

Effects of fexofenadine on T-cell function in a murine model of allergen-induced airway inflammation and hyperresponsiveness

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There is renewed interest in antihistamines for the treatment of allergic asthma. A growing body of literature has shown that the newer compounds can affect inflammatory cell accumulation and cytokine/chemokine production. In a murine model of allergen-induced airway inflammation and hyperresponsiveness, the ability of fexofenadine to affect these outcomes was tested in a primary sensitization and challenge model and after treatment of donor mice before the adoptive transfer of T cells into recipients receiving limited allergen exposure. Mice were sensitized and challenged with allergen (ovalbumin). Airway function after inhaled methacholine was monitored in parallel to the assessment of tissue and airway inflammation and cytokine production. In further experiments, lung T lymphocytes from sensitized/challenged donor mice were transferred into naive recipients before limited airway challenge with the allergen. Administration of fexofenadine before challenge but after sensitization was effective in preventing tissue eosinophilia and airway hyperresponsiveness. Moreover, the treatment of donor mice with fexofenadine before transfer of lung T cells effectively prevented airway hyperresponsiveness and eosinophilia in naive mice exposed to limited airway challenge. These data therefore support the potential for fexofenadine in the treatment of allergen-induced airway hyperresponsiveness and inflammation. (*J Allergy Clin Immunol* 2003;112:S89-95.)

Key words: Antihistamines, airway hyperresponsiveness, inflammation, allergen

A substantial body of evidence is accumulating that supports the hypothesis that antihistamines, generally used in the treatment of allergic rhinitis, might also exhibit anti-inflammatory properties. It is not clear what underlying mechanisms might be responsible, or if the effects go beyond H₁-receptor antagonism.

Histamine is known to downregulate several immunologic functions, presumably through its interaction with H₂-receptors. Histamine stimulates CD8⁺ T cells, resulting in the inhibition of antibody synthesis, cytokine production, and lymphocyte proliferation.¹⁻³ Histamine

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
EPO: Eosinophil peroxidase
ICAM: Intercellular adhesion molecule
Mch: Methacholine
OVA: Ovalbumin
PAS: Periodic acid Schiff
Penh: Enhanced pause

might also suppress the release of IL-12 and stimulate the release of IL-10.⁴ Such studies indicate the potential of histamine to skew the response to a T_H2 phenotype.

Histamine also stimulates parasympathetic nerves, increasing the release of a number of cytokines that might contribute to allergic inflammation, including GM-CSF, IL-1, IL-6, IL-8, IL-10, IL-11, eotaxin, RANTES, and TNF- α .⁵ Furthermore, histamine increases the expression of HLA-DR and intercellular adhesion molecule 1 (ICAM-1) receptors on epithelial cells, presumably via H₁-receptors.⁶ As summarized in this supplement and elsewhere, the data imply that antihistamines might have effects other than simple antagonism of histamine receptors, with important actions on the inhibition of mediator release and blockade of cellular recruitment and activation. In addition, these inhibitory effects of antihistamines appear not to directly correlate with H₁-receptor antagonism.

Prompted by these observations, as well as the suggestion of antihistamine effects in asthma (especially with terfenadine),^{7,8} we used a well-characterized murine model to determine the activity of fexofenadine (the active metabolite of terfenadine) in preventing allergen-induced airway inflammation and airway hyperresponsiveness (AHR). The data demonstrate the effectiveness of this antihistamine in modulating the response to allergen sensitization and challenge, particularly in preventing tissue eosinophilia and AHR.

METHODS

Animals

Female BALB/c mice, free of murine-specific pathogens, were obtained from Jackson Laboratories (Bar Harbor, Me). The mice were maintained on an ovalbumin (OVA)-free diet. All experimental animals used in this study were under a protocol approved by the

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Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center (Denver, Colo).

