) The expression of the TSLP receptor subunits TSLPR and IL-7R α on CD11c⁺ and CD123⁺ cells in bone marrow aspirates 24 hours after allergen compared with postdiluent values. Both components of the TSLP receptor were significantly increased on cDCs and pDCs after allergen compared with before allergen. * $P < 0.05$ compared with before allergen; $\Delta^{\#}P < 0.05$ compared with diluent challenge.

activation of DCs into the airways is TSLP. In contrast to IL-4R α , the numbers of cDCs and pDCs expressing both components of the TSLP receptor (TSLPR and IL7R α) was increased in bone marrow aspirates 24 hours after allergen compared with diluent challenge. The importance of TSLP in the maturation and egress of DCs from the bone marrow has not been studied in subjects with asthma; however, previous work from our laboratory has shown that trafficking of eosinophil-lineage committed progenitors may be orchestrated by TSLP (22), and blocking TSLP with a monoclonal antibody effectively attenuates allergen-induced

airway eosinophilia and constrictor responses (23).

The trafficking of DCs into the site of airway inflammation was also evaluated after allergen and diluent challenge. In the peripheral blood, no change in the numbers of cDCs or pDCs was observed at any time point after allergen challenge when compared with diluent challenge. This is consistent with a previous study done in our laboratory showing that there was no change in circulating cDCs after allergen challenge (12); however, in other studies we have reported an efflux of circulating cDCs from the blood 6 and 24 hours after allergen compared with

diluent during the asthmatic response (10, 11). There is no obvious explanation for this discrepancy, because the subjects studied and the methodology airway challenges were similar between these studies.

All subjects enrolled into the study had mild, allergic asthma and were not taking any regular asthma treatment. This is necessary to reproducibly study the effects of inhaled environmental allergens on immunologic responses in asthmatic airways. This means, however, that the results obtained in the study cannot be extrapolated to other asthmatic phenotypes, such as nonallergic asthma.

