Fexofenadine modulates T-cell function, preventing allergen-induced airway inflammation and hyperresponsiveness

Erwin W. Gelfand, MD, Zhi-Hua Cui, MD, PhD, Katsuyuki Takeda, MD, Arihiko Kanehiro, MD, and Anthony Joetham, BSc *Denver, Colo*

Background: Antihistamines have been evaluated for usefulness in the treatment of asthma for more than 50 years. Interest was limited until the introduction of newer compounds that were free of much of the dose-limiting sedation associated with the earlier drugs.
Objective: In a murine model of allergen-induced airway inflammation and hyperresponsiveness, the efficacy of an H1 receptor antagonist to prevent allergic inflammation and altered airway function was evaluated.
Methods: Mice were sensitized and challenged to an allergen, ovalbumin, which elicited marked airway and tissue eosinophilia and airway hyperresponsiveness. Fexofenadine was administered before challenge, and airway responsiveness to inhaled methacholine, airway and tissue eosinophilia, bronchoalveolar lavage fluid cytokine levels, and serum IgE levels were assayed. In a second group of experiments, sensitized and challenged mice were treated or not treated with fexofenadine before challenge. T cells were isolated from the lungs and adoptively transferred into naive recipients before exposure to limited airway allergen challenge, and lung function and inflammation were evaluated.
Results: Fexofenadine treatment of sensitized mice prevented the development of airway hyperresponsiveness in both the primary sensitization and challenge, as well as in the adoptive transfer experiments. These changes were accompanied by decreases in bronchoalveolar lavage and tissue eosinophilia, lymphocyte numbers, and T_H2 cytokine production.
Conclusion: The results demonstrate the efficacy of an H1 receptor antagonist in preventing allergen-induced alterations in pulmonary inflammation and airway function. The data support the evaluation of drugs such as fexofenadine in the treatment of allergic asthma. (J Allergy Clin Immunol 2002;110:85-95.)

Key words: *Fexofenadine, allergen, inflammation, airway hyperresponsiveness*

Abbreviations used
AHR: Airway hyperresponsiveness
APC: Antigen-presenting cell
BAL: Bronchoalveolar lavage
EPO: Eosinophil peroxidase
MNC: Mononuclear cell
OVA: Ovalbumin
PAS: Periodic acid–Schiff
Penh: Enhanced pause

Antihistamines have been evaluated for asthma therapy for almost 5 decades. Interest in this approach waned until the newer antihistamines were introduced, which were not limited by the sedation induced by earlier preparations. The possible bronchoprotective effects of the second- and third-generation compounds have been examined in a number of situations, including specific airway challenge with allergen,[1-5] exercise-induced bronchospasm,[6,7] and double-blind placebo-controlled trials in asthma.[8-10] Although not conclusive, it appears that there might be a role for these drugs in the treatment of some forms of asthma (eg, mild-to-moderate asthma).

The resurgence of interest in using antihistamines as part of asthma therapy is stimulated by several factors. First, histamine is an important mediator of inflammation and of airway smooth muscle contraction.[11-14] Second, antihistamines with specificity for the H1 receptor have been shown to be potent inhibitors of a number of pathways that contribute to inflammatory cell recruitment and accumulation.[15-17] Finally, the immediate actions of histamine on vascular endothelium, as well as on bronchial and vascular smooth muscle cells, are activities responsible for some of the acute symptoms of asthma. They are potentially antagonized by H1 receptor blockers and are mediated through the H1 receptor primarily.[18] There is also a growing appreciation of the "one airway, one disease" concept and the need to define agents that can effectively target both the upper and lower airways.

The pathophysiology of asthma is complex, with allergens triggering a cascade of cellular interactions and the release of cytokines and mediators resulting in acute and delayed (late) symptoms. Central in this cascade are antigen-presenting cells (APCs) and T lymphocytes, which together release important cytokines and chemokines

From the Program in Cell Biology, Department of Pediatrics, National Jewish Medical and Research Center, Denver.
Supported by National Institutes of Health grant HL-36577 and Aventis Pharmaceuticals.
Received for publication October 12, 2001; revised January 17, 2002; accepted for publication March 12, 2002.
Reprint requests: Erwin W. Gelfand, MD, National Jewish Medical and Research Center, 1400 Jackson St, Denver, CO 80206.