Experimental protocol

Mice, 10 to 12 weeks of age, were sensitized by intraperitoneal injection of 20 μ g OVA (Grade V; Sigma Chemical Co, St Louis, Mo) emulsified in 2.25 mg aluminum hydroxide (AlumImject; Pierce, Rockford, Ill) in a total volume of 100 μ L on days 1 and 14. Mice were challenged with OVA (1% in saline) via the airways for 20 minutes daily for 3 days (days 26, 27, and 28) by using ultrasonic nebulization (AeroSonic ultrasonic nebulizer; DeVilbiss, Somerset, Pa).⁹

Response to primary sensitization and challenge

The primary challenge protocol was as follows. Mice were sensitized to OVA (together with alum) on days 1 and 14. Aerosolized OVA challenge was administered on days 26, 27, and 28. Lung function, inflammation, and cytokine response were monitored 48 hours later (day 30).

Drug treatment

Fexofenadine was suspended in saline and administered by gavage twice daily at a dose of 5 mg, beginning 3 days before allergen challenge and on each of the days of allergen challenge.

Airway responsiveness

AHR was assessed by using a single chamber, whole-body plethysmograph obtained from Buxco (Troy, NY).¹⁰ In this system an unrestrained, spontaneously breathing mouse was placed into the main chamber of the plethysmograph, and pressure differences between this chamber and a reference chamber were recorded. The box pressure signal is caused by volume and resultant changes in pressure during the respiratory cycle of the animal. A low-pass filter in the wall of the main chamber allows for thermal compensation. From these box pressure signals, the phases of the respiratory cycle, tidal volumes, and the enhanced pause (Penh) can be calculated. Penh is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and of the timing of expiration. It correlates closely with pulmonary resistance measured by conventional two-chamber plethysmography in ventilated animals.¹⁰ Penh was used as a measure of airway responsiveness to methacholine (MCh). In the plethysmograph, mice were exposed for 3 minutes to nebulized PBS and subsequently to increasing concentrations of nebulized MCh (in PBS).

Determination of cell numbers and cytokine levels in bronchoalveolar lavage fluid

Immediately after assessment of AHR, lungs were lavaged via the tracheal tube with Hank's balanced salt solution (1×1 mL, 37°C). Total leukocyte numbers were measured (Coulter Counter; Coulter Corporation, Hialeah, Fla). Differential cell counts were performed by counting at least 300 cells on cytocentrifuged preparations (Cytospin 2, Cytospin; Shandon Ltd, Runcorn, Cheshire, United Kingdom), stained with Leukostat (Fisher Diagnostics, Pittsburgh, Pa) and differentiated by standard hematologic procedures. Bronchoalveolar lavage (BAL) supernatants were collected and kept frozen at -70°C. Cytokine levels were measured by ELISA.

Determination of cell numbers in tissue

Lung tissue was subjected to collagenase digestion, and single-cell preparations were obtained; cytospin preparations were stained and analyzed.¹¹

Goblet cell and tissue eosinophil quantitation

Lungs were inflated through the tracheal tube with 2 mL formalin and fixed in 10% formalin. Blocks of lung tissue were cut around the main bronchus and embedded in paraffin blocks. Tissue sections, 5 μ m thick, were affixed to microscope slides and deparaffinized. The slides were stained with hematoxylin and eosin and periodic acid Schiff (PAS) for identification of mucus-containing cells and examined by using light microscopy.

The number of goblet cells in the airway epithelium was counted in at least 20 sections by measuring the length of epithelium defined as basement membrane and the luminal area by using a National Institutes of Health Image analysis system (Bethesda, Md). Mucus-containing cells were expressed as the number of goblet cells per 100 μ m epithelium.¹²

Eosinophil peroxidase

In a separate series of studies, levels of eosinophil peroxidase (EPO) in BAL fluid supernatant were determined as previously described.¹³ Briefly, 100 μ L of the substrate solution (0.1 mmol/L *o*-phenylene-diamine-dihydrochloride, 0.1% Triton X-100, 1 mmol/L hydrogen peroxide in 0.05 mol/L Tris-HCl; all reagents from Sigma) was added to 100 μ L of supernatant in microtiter plates and incubated for 30 minutes at 37°C. The reaction was stopped by adding 50 μ L of 4 mol/L sulfuric acid, and the optical densities were read in a microtiter auto reader at 492 nm. This method has been shown to be specific for the peroxidase activity of eosinophils.

