

Role for cysteinyl leukotrienes in allergen-induced change in circulating dendritic cell number in asthma

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Background: Dendritic cells are important antigen-presenting cells. After an allergen inhalation, their numbers rapidly decrease in circulation and increase in the airway mucosa.

Objective: To investigate whether allergen-induced changes in the number of circulating dendritic cells are mediated by cysteinyl leukotrienes.

Methods: In a randomized, double-blind, crossover study, we examined the effects of 2 weeks of treatment with pranlukast (a cysteinyl leukotriene 1 [CysLT₁] receptor antagonist) 300 mg twice daily and placebo on allergen-induced changes in airway responses and circulating dendritic cells in 15 subjects with mild asthma. We examined by flow cytometry, before and at 3 hours and 24 hours after allergen inhalation, the proportion of myeloid (CD33⁺) and plasmacytoid (CD123⁺) dendritic cells (HLA-DR⁺, CD14⁺, CD16⁺) among all peripheral blood mononuclear cells. The fraction of dendritic cells expressing CysLT₁ receptor was also determined.

Results: Compared with placebo, pranlukast significantly attenuated both the maximum early (by 55%) and the late (by 39%) asthma responses, the allergen-induced methacholine airway hyperresponsiveness, and the increase in macrophage inflammatory protein 1 α and 3 α in induced sputum. A significantly greater proportion of CD33⁺ cells (55%) expressed CysLT₁ receptor compared with CD123⁺ cells (11%). Consistent with this, pranlukast prevented the allergen-induced decrease in CD33⁺ dendritic cells at 3 hours postallergen (mean Δ from baseline, +4.4%) compared with placebo (mean Δ , -8.4; $P < .05$), but not CD123⁺ cells.

Conclusion: Pretreatment with pranlukast attenuated allergen-induced airway responses and the decrease in circulating myeloid dendritic cells, demonstrating a novel role of cysteinyl leukotrienes in dendritic cell trafficking. (*J Allergy Clin Immunol* 2004;114:73-9.)

Key words: Cysteinyl leukotrienes, pranlukast, dendritic cells, allergen inhalation, asthma

Abbreviations used

CysLT₁: Cysteinyl leukotriene 1

EAR: Early asthma response

LAR: Late asthma response

MIP: Macrophage inflammatory protein

Inhalation of allergens by atopic asthmatic patients causes an immediate bronchoconstriction (early asthma response [EAR]), which is followed in approximately half the subjects by a late bronchoconstriction (late asthma response [LAR]) and an increase in airway hyperresponsiveness.¹ The EAR is a result of the release of mediators such as histamine and cysteinyl leukotrienes.² These mediators and other cytokines facilitate an inflammatory cell infiltration of the airway that is responsible for the LAR and changes in airway hyperresponsiveness.

Allergen inhalation is able to trigger this response only if the allergens are initially processed by airway dendritic cells that possess pattern recognition receptors to present them to T cells after their migration to regional lymph nodes. This migration through the lymphatic system is mediated by chemoattractants such as CCL19 (macrophage inflammatory protein (MIP)-3 β , Exodus-3) and CCL21 (6Ckine, Exodus-2, secondary lymphoid tissue chemokine), which are ligands for CCR7 receptors expressed on the dendritic cells.^{3,4} Leukotriene C₄, produced from the dendritic cells themselves or from other inflammatory cells, is necessary for the migration of dendritic cells toward CCL19.⁵ It is generally believed that the myeloid dendritic cells (CD33⁺) are responsible for antigen presentation in the lymph nodes, whereas the lymphoid or plasmacytoid cells (CD123⁺IL-3R α) are natural interferon-producing cells.⁶

There is little known about the chemoattractants that mediate the migration of dendritic cells from blood to the airway. The newly described C-C chemokine, MIP-3 α (liver and activated-regulated chemokine, Exodus-1),⁷ which is produced by airway epithelial cells,⁸ is a likely chemoattractant. We have previously observed that the numbers of myeloid dendritic cells in circulation decrease rapidly within 3 hours of an allergen inhalation.⁹ Because cysteinyl leukotriene levels increase at this time in the airway¹⁰ and because cysteinyl leukotriene antagonist can attenuate allergen-induced airway responses,¹¹⁻¹³ we