0091-6749/2002 $35.00 + 0 **1/83/124770**
doi:10.1067/mai.2002.124770

responsible for inflammatory cell accumulation. Because histamine has been shown to alter APC and T-cell function,[16,19-21] the ability of antihistamines to alter allergen-induced changes in an in vivo model was investigated. In the present study we evaluated the ability of fexofenadine, a potent third-generation antihistamine both specific for the H1 receptor and unencumbered by dose-limiting sedation, to interfere with allergen-induced airway hyperresponsiveness (AHR) and inflammation. In both a primary sensitization and challenge model and in an adoptive transfer model, fexofenadine demonstrated potency in preventing the allergen-induced development of airway inflammation and alterations in airway function.

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 subject to a protocol approved by the Institutional Animal Care and Use Committee of the National Jewish Medical and Research Center.

Experimental protocol

Mice 10 to 12 weeks of age were sensitized by means of intraperitoneal injection of 20 μg of OVA (Grade V; Sigma Chemical Co, St. Louis, Mo) emulsified in 2.25 mg of aluminum hydroxide (AlumImuject; Pierce, Rockford, Ill) in a total volume of 100 μL on days 1 and 14. Mice were challenged for 20 minutes through the airways daily with OVA (1% in saline) for 3 days (days 26, 27, and 28) by using ultrasonic nebulization (AeroSonic ultrasonic nebulizer; DeVilbiss, Sommerset, Pa; Fig 1, *A*).[22]

Airway responsiveness

Airway responsiveness was assessed with a single-chamber, whole-body plethysmograph obtained from Buxco (Troy, NY).[23] From the box-pressure signals, the phases of the respiratory cycle, tidal volumes, and enhanced pause (Penh) can be calculated. Penh correlates closely with pulmonary resistance measured by means of conventional 2-chamber plethysmography in ventilated animals.[23] In the plethysmograph mice were exposed for 3 minutes to nebulized PBS and subsequently to increasing concentrations of nebulized methacholine (in PBS). There were no significant differences in baseline Penh values between any of the treated or control groups.

Determination of cell numbers and cytokine levels in bronchoalveolar lavage fluid

Immediately after assessment of AHR, lungs were lavaged through the tracheal tube with HBSS (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) and differentiated by using standard hematologic procedures. Bronchoalveolar lavage (BAL) supernatants were collected and kept frozen at –70°C. Cytokine levels were measured by means of 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.[24]

Goblet cell and tissue eosinophil quantitation

Lungs were inflated through the tracheal tube with 2 mL of formalin and fixed in 10% formalin. Blocks of lung tissue were cut around the main bronchus and embedded in paraffin blocks. Five-micrometer-thick tissue sections 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 under light microscopy.

The number of goblet cells in the airway epithelium of all central airways present in all lobes was counted by using at least 20 sections and measuring the length of epithelium defined as basement membrane and the luminal area with an NIH Image analysis system. Mucus-containing cells were expressed as the number of goblet cells per 100 μm of epithelium.[25]

Eosinophil peroxidase

In a separate series of studies, levels of eosinophil peroxidase (EPO) in BAL fluid supernatants were determined as previously described.[26] 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 challenge from mice that were or were not treated with fexofenadine (Fig 1, *B*). 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 on 6 consecutive days.

Drug treatment

The drug fexofenadine (terfenadine acid metabolite) was suspended in saline and administered by means of gavage (without anesthesia) at a dose of 2.5 mg twice daily beginning 3 days before allergen challenge and on each of the days of allergen challenge (Fig 1).

Data analysis

All results are expressed as means ± SE. ANOVA was used to determine the levels of difference among all groups. Pairs of groups were compared with the unpaired 2-tailed Student *t* test or the Tukey-Kramer honest significant difference test, with a *P* value for significance set at .05.