Adoptive transfer

Single-cell preparations from the lungs were obtained 2 days after the last allergen challenge from mice that were treated or were not treated with fexofenadine. The cells were passed over nylon wool columns and eluted, providing a population of cells in which greater than 85% stained for CD3. Approximately 1×10^6 cells were injected intravenously into naive recipients before initiation of airway challenges with allergen.

RESULTS

Intraperitoneal sensitization and nebulized challenge of mice resulted in a dose-dependent increase in AHR to inhaled MCh, as shown by up to 6-fold increases in Penh (Fig 1).¹⁴ Mice exposed to allergen challenge alone did not develop increases in AHR. Treatment of the mice with fexofenadine eliminated the increased responsiveness to MCh at all concentrations of the bronchoconstrictor. Sensitization and challenge of mice resulted in significant increases in total cell numbers as well as the number and percentage of BAL eosinophils. Pretreatment with fexofenadine had a modest effect on the number of BAL fluid eosinophils, whereas the effect was even more dramatic in lung digests.

Examination of lung digests showed that sensitization and challenge resulted in an increase in total cell number, particularly the numbers of eosinophils (Fig 2). Whereas total tissue cell numbers were unaffected by fexofenadine, the number of eosinophils in lung digests was significantly reduced (Fig 2). In parallel, levels of EPO in the BAL fluid were also significantly reduced.

BAL fluid supernatants were also examined for levels of IL-4, IL-5, IL-10, and IFN- γ by ELISA. As illustrated in Table I, sensitization and challenge resulted in increases in IL-4 and IL-5 levels, consistent decreases in IL-10

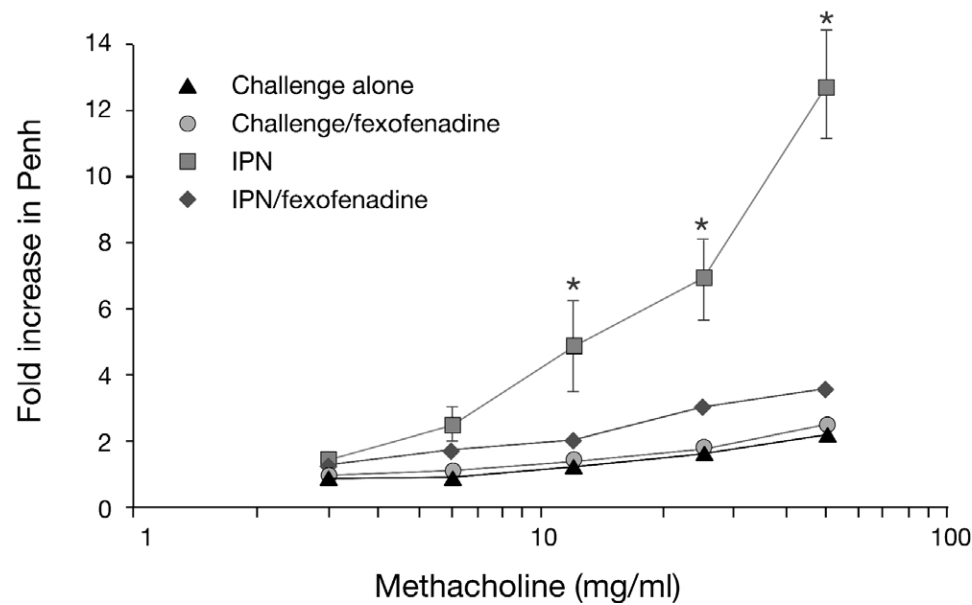


FIG 1. Airway hyperresponsiveness: Effect of fexofenadine, $n = 12$ in each group. $*P < .05$ compared with IPN/fexofenadine or challenge alone groups. Reproduced with permission from Gelfand E et al. J Allergy Clin Immunol 2002;110:85-95.¹⁴