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TABLE I. Baseline subject characteristics

	Mean (minimum, maximum)
Age, y	27 (19, 53)
FEV ₁ , L	3.3 (2.3, 4.6)
FEV ₁ , % predicted	91 (71, 105)
PC ₂₀ methacholine, mg/mL*	2.4 (0.1, 18.4)

*Geometric mean.

hypothesized that leukotrienes might also regulate the disappearance of dendritic cells from blood after an allergen inhalation and that this effect would be more on the myeloid cells than the plasmacytoid dendritic cells. In this study, we investigated the proportion of myeloid and plasmacytoid dendritic cells that expressed the cysteinyl leukotriene 1 (CysLT₁) receptor and the effect of pre-treatment with a cysteinyl leukotriene receptor antagonist, pranlukast, on allergen-induced airway responses and dendritic cell chemoattractants and circulating dendritic cell numbers.

METHODS

Subjects

The subjects were 16 atopic nonsmokers with mild asthma (Table I) who were using short-acting β -agonists infrequently. Asthma had been previously diagnosed by a physician on the basis of typical episodic symptoms and bronchoconstriction induced by allergen inhalation. Subjects were studied out of their allergy season and had not had any respiratory tract infections for at least 6 weeks before the study. All medications except the study medication were withheld for at least 8 hours before each visit. The subjects refrained from vigorous exercise and from caffeine-containing beverages in the morning before visits to the laboratory. All subjects gave written informed consent, and the Research Ethics Committee of Hamilton Health Sciences Corp approved the study.

Study design

This was a randomized, double-blind, crossover, 2-period study comparing 2 weeks of treatment with pranlukast 300 mg tablet twice daily or identical placebo with at least 2 weeks of washout period between the treatment periods. The subjects underwent a screening allergen inhalation to identify those with a dual asthma response (at least 15% fall in FEV₁ within the 120 minutes followed by a similar drop between 3 and 7 hours after allergen inhalation). After 2 to 4 weeks, they were randomized to 1 of the 2 treatment arms by using computer-derived codes that were maintained off-site by an independent third-party pharmacist. The medications were taken at 8 PM and 8 AM starting in the evening of the first day. On the 13th day (± 2 days), spirometry and methacholine inhalation test were performed, sputum was induced for measurement of chemokines, and 5 mL blood was collected in EDTA tube for dendritic cell assay. The next day, an allergen inhalation test was performed. At 3 hours after allergen inhalation, blood was drawn for dendritic cell measurement, and at 7 hours, sputum was induced for chemokine measurement. The following day, approximately 24 hours after allergen inhalation, subjects attended the laboratory for spirometry, methacholine inhalation test, and blood sampling for dendritic cell measurement. Compliance was evaluated weekly by pill counting. Any adverse effect was evaluated by self-reported symptoms, physical examination, blood chemistry, and urine examination.

Airway responses

FEV₁ was measured by using a Collins water-sealed spirometer (Warren E. Collins, Braintree, Mass) and kymograph according to the American Thoracic Society recommendations.¹⁴ Methacholine inhalation test was performed by the tidal breathing method of Cockcroft et al¹⁵ by inhaling doubling concentrations of methacholine (Methapharm, Mississauga, Ontario, Canada) from a Wright nebulizer (Roxon Medi-Tech, Montreal, Quebec, Canada). The concentration of methacholine required to achieve a decrease in FEV₁ of 20% (PC₂₀) was calculated through linear interpolation of percent fall in FEV₁ against the log-transformed methacholine concentration.

Allergen inhalation

Allergen inhalation was performed as previously described.¹⁶ The allergen producing the largest diameter skin wheal was diluted in 0.9% saline for inhalation. Allergens used were extracts of house dust mite (n = 7), cat (n = 2), ragweed (n = 3), *Alternaria* (n = 1), and grass (n = 2). The concentration of allergen extract for inhalation was determined from a formula described by Cockcroft et al.¹⁷ The early bronchoconstrictor response was the largest percent fall in FEV₁ within the first 2 hours after allergen inhalation, and the late bronchoconstrictor response was the largest fall in FEV₁ between 3 and 7 hours after the allergen inhalation.