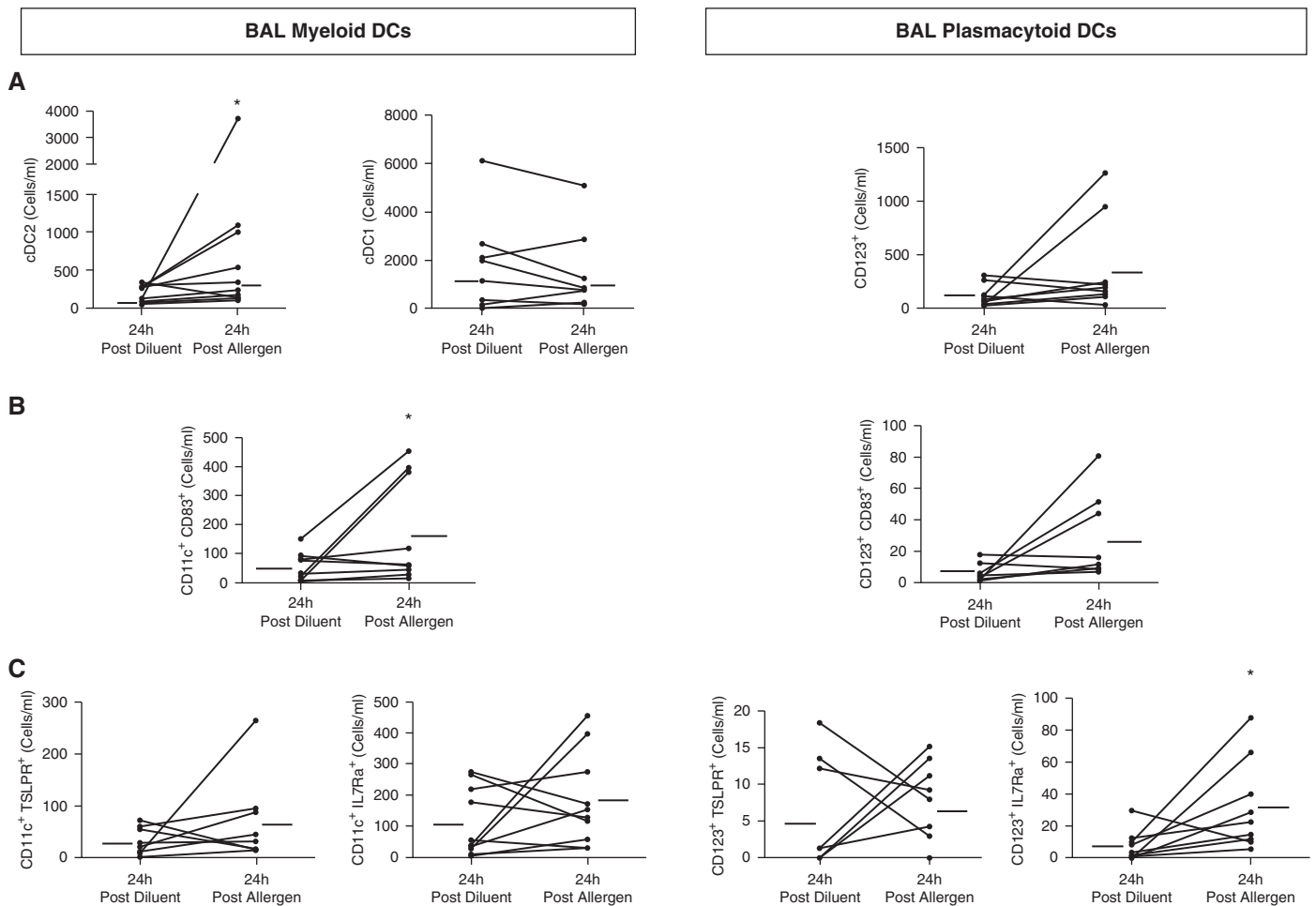


Figure 5. Allergen inhalation induced changes in bronchoalveolar lavage (BAL) dendritic cells (DCs). Enumeration, phenotypic characterization, and thymic stromal lymphopoietin receptor (TSLPR) expression of CD11c⁺ (classical DCs [cDC]) and CD123⁺ (plasmacytoid DCs [pDC]) cells 24 hours after allergen or diluent challenge. (A) There was a significant increase in cDC2s numbers but not cDC1s or pDCs after allergen compared with after diluent. * $P < 0.05$. (B) There was a significant increase in CD11c⁺ CD83⁺ but not CD123⁺ CD83⁺ after allergen compared with after diluent. * $P < 0.05$. (C) The expression of the TSLP receptor subunits TSLPR and IL-7Rα on CD11c⁺ and CD123⁺ cells showing significant increase in IL-7Rα on pDCs after allergen compared with after diluent. * $P < 0.05$.

Both cDC1s and cDC2s increased significantly in BAL 24 hours after allergen challenge, when expressed as a percentage of BAL cells, whereas only the absolute number of cDC2s significantly increased. We have previously shown a significant allergen-induced increase in both subtypes in induced sputum (12). BAL DCs after inhaled allergen had a more developmentally mature phenotype compared with BAL DCs after diluent challenge. By contrast, Bratke and colleagues (14) showed that after segmental allergen challenge, BAL DCs have a less developmentally mature phenotype compared with BAL DCs

from control segments. cDC2s, but not cDC1s, were also significantly increased after inhaled allergen in bronchial tissues. Jahnsen and coworkers (17) have previously shown that DCs increase in bronchial tissues after allergen inhalation, but did not segregate the DC subtypes.

The number of cDC1s was higher in BAL when compared with cDC2s, but the converse was true in the bronchial tissues. A role for cDC1s in allergic disease was initially shown by Yerkovich and colleagues (24). cDC1s are more prevalent in the peripheral blood of subjects with allergy and asthma compared with healthy control subjects (25, 26). In the lungs,

cDC1s have been identified in the airway tissue (27), in BAL (28), and in sputum (13), with higher numbers in subjects with atopic asthma compared with healthy control subjects (13, 29). Although cDC2s and cDC1s share many characteristics, cDC1s have a unique ability to present necrotic viral cell antigens to CD8 T cells (30).