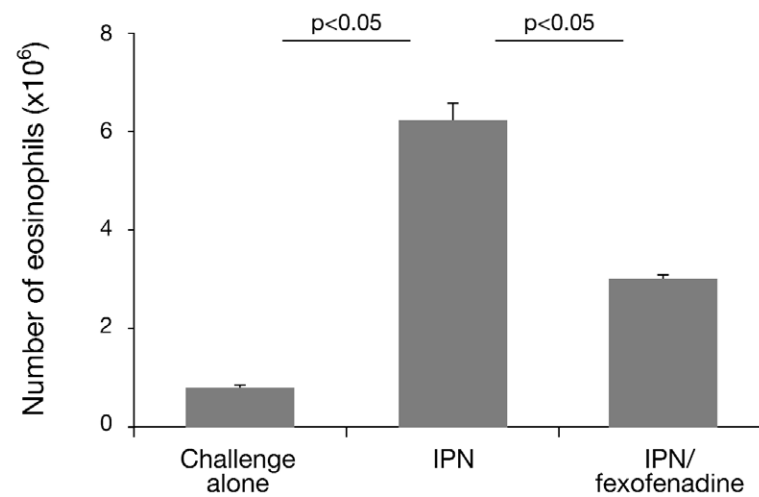


FIG 2. Effect of fexofenadine on eosinophil numbers in lung digests, $n = 12$ in each group.

TABLE I. Effect of fexofenadine on BAL cytokine levels

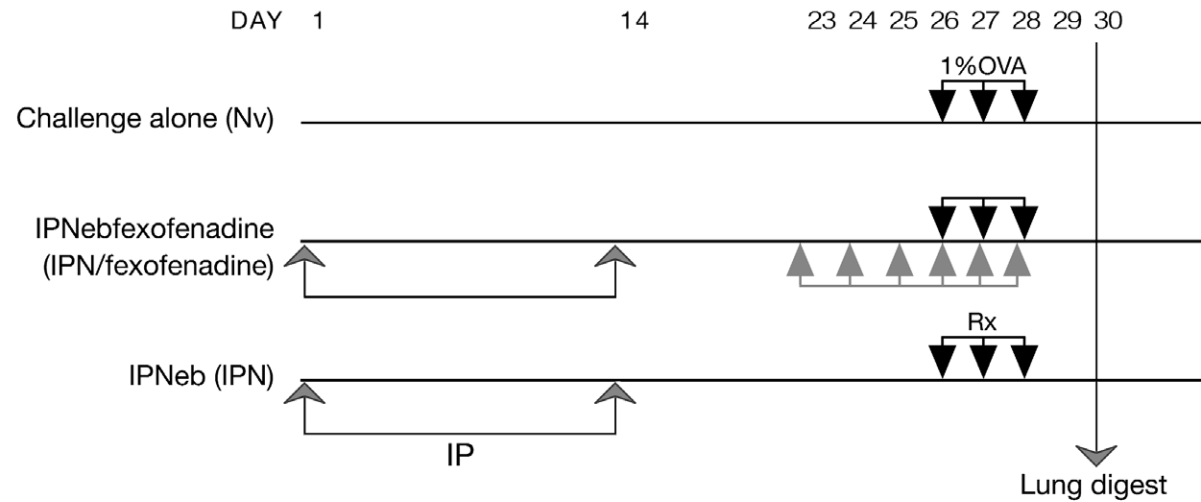
Group	pg/mL			
	IL-4	IL-5	IL-10	IFN- γ
Challenged alone	38 $\pm$ 9	120 $\pm$ 14	1560 $\pm$ 280	810 $\pm$ 37
Sensitized/challenged	141 $\pm$ 12*	283 $\pm$ 8*	680 $\pm$ 110*	720 $\pm$ 108
Sensitized/challenged/treated	48 $\pm$ 7†	175 $\pm$ 11†	1420 $\pm$ 180†	680 $\pm$ 61

BAL fluid was collected 2 days after the last airway challenge, and cytokine levels were measured by ELISA. $n = 10$ -12 for each group.