Immunofluorescent staining

The following directly conjugated mAbs were used: mouse antihuman CD14-fluorescein isothiocyanate, CD16- fluorescein isothiocyanate, CD123-phycoerythrin, CD33-allophycocyanin, and HLA-DR—CyChrome (PharMingen, San Diego, Calif). CysLT₁ receptor was stained by using goat anti-CysLT₁ receptor specific antisera, swine biotinylated anti-goat IgG antibody (1/250 dilution), and Streptavidin—energy-coupled dye (Beckman Coulter, Mississauga, Ontario, Canada). Dendritic cells were stained as previously described.¹⁸ This method has been shown to be valid, reliable, and reproducible. Immunofluorescent staining of dendritic cells for CysLT₁ receptor expression was performed as previously described¹⁹ with slight modification. Briefly, 200 μ L blood was added to each 5-mL polystyrene tube. Red cells were lysed by using NH₄Cl-EDTA lysing solution, and remaining leukocytes were stained with the directly conjugated antibodies against CD14, CD16, CD33, CD123, and HLA-DR or with indirectly conjugated antibodies against CysLT₁ receptor. Myeloid dendritic cells were identified as CD33⁺HLA-DR⁺ mononuclear cells that were CD14⁺CD16⁺. The myeloid dendritic cells were all CD11c⁺.¹⁸ Plasmacytoid dendritic cells were identified as CD123⁺HLA-DR⁺ mononuclear cells that were weakly CD33⁺. Duplicate tubes were stained with these antibodies for analysis of dendritic cell numbers.

Flow cytometry

Stained and control cell preparations were analyzed within 24 hours by using a 6-color FACSVantageSE flow cytometer equipped with a 488-nm argon laser and a 635-nm helium-neon laser (Becton Dickinson Instrument Systems, Mississauga, Ontario, Canada) and operated with CellQuest software (Becton Dickinson Instrument Systems). Dendritic cell count was based on total leukocyte count of 300,000 to 400,000 cells, of which approximately 100,000 were mononuclear cells. Dendritic cell count for CysLT₁ receptor staining was based on total leukocyte count of 700,000 to 800,000 cells, of which approximately 400,000 were mononuclear cells. Off-line analysis was performed with WinList software, version 5.0 (Verity Software House, Topsham, Me), as previously described.^{9,18}

Sputum measurements

Sputum was induced with hypertonic saline, separated from saliva, and processed as described by Pizzichini et al.²⁰ MIP-1 α and

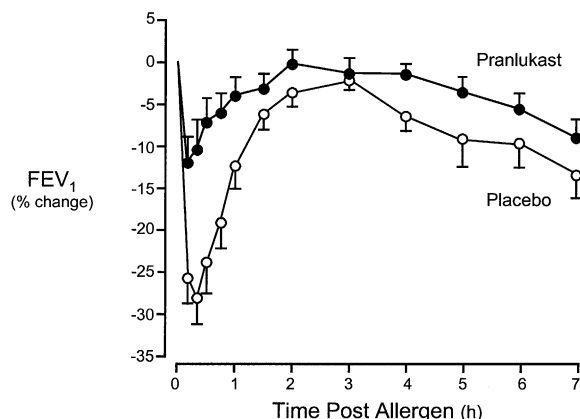


FIG 1. Effect of pranlukast on allergen-induced early and late asthma responses.

MIP-3 α in sputum supernatant were measured by ELISA (R&D Systems, Minneapolis, Minn).

Analysis

All data except methacholine PC₂₀ are expressed as means and SDs. Nonnormally distributed data were log-transformed for analysis. PC₂₀ data are expressed as geometric means and SDs. The differences in maximum early and late allergen responses and airway hyperresponsiveness between pranlukast and placebo treatment were compared by paired *t* test. Dendritic cells were expressed as a percentage of the total mononuclear cells. This has been shown to be as repeatable as their absolute numbers.¹⁸ The percentage change in dendritic cell number at 3 hours and 24 hours after allergen challenge and the preallergen baseline for the 2 treatment arms were compared by repeated-measures ANOVA. Correlations between variables were examined by using the Pearson coefficient. The proportion of myeloid and plasmacytoid dendritic cells expressing the CysLT₁ receptor was compared by unpaired *t* test. A *P* value < .05 was considered statistically significant. Analysis was performed by using the Statistical Package for Social Sciences, version 10.0 (SPSS, Chicago, Ill).