RESULTS

Fexofenadine treatment prevents AHR and airway eosinophilia in a primary challenge model

We have previously shown, in this primary sensitization and challenge model, that airway challenge of sensitized mice results in AHR and airway eosinophilia, both peaking 48 hours after the last of the 3 airway challenges.[27,28] In the present experiments sensitization and challenge (Fig 1, *A*) of mice to OVA resulted in an increase in airway responsiveness to inhaled methacholine (increased Penh) in a dose-dependent manner (Fig 2, *A*). Mice challenged alone (nonsensitized) did not have AHR. Sensitization and challenge also elicited a 35% increase in cell number compared with that of mice challenged alone or sensitized alone (data not shown). In

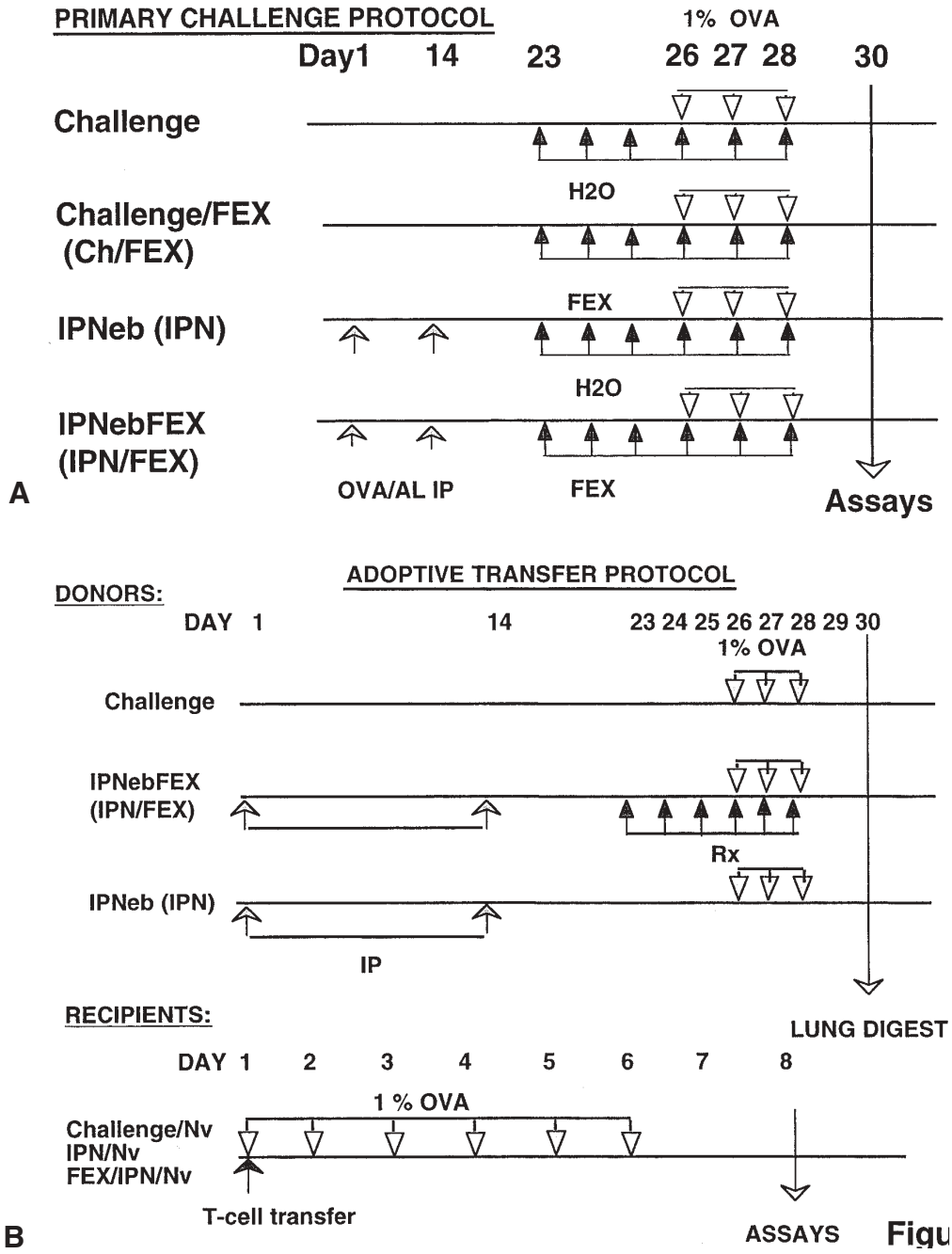

FIG 1. Protocols for eliciting airway inflammation and hyperresponsiveness. **A,** Primary sensitization and challenge protocol. On days 1 and 14, mice were sensitized by means of intraperitoneal injections of OVA-alum. On days 26, 27, and 28, airway challenge was carried out. Fexofenadine or the vehicle (H_2O) was administered twice daily before and on the days of challenge. Assays were carried out 48 hours after the last challenge. **B,** Adoptive transfer protocol. Mice were sensitized and challenged as in Fig 1, *A*; lung digests were prepared on day 30; and T cells were isolated. T cells were transferred into naive recipients who were then exposed to 6 daily challenges. Assays were carried out 48 hours after the last challenge. *FEX,* Fexofenadine; *IPNeb (IPN),* sensitized and challenged; *Nv,* naive.

parallel to changes in airway function, sensitized and challenged mice had significant airway (BAL fluid; Fig 2, *B*) and tissue (Fig 2, *C*) eosinophilia; the eosinophils comprised 58% of the cells in the BAL fluid. Treatment of the mice with fexofenadine after sensitization but before and during challenge significantly inhibited the development of AHR (Fig 2, *A*). The Penh responses to increasing concentrations of inhaled methacholine were similar to those in control animals. Fexofenadine treatment reduced the number of BAL eosinophils (Fig 2, *B*),