* $P < .05$, sensitized/challenged group compared with challenged alone group.

† $P < .05$, fexofenadine-treated group compared with nontreated group.

DONORS:



RECIPIENTS:

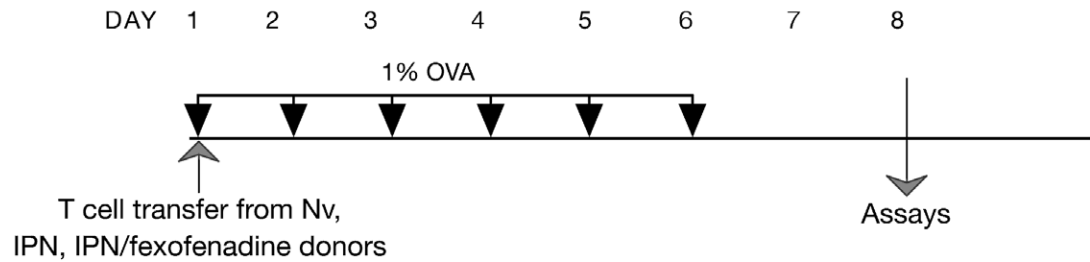


FIG 3. Adoptive transfer protocol. Reproduced with permission from Gelfand E et al. J Allergy Clin Immunol 2002;110:85-95.¹⁴

TABLE II. Effect of fexofenadine on allergen-specific serum IgE levels

Group	OVA-specific Ig (EU/mL)
Challenged alone	<10
Sensitized/challenged	410 ± 53*
Sensitized/challenged/treated	200 ± 31†

Serum was obtained from mice 48 hours after the last challenge, and antigen-specific IgE levels were assayed with ELISA. n = 10-12 in each group. EU, Endotoxin unit.
*P < .05, sensitized/challenged group compared with challenged alone group.
†P < .05, fexofenadine-treated group compared with nontreated group.

concentrations, but little change in IFN-γ levels. Treatment of the mice with fexofenadine prevented the increases in IL-4 and IL-5 and normalized the levels of IL-10; there was little effect on the concentrations of IFN-γ.

When serum levels of IgE and allergen-specific IgE were examined, fexofenadine was shown to significantly reduce serum concentrations of OVA-specific IgE (Table II) without affecting total serum IgE levels or allergen-specific IgG1.

Adoptive transfer

To identify the effector cells mediating the fexofenadine-induced suppression of allergen-induced responses, cells were isolated from the lungs of sensitized and challenged mice. T cells were purified and injected intravenously into naive recipients before allergen exposure (6 days of aerosolized allergen). The protocol is illustrated in Fig 3.¹⁴ Donor mice were sensitized and challenged to 1% OVA (intraperitoneal sensitization, nebulizer challenge) as in the primary protocol or challenged alone (Nv). One group of sensitized and challenged donor mice was also treated with fexofenadine on 6 consecutive days (days 23 to 28) at an oral dose of 5 mg twice a day. Forty-eight hours after the last challenge, lung digests were prepared and purified over nylon wool columns, resulting in a population of cells that were stained for CD3 (~85%). Approximately 1 × 10⁶ cells were injected intravenously into naive recipients that were then exposed to 6 consecutive days of 1% OVA-aerosolized challenge for 20 minutes each day. Airway function, inflammation, and cytokine levels were assessed 48 hours later.