RESULTS

One subject was withdrawn from the study after developing an urticarial rash after the first dose of the medication. Fifteen subjects completed the study without any significant adverse events.

Airway responses

The mean FEV₁ values after placebo and pranlukast treatments (preallergen challenge) were identical (3.3 L). The mean (SD) maximum EAR was 30.3% (12.1) after placebo treatment and 13.5% (12.2) after pranlukast treatment (mean attenuation, 55%; *P* < .05). The maximum LAR was 15.2% (10.0) after placebo treatment and 9.3% (8.1) after pranlukast treatment (mean attenuation, 39%; *P* < .05; Fig 1). Allergen inhalation after placebo treatment caused a 0.8-fold increase in PC₂₀ at 24 hours. This was significantly decreased after pranlukast treatment (Fig 2).

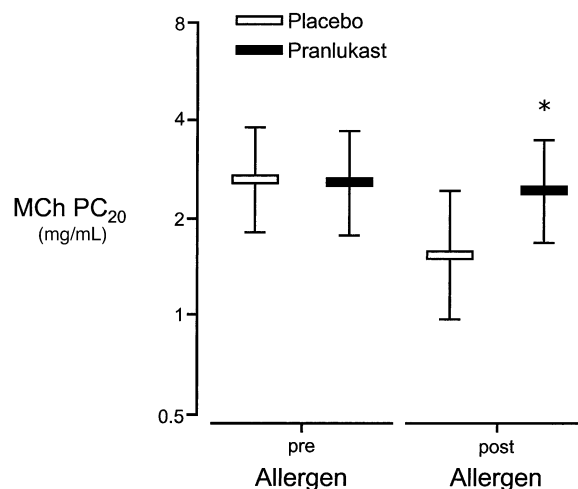


FIG 2. Effect of pranlukast on allergen-induced airway hyperresponsiveness. **P* < .05 compared with placebo.

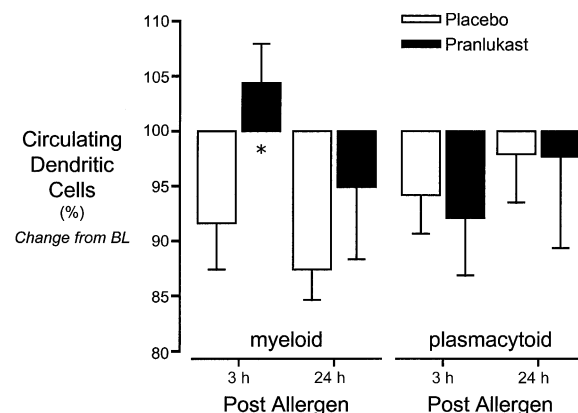


FIG 3. Effect of pranlukast on circulating dendritic cell number. BL, Baseline. **P* < .05 compared with placebo.

Effect on circulating myeloid and plasmacytoid dendritic cell numbers

One subject had a negligible number of dendritic cells and was therefore excluded from the analysis. This decision was made before the randomization code was broken. Analysis is thus based on data from 14 subjects. At baseline, the mean ratio of myeloid to plasmacytoid dendritic cells was 1.2. After placebo treatment, the proportion of myeloid dendritic cells in blood decreased by a mean of 8.4% at 3 hours after allergen inhalation compared with preallergen value. This was abolished by pretreatment with pranlukast. The mean change was an increase by 4.4% (*P* < .05). Pranlukast also attenuated the decrease in myeloid cells at 24 hours. This was not statistically significant (Fig 3). The proportion of plasmacytoid cells also decreased 3 hours after allergen inhalation (mean change, 5.8%); however, this was not prevented by pranlukast (mean decrease, 7.8%). The correlation between the changes in dendritic cell number,

TABLE II. Effect of placebo and pranlukast treatment on RANTES, MIP-1 α , and MIP-3 α in sputum before and after an allergen inhalation

Chemoattractant, mean (SD)	Placebo		Pranlukast	
	Preallergen	Postallergen	Preallergen	Postallergen
MIP-1 α (pg/mL)	3.4 (4.8)	8.6 (11.2)	4.5 (8.1)	1.5 (1.5)
MIP-3 α (pg/mL)	121 (231)	176 (182)	122 (162)	108 (226)