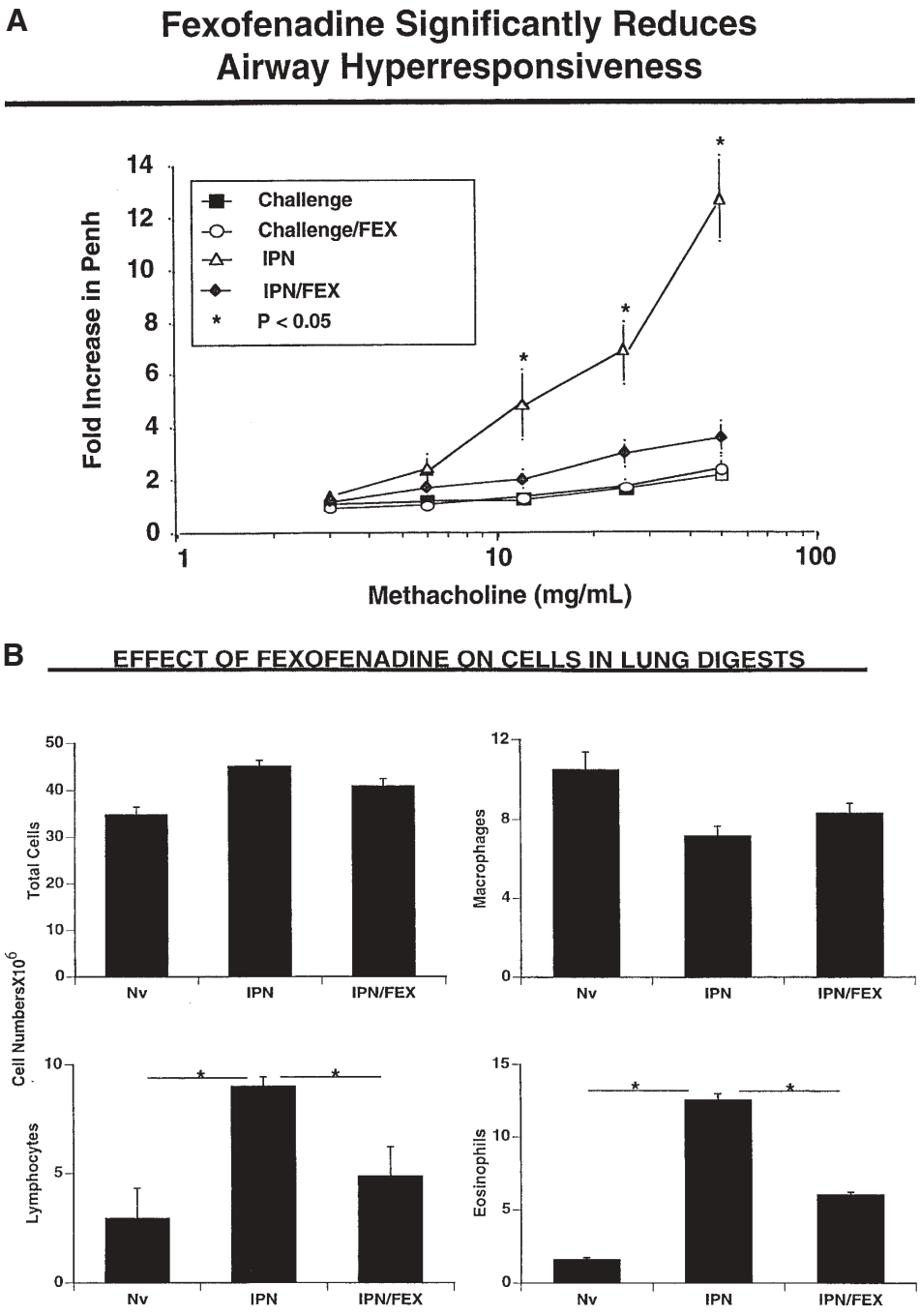

FIG 2. Effects of fexofenadine (n = 12 in each group; $*P < .05$). **A,** Fexofenadine *(FEX)* reduces AHR. Airway responsiveness (Penh) to inhaled methacholine was monitored in mice challenged alone or after sensitization and challenge *(IPNeb, IPN),* as illustrated in Fig 1, *A.* Parallel groups received fexofenadine. **B,** BAL fluid cell numbers. **C,** Tissue cell numbers. **D,** Eosinophil peroxidase levels in BAL fluid. Fig 2 continued on next page.

as well as the numbers of tissue eosinophils, which were reduced by more than 50% (Fig 2, *C*). Both BAL and tissue lymphocyte numbers were reduced as well (Figs 2, *B* and *C*). Levels of BAL EPO (Fig 2, *D*) were significantly reduced by treatment with fexofenadine, suggesting perhaps some inhibition of eosinophil activation and release of mediators.

Several groups have shown a skewing toward a T_H2-like cytokine profile in sensitized and challenged mice.[26,29] The supernatants of BAL fluid were analyzed for levels of IL-4, IL-5, IL-10, and IFN-γ. Sensitization and challenge resulted in increases in IL-4 and IL-5 levels and, at the same time, a decrease in IL-10 but no change in IFN-γ levels when compared with mice chal-