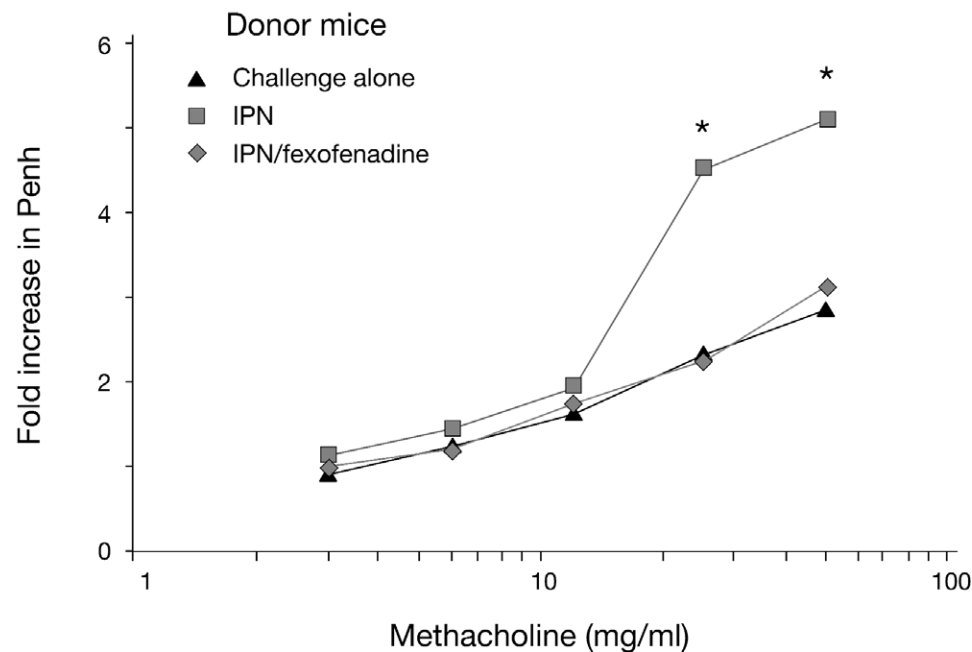


FIG 4. Effect of adoptive transfer of T cells on airway hyperresponsiveness, $n = 12$ in each group. $*P < .05$ comparing IPN donors with IPN/fexofenadine donors. Reproduced with permission Gelfand E et al. *J Allergy Clin Immunol* 2002;110:85-95.¹⁴

TABLE III. Effect of adoptive transfer on eosinophil and lymphocyte numbers in BAL fluid

Donor	Recipient	% Eosinophils	% Lymphocytes
Challenged	Challenged	<1	3.6
Sensitized/challenged	Challenged	9.8*	15.1*
Sensitized/challenged	Treated/challenged	2.2†	7.2†

Lung T cells were obtained from mice that were challenged alone, sensitized and challenged, or sensitized and challenged but treated for 6 days with fexofenadine (5 mg twice a day). Cells were injected intravenously into naive recipients that were then exposed to 6 consecutive days of aerosolized OVA (1%, 20 minutes each day). $n = 10$ -12 each group.

* $P < .05$, sensitized/challenged group compared with challenged alone group.

† $P < .05$, treated group compared with nontreated group.

Naive mice exposed to allergen alone via the airways for 6 consecutive days failed to develop any change in airway responsiveness or airway inflammation. Recipients of T cells from sensitized and challenged mice developed MCh dose-dependent increases in airway responsiveness as illustrated in Fig 4.¹⁴ In contrast, adoptive transfer of T cells from sensitized, challenged, fexofenadine-treated mice failed to increase airway responsiveness in the recipient, challenged mice. Airway responsiveness was similar to that in mice receiving T cells from mice that were challenged alone. Moreover, mice receiving cells from fexofenadine-treated mice did not show any increase in numbers of BAL eosinophils and had fewer BAL lymphocytes compared with mice receiving cells from sensitized and challenged but untreated animals.

In addition, adoptive transfer of cells from sensitized/challenged but treated mice did not demonstrate the marked increase in goblet cell numbers, IL-4 or IL-5 levels in the BAL fluid that were observed in recipients of

cells from untreated, sensitized, and challenged mice. For example, the number of PAS⁺ cells/mm basement membrane decreased from 84 cells to approximately 20 cells in recipients of cells from treated mice, and IL-4 and IL-5 levels were reduced by 70% and 33%, respectively (Table III).