TABLE III. Expression of CysLT₁ receptor on circulating myeloid and plasmacytoid dendritic cells and the effects of placebo and pranlukast treatment

% Dendritic cells with CysLT ₁ receptor, mean (SD)	Placebo			Pranlukast		
	Pre	+3 h	+24 h	Pre	+3 h	+24 h
Myeloid	55 (27)	39 (22)	39 (27)	38 (29)	43 (36)	28 (25)
Plasmacytoid	11 (6.8)	10 (10)	8 (11)	13 (17)	18 (32)	5 (4)

percentage fall in FEV₁, or PC₂₀ methacholine was not statistically significant.

Effect on dendritic cell chemoattractants in the airway

Levels of MIP-1 α and MIP-3 α in sputum increased 7 hours after allergen inhalation in the placebo treatment arm. This was prevented by pranlukast treatment (Table II).

Effect on CysLT₁ receptor expression

A significantly greater proportion of the myeloid dendritic cells expressed the CysLT₁ receptor (mean, 55%) compared with the plasmacytoid dendritic cells (mean, 11%; $P < .05$; Table III; Fig 4). This was not significantly altered by allergen inhalation or pranlukast treatment (Table III).

DISCUSSION

This study confirmed that a cysteinyl leukotriene receptor antagonist, pranlukast, can attenuate allergen-induced early and late asthma responses and airway hyperresponsiveness. The study also showed that circulating dendritic cells express CysLT₁ receptor. Pranlukast prevented an allergen-induced decrease in circulating myeloid dendritic cells. This was accompanied by a reduction in the levels of dendritic cell chemoattractants MIP-1 α and MIP-3 α in sputum. The results suggest a role for cysteinyl leukotrienes in the migration of myeloid dendritic cells from blood, presumably to the airway.

We confirmed previous observations that a cysteinyl leukotriene receptor antagonist can attenuate allergen-induced early and late asthma responses and increase in airway hyperresponsiveness.¹¹⁻¹³ The degree of protection is comparable with that of previous reports. As in previous reports, we observed a greater attenuation of the early asthma response than the late asthma response. This is consistent with the facts that the early response is mainly mediated by cysteinyl leukotrienes and that the late asthma

response is caused by a cellular inflammatory response that is less affected by cysteinyl leukotrienes.

Airway dendritic cells play a fundamental role in allergen-induced airway responses and inflammation.²¹ Inhalation of allergen causes a rapid decrease in their number in peripheral blood⁹ and their accumulation in the airway mucosa.²² The migration of dendritic cells has been the subject of great interest and investigation.²³ Most of the studies have focussed on Langerhans cells in the skin or trafficking in animals. Dendritic cell precursors are derived from the bone marrow. They migrate through the blood and enter nonlymphoid tissues such as skin and lung, where they may become resident cells. On contact with an allergen, they migrate to the lymph node, become mature, and present the allergens to the lymphocyte subsets to polarize them to either the T_H1 or the T_H2 phenotype. Leukotriene C₄ plays a critical role in the migration of immature dendritic cells to the lymph node. Mobilization of dendritic cells from the epidermis and trafficking to lymphatics was greatly reduced in mice deficient in the multidrug resistance-associated protein, which is necessary for the transport of leukotriene C₄ from inside a cell to its outside. This was restored by exogenous leukotriene C₄ and D₄.⁵ Our experiments suggest that leukotrienes may also be involved in the mobilization of immature dendritic cells from blood after an allergen inhalation. One of the mechanisms may be an increase in the levels of dendritic cell chemoattractants such as RANTES,²³ MIP-1 α ,²⁴ and MIP-3 α .²⁵ Consistent with this, we observed that allergen-induced increase in the levels of RANTES,²⁶ MIP-1 α , and MIP-3 α were prevented by pranlukast treatment. Cysteinyl leukotrienes may also have a direct effect on dendritic cells by up-regulating the CCR6 receptors that are necessary for their migration toward MIP-3 α .²⁵ Alternatively, cysteinyl leukotrienes produced by inflammatory cells after an allergen inhalation may augment the chemotactic activity of MIP-3 α , similar to its potentiating effect on alveolar macrophage-derived MIP-1 α .²⁷