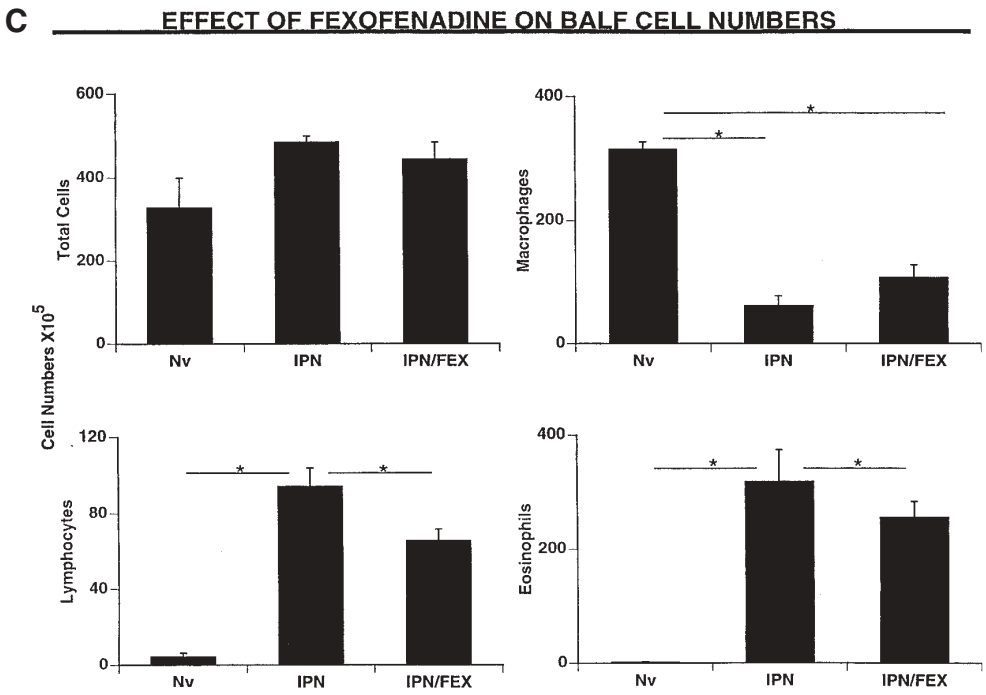

FIG 2. Continued.

lenged alone (Fig 3) or naive mice (data not shown). Treatment with fexofenadine prevented the increases in IL-4 and IL-5 levels but normalized the levels of IL-10; IFN-γ levels were little affected.

The consequences of allergen sensitization and challenge resulting in airway eosinophilia and the increases in IL-4 and IL-5 levels all point to induction of a T_H2-like response, with fexofenadine treatment preventing this T_H2-driven response. This was supported by examination of allergen-specific IgE levels in the serum. Sensitization and challenge with OVA induced a significant increase in serum OVA-specific IgE levels (Fig 4). Treatment with fexofenadine prevented these increases in serum anti-OVA IgE levels.

Effect of fexofenadine on the function of adoptively transferred T cells

On the basis of these data in the primary challenge model and the inhibition of the induction of T_H2 responses after allergen sensitization and challenge, we began to