Allergen challenge also increased the numbers of pDCs in bone marrow aspirates, and these also had increased expression of TSLPR and IL-7Rα. There was a nonsignificant trend for an increase in BAL, and pDCs were not examined for in bronchial tissues. In animal models of allergic asthma, the

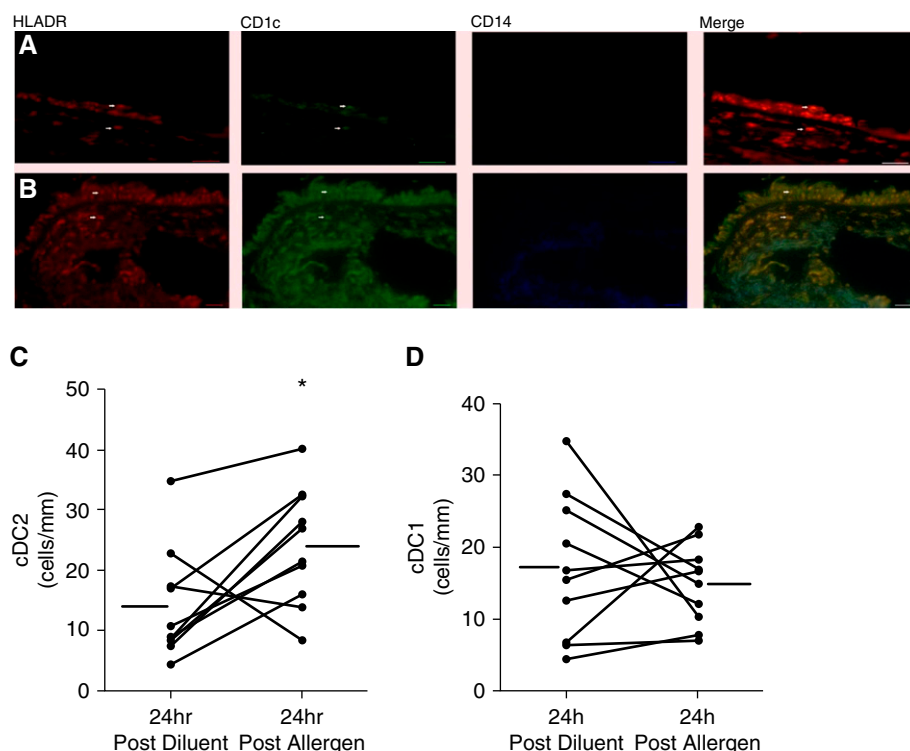


Figure 6. Allergen inhalation induced changes in airway biopsy dendritic cells. Immunofluorescence staining of bronchial mucosa and submucosa in postdiluent (A) and postallergen (B) bronchial biopsies. cDC2s express HLA-DR (red) and CD1c (green) but are negative for CD-14 (blue). Arrows indicate cDC1s in bronchial epithelium and submucosa. Scale bar = 25 μ m. Magnification, $\times 40$, oil immersion. Postallergen bronchial biopsies show higher cDC1 count per square millimeter compared with postdiluent biopsies. (C) Significantly (asterisk) higher numbers of cDC2s per square millimeter in postallergen samples compared with after diluent. (D) No significant increases in cDC1s. cDC = classical dendritic cell.

induction and maintenance of airway inflammation is primarily a function of cDCs (7). In contrast, tolerization to inhaled allergens is likely a function of

pDCs (8, 31). It remains uncertain whether the concept of proallergic cDCs and antiallergic pDCs translates from animal to humans.

In light of the increasing evidence for a role of epithelium-derived cytokines in orchestrating type 2 immune responses (18), the current study explored the role of TSLP and IL-33 in orchestrating the trafficking of DC populations to the airways, by measuring the expression of their receptors on DCs. The results from the present study suggest that TSLP, but not IL-33, may be important in the recruitment of both DC subtypes from the bone marrow. This is consistent with evidence that TSLP may play an important role in DC migration into the skin after cutaneous allergen challenge (32).

Accumulation of airway DCs is most likely a combination of migration, prolonged survival, and an increased production of these cells in the bone marrow. This study has demonstrated increases in human DCs in the bone marrow and in bone marrow culture associated with the development of airway inflammation after allergen inhalation in sensitized subjects with asthma.