There is an apparent difference in the ability of the myeloid and plasmacytoid cells to migrate. Although

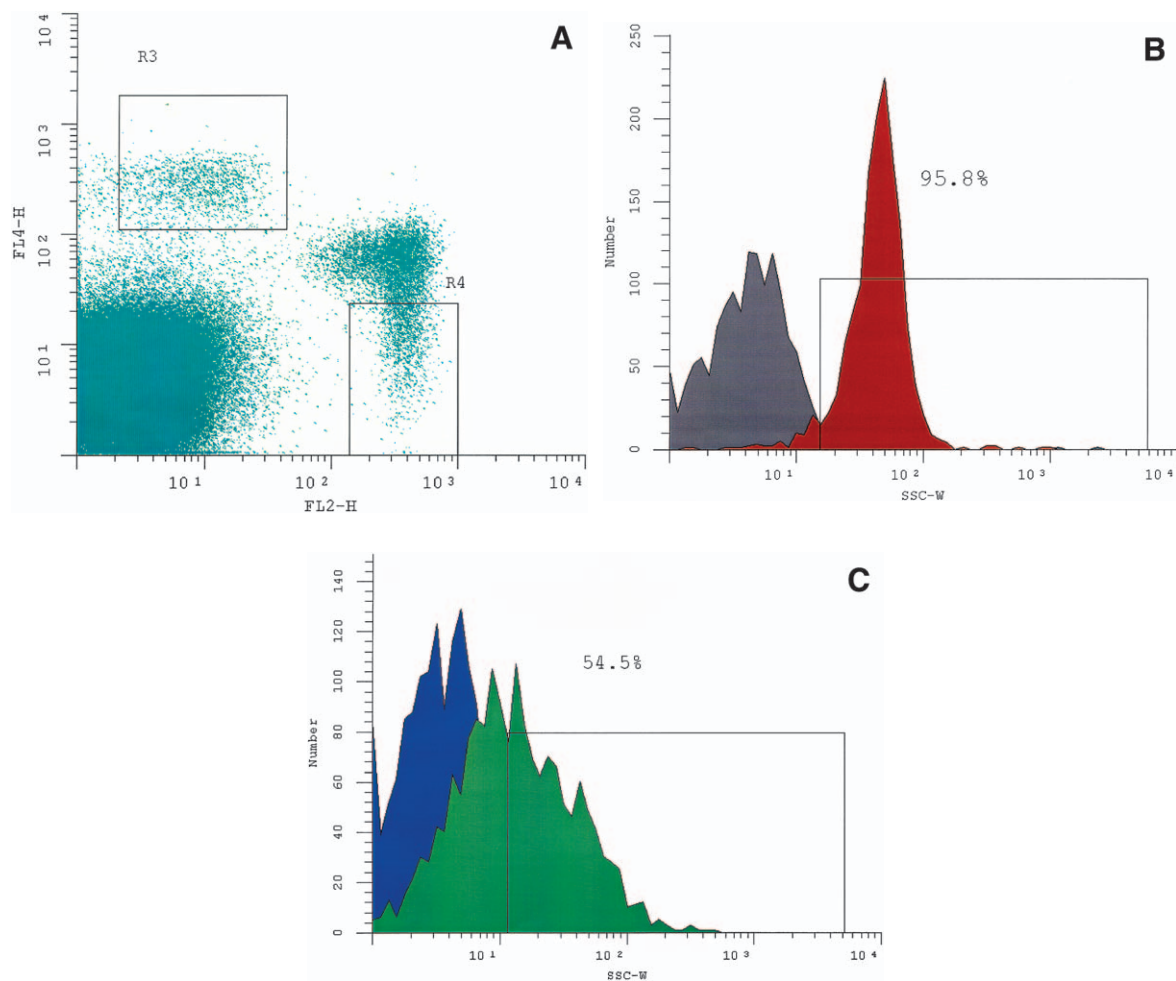


FIG 4. Representative data of CysLT₁ receptor expression on 2 dendritic cell subsets in blood. **A**, Two-color analysis of gated (HLA-DR⁺, CD14/16⁺) blood mononuclear cells, more expression of CD33 (y-axis) and CD123 (x-axis). The myeloid CD33⁺ dendritic cells are identified within the R3 region (CD33⁺, CD123⁻), and the plasmacytoid dendritic cells are identified within the R4 region (CD33⁻, CD123⁺). **B**, All cells identified as myeloid dendritic cells in **A** were further analyzed for expression of the CysLT₁ receptor (brown histogram) compared with background control stain (gray histogram). **C**, All cells identified as plasmacytoid dendritic cells in **A** were further examined for expression of the CysLT₁ receptor (green histogram) compared with background control stain (blue histogram). FL2-H, Orange-red fluorescence; FL4-H, red-orange fluorescence; SSC, side scatter.

more myeloid cells appear in the bronchial mucosa after an allergen inhalation,²² more plasmacytoid cells seem to migrate to the nasal mucosa after nasal allergen challenge.²⁸ The reason for this difference is not known. *In vitro*, plasmacytoid cells show markedly less chemotaxis than myeloid dendritic cells toward classical agonists.²⁹ One of the reasons may be because most of the chemokine receptors expressed on the plasmacytoid cells are not functional in circulating cells.²⁹ In keeping with these observations, we noted a significant decrease in circulating myeloid dendritic cells compared with plasmacytoid cells immediately after the allergen inhalation. Also, the effect of pretreatment with a CysLT₁ receptor antagonist was observed only in myeloid cell numbers. A possible explanation is the greater expression of CysLT₁ receptor expression on myeloid cells than on plasmacytoid

cells. Although, immature dendritic cells express 5-lipoxygenase and 15-lipoxygenase activity,³⁰ very little is known about the regulation of CysLT₁ receptor expression on circulating dendritic cells.

The current study does not provide information on the destination of the cells that disappear from circulation. On the basis of previous evidence,^{9,22} we believe that they migrate to the site of allergic inflammation, ie, the airway mucosa. It is also plausible that they may move back into the bone marrow or into the lymphatic system. This can only be studied if bone marrow, blood, and bronchial mucosal biopsies are obtained simultaneously. We considered it in this study but were unable to perform this step. However, we plan to do this in a future study. As with the observations of Jahnsen et al²² and Upham et al,⁹ we did not observe a significant correlation between the