DISCUSSION

These studies demonstrate the effectiveness of the second-generation antihistamine fexofenadine in modulating an allergic inflammatory response and AHR. Sensitization and challenge with allergen elicit a T_H2 cytokine response, characterized primarily by increased production of IL-4, IL-5, and IL-13.¹³ This is accompanied by, or is the downstream result of, the production of these cytokines, an increase in allergen-specific IgE, lung eosinophilia, BAL eosinophilia, and alterations in airway function with increased responsiveness to the inhaled bronchoconstrictor agent MCh. In the primary sensitiza-

tion and challenge model, oral administration of fexofenadine, after sensitization but before and during allergen challenge, prevented these changes. Thus, fexofenadine not only reduced T_H2 cytokine production but also prevented eosinophil accumulation in the BAL and lung, inhibited antigen-specific IgE production, and resulted in normal airway responsiveness. There was a greater reduction in tissue eosinophils than in the numbers of BAL eosinophils. The accumulation of tissue eosinophils precedes their appearance in the BAL fluid.¹³ Moreover, AHR is better correlated to the tissue eosinophil number than the BAL fluid number.¹³ Conceivably, the accumulation of these cells in the lung tissue is under a different regulatory pathway than the BAL fluid component, with fexofenadine differentially targeting these pathways.

Antihistamines in general have been associated with changes in cellular functions that might contribute to allergic inflammatory responses. These include effects on ICAM-1 expression and epithelial cell function. H_1 -receptor antagonists inhibit histamine-induced ICAM-1 and HLA-DR expression.⁶ Fexofenadine reduced the release of IL-8, RANTES, and soluble ICAM-1 from stimulated nasal epithelial cells, indicating modulatory effects on the release of pro-inflammatory mediators and adhesion molecules,¹⁵ and terfenadine also reduced ICAM-1 expression on nasal epithelial cells.¹⁶

Histamine downregulates several immunologic functions through its interaction with histamine receptors. For example, histamine stimulates $CD8^+$ T cells, causing inhibition of antibody synthesis, T-cell proliferation and cytotoxicity, and cytokine production.^{1,2} Histamine can also suppress IL-10 and IL-12 production.⁴ These effects were generally shown to be mediated via H_2 -receptors.¹⁷ However, recently histamine has been shown to activate lung macrophages, increasing the synthesis of IL-6, and these effects can be antagonized by fexofenadine.¹⁸ IL-6 is involved in mast cell proliferation and differentiation, T_H2 responses, and IgE production. It stimulates airway epithelial cells to release IL-8. Histamine has also been shown to affect (augment) antigen receptor-mediated immune responses of T and B cells via signals from H_1 -receptors. T- and B-cell function was reduced in H_1 -receptor-deficient mice.¹⁹ Furthermore, in the receptor-deficient mice, IL-2 and IFN- γ were significantly reduced, whereas IL-4 levels were enhanced.¹⁹

To further establish the effects of fexofenadine in modifying allergic responsiveness, T cells obtained from sensitized and challenged, fexofenadine-treated or untreated mice were adoptively transferred into naive recipients before airway challenge. Transfer of T cells from untreated, sensitized, and challenged mice conferred responsiveness in the recipients, including eosinophilic inflammation and AHR. This was not observed when cells from fexofenadine-treated mice were transferred. This suggests that in the donor mice, fexofenadine treatment reduced the ability of the transferred cells to enhance allergic responsiveness. It is unclear at present whether the effects were on the T cells themselves, or whether the effects were mediated

through intermediates to prevent their ability to augment allergen-induced eosinophilia and AHR. The transferred cells were highly purified; nonetheless, we cannot rule out the possibility that a non-T-cell component mediated these inhibitory effects.

This ability of fexofenadine, a selective H_1 -blocker, to inhibit many of the characteristic immune/inflammatory responses that follow allergen challenge of sensitized mice shows a potential opportunity for the use of H_1 -receptor antagonists in the treatment of allergic lung diseases.

SUMMARY

These data in an animal model of allergen-induced airway inflammation and AHR together with increasing in vitro data on cytokine/chemokine production and expression of key inflammatory cell adhesion molecules all support the potential role for fexofenadine in the treatment of some forms of allergic asthma. Additional studies appear warranted to further evaluate this potential.

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