Furthermore, allergen challenge is associated with up-regulation of TSLP receptors on DCs, the mechanism of which is still unidentified. Further studies to uncover the specific mechanisms underlying this trafficking of DCs should help the understanding of the mechanisms of allergic asthma and potentially uncover new targets for therapeutic intervention. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

- Diamant Z, Gauvreau GM, Cockcroft DW, Boulet LP, Sterk PJ, de Jongh FH, Dahlén B, O'Byrne PM. Inhaled allergen bronchoprovocation tests. *J Allergy Clin Immunol* 2013;132:1045–1055.e6.
- Holt PG, Upham JW. The role of dendritic cells in asthma. *Curr Opin Allergy Clin Immunol* 2004;4:39–44.
- de Heer HJ, Hammad H, Kool M, Lambrecht BN. Dendritic cell subsets and immune regulation in the lung. *Semin Immunol* 2005;17:295–303.
- Szabolcs P, Ciocon DH, Moore MA, Young JW. Growth and differentiation of human dendritic cells from CD34⁺ progenitors. *Adv Exp Med Biol* 1997;417:15–19.
- Ratta M, Rondelli D, Fortuna A, Curti A, Fogli M, Fagnoni F, Martinelli G, Terragna C, Tura S, Lemoli RM. Generation and functional characterization of human dendritic cells derived from CD34 cells mobilized into peripheral blood: comparison with bone marrow CD34⁺ cells. *Br J Haematol* 1998;101:756–765.
- Guilliams M, Ginhoux F, Jakubczik C, Naik SH, Onai N, Schraml BU, Segura E, Tussiwand R, Yona S. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* 2014;14:571–578.
- Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000;106:551–559.
- Kool M, van Nimwegen M, Willart MA, Muskens F, Boon L, Smit JJ, Coyle A, Clausen BE, Hoogsteden HC, Lambrecht BN, et al. An anti-inflammatory role for plasmacytoid dendritic cells in allergic airway inflammation. *J Immunol* 2009;183:1074–1082.
- Möller GM, Overbeek SE, Van Helden-Meeuwse CG, Van Haarst JM, Prens EP, Mulder PG, Postma DS, Hoogsteden HC. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: downregulation by inhaled corticosteroids. *Clin Exp Allergy* 1996;26:517–524.
- Upham JW, Denburg JA, O'Byrne PM. Rapid response of circulating myeloid dendritic cells to inhaled allergen in asthmatic subjects. *Clin Exp Allergy* 2002;32:818–823.
- Farrell E, O'Connor TM, Duong M, Watson RM, Strinich T, Gauvreau GM, O'Byrne PM. Circulating myeloid and plasmacytoid dendritic cells after allergen inhalation in asthmatic subjects. *Allergy* 2007;62:1139–1145.