changes in circulating dendritic cell number and airway responses. This may be a reflection of the complex interplay between dendritic cells and various inflammatory and structural cells in orchestrating the airway inflammatory responses. The study also does not confirm that the effects of pranlukast are unique to dendritic cells. In this study, we did not enumerate the numbers of lymphocytes and monocytes by flow cytometry in all of the subjects at 3 hours after allergen inhalation. However, we had previously observed that the number of monocytes in blood is unchanged after an allergen inhalation.⁹ Inhaled corticosteroids may also affect the recruitment of dendritic cells to the airway after an allergen inhalation. However, this has not been investigated. In a non-randomized clinical trial,³¹ the number of dendritic cells in the airway mucosa of asthmatic patients treated with beclomethasone for 2.5 years was significantly lower than in patients treated with bronchodilators. The mechanism was not investigated.

Despite the limitations, this study has important clinical implications. A prevention of mobilization of dendritic cells from blood to the airway may prevent the initiation of the allergic process and perpetuation of eosinophilic inflammation.³² Although inhaled corticosteroids have also been shown to decrease the number of dendritic cells in the airway,³¹ there is concern about increased IgE synthesis with long-term corticosteroid therapy.³³ Cysteinyl leukotrienes have recently shown to regulate the vesicular-mediated, extracellular transport of the B-cell switch-regulating cytokine, IL-4, from eosinophils.³⁴ This effect, together with their effect on dendritic cell mobilization from blood to the airway, make them attractive targets for preventing the development of allergy. Long-term clinical trials similar to the study of cetirizine³⁵ are necessary to determine whether cysteinyl leukotriene antagonists are effective in the prevention of allergies.

In conclusion, the cysteinyl leukotriene antagonist pranlukast attenuated allergen-induced airway responses and the allergen-induced decrease in circulating myeloid dendritic cells, demonstrating a novel role for cysteinyl leukotrienes in dendritic cell trafficking.

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