12. Dua B, Watson RM, Gauvreau GM, O'Byrne PM. Myeloid and plasmacytoid dendritic cells in induced sputum after allergen inhalation in subjects with asthma. *J Allergy Clin Immunol* 2010;126:133–139.
13. Dua B, Tang W, Watson R, Gauvreau G, O'Byrne PM. Myeloid dendritic cells type 2 after allergen inhalation in asthmatic subjects. *Clin Exp Allergy* 2014;44:921–929.
14. Bratke K, Lommatzsch M, Julius P, Kuepper M, Kleine HD, Luttmann W, Christian Virchow J. Dendritic cell subsets in human bronchoalveolar lavage fluid after segmental allergen challenge. *Thorax* 2007;62:168–175.
15. Lambrecht BN, Carro-Muino I, Vermaelen K, Pauwels RA. Allergen-induced changes in bone-marrow progenitor and airway dendritic cells in sensitized rats. *Am J Respir Cell Mol Biol* 1999;20:1165–1174.
16. van Rijt LS, Prins JB, Leenen PJ, Thielemans K, de Vries VC, Hoogsteden HC, Lambrecht BN. Allergen-induced accumulation of airway dendritic cells is supported by an increase in CD31(hi)Ly-6C (neg) bone marrow precursors in a mouse model of asthma. *Blood* 2002;100:3663–3671.
17. Jahnsen FL, Moloney ED, Hogan T, Upham JW, Burke CM, Holt PG. Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. *Thorax* 2001;56:823–826.
18. Lambrecht BN, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. *J Allergy Clin Immunol* 2014;134:499–507.
19. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235–243.
20. Collins AM, Rylance J, Wootton DG, Wright AD, Wright AK, Fullerton DG, Gordon SB. Bronchoalveolar lavage (BAL) for research; obtaining adequate sample yield. *J Vis Exp* 2014;(85):4345.
21. Lardon F, Snoeck HW, Berneman ZN, Van Tendeloo VF, Nijs G, Lenjou M, Henckaerts E, Boeckxtaens CJ, Vandenabeele P, Kestens LL, et al. Generation of dendritic cells from bone marrow progenitors using GM-CSF, TNF-alpha, and additional cytokines: antagonistic effects of IL-4 and IFN-gamma and selective involvement of TNF-alpha receptor-1. *Immunology* 1997;91:553–559.
22. Smith SG, Gugilla A, Mukherjee M, Merim K, Irshad A, Tang W, Kinoshita T, Watson B, Oliveria JP, Comeau M, et al. Thymic stromal lymphopoietin and IL-33 modulate migration of hematopoietic progenitor cells in patients with allergic asthma. *J Allergy Clin Immunol* 2015;135:1594–1602.
23. Gauvreau GM, O'Byrne PM, Boulet LP, Wang Y, Cockcroft D, Bigler J, FitzGerald JM, Boedigheimer M, Davis BE, Dias C, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 2014;370:2102–2110.
24. Yerkovich ST, Roponen M, Smith ME, McKenna K, Bosco A, Subrata LS, Mameless E, Wikström ME, Le Souef P, Sly PD, et al. Allergen-enhanced thrombomodulin (blood dendritic cell antigen 3, CD141) expression on dendritic cells is associated with a TH2-skewed immune response. *J Allergy Clin Immunol* 2009;123:209–216.e4.
25. Spears M, McSharry C, Donnelly I, Jolly L, Brannigan M, Thomson J, Lafferty J, Chaudhuri R, Shepherd M, Cameron E, et al. Peripheral blood dendritic cell subtypes are significantly elevated in subjects with asthma. *Clin Exp Allergy* 2011;41:665–672.
26. Hayashi Y, Ishii Y, Hata-Suzuki M, Arai R, Chibana K, Takemasa A, Fukuda T. Comparative analysis of circulating dendritic cell subsets in patients with atopic diseases and sarcoidosis. *Respir Res* 2013;14:29.
27. Demedts IK, Brusselle GG, Vermaelen KY, Pauwels RA. Identification and characterization of human pulmonary dendritic cells. *Am J Respir Cell Mol Biol* 2005;32:177–184.
28. Tsoumakidou M, Tzanakis N, Papadaki HA, Koutala H, Siafakas NM. Isolation of myeloid and plasmacytoid dendritic cells from human bronchoalveolar lavage fluid. *Immunol Cell Biol* 2006;84:267–273.
29. Kayserova J, Zentsova-Jaresova I, Budinsky V, Rozkova D, Kopecka J, Vernerova E, Pohunek P, Skalicka V, Spisek R, Sediva A. Selective increase in blood dendritic cell antigen-3-positive dendritic cells in bronchoalveolar lavage fluid in allergic patients. *Scand J Immunol* 2012;75:305–313.
30. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, Chen CJ, Dunbar PR, Wadley RB, Jeet V, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* 2010;207:1247–1260.
31. de Heer HJ, Hammad H, Soulié T, Hijdra D, Vos N, Willart MA, Hoogsteden HC, Lambrecht BN. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004;200:89–98.
32. Corrigan CJ, Jayaratnam A, Wang Y, Liu Y, de Waal Malefyt R, Meng Q, Kay AB, Phipps S, Lee TH, Ying S. Early production of thymic stromal lymphopoietin precedes infiltration of dendritic cells expressing its receptor in allergen-induced late phase cutaneous responses in atopic subjects. *Allergy* 2009;64:1014–1022.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.