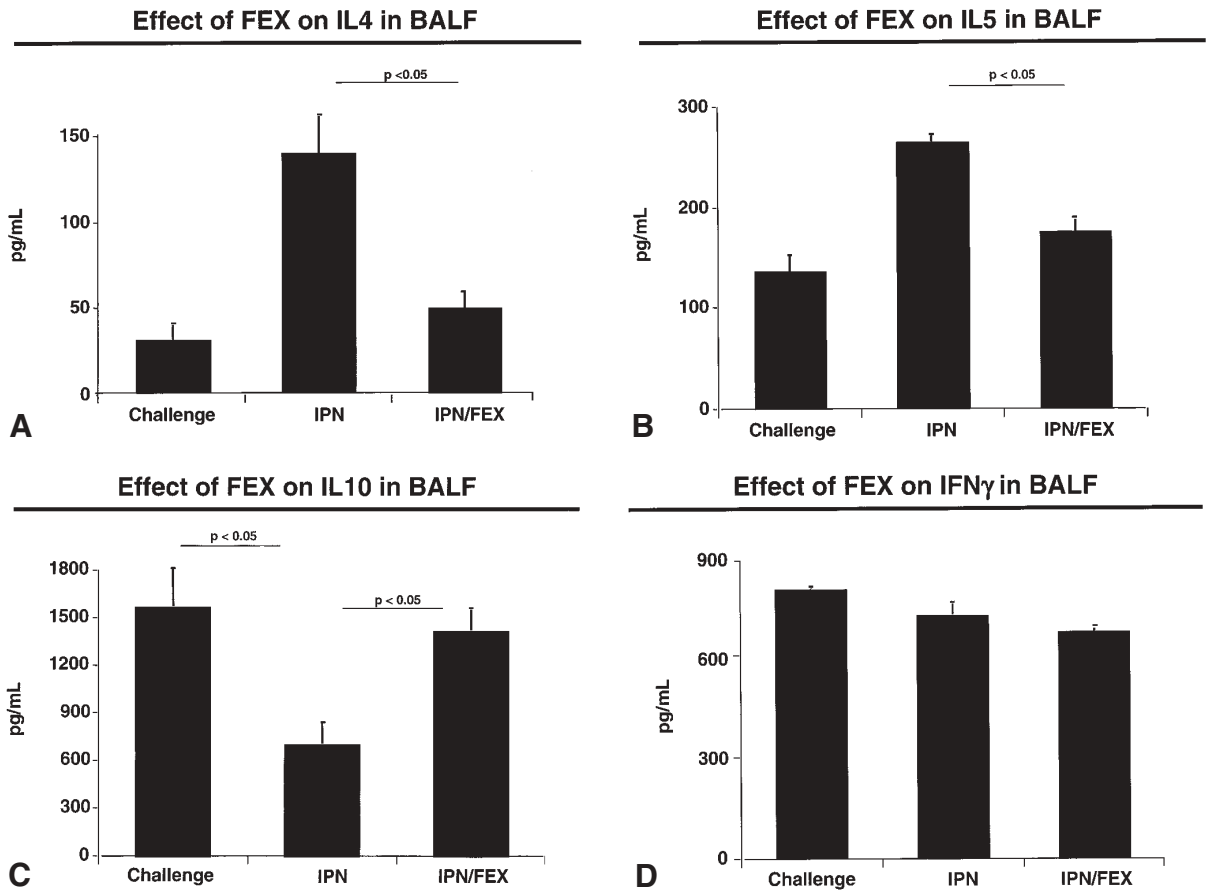

FIG 3. Effect of fexofenadine on BAL fluid cytokine levels. All groups are as in Fig 2: **A,** IL-4; **B,** IL-5; **C,** IL-10; **D,** IFN-γ. *FEX,* Fexofenadine; *IPNeb, IPN,* sensitization and challenge.

define the nature of the effector cells mediating fexofenadine-induced suppression. In preliminary experiments in the adoptive transfer protocol (Fig 1, *B*), mononuclear cells (MNCs) were isolated from the lungs of sensitized and challenged mice and administered intravenously into naive recipients before 6 daily exposures to aerosolized OVA. Initial results indicated that MNCs from sensitized-challenged mice were capable of triggering AHR in the recipient mice exposed to allergen through the airways alone; exposure to allergen alone for 6 days did not induce AHR nor did transfer of MNCs from nonsensitized mice. Moreover, transfer of MNCs from sensitized and challenged mice that had been treated with fexofenadine also failed to induce AHR.

Lung T cells were isolated to approximately 85% to 90% purity before transfer to further define the effector cells. In vitro culture of these lung T cells with OVA demonstrated a marked decrease in amounts of IL-4 and IL-5 produced when T cells from fexofenadine-treated mice were compared with T cells from nontreated mice (180 vs 40 pg/mL IL-4; 510 vs 310 pg/mL IL-5). Adoptive transfer of lung T cells (1×10^6 per mouse) from sensitized and challenged mice induced development of AHR in recipient mice to inhaled methacholine in a dose-dependent manner (Fig 5). The level of AHR that develops is lower than that after the primary challenge because no primary sensitization is carried out.[25] When T cells were isolated from fexofenadine-treated mice and transferred, there was no induction of AHR; the methacholine dose-response curve was identical to that in mice simply exposed to allergen inhalation after transfer of cells from mice challenged alone.

Fig 6 illustrates the changes in BAL cell composition after transfer of lung T cells. Adoptive transfer of lung T cells from sensitized-challenged mice resulted in a significant increase in BAL eosinophil numbers in recipient mice challenged on 6 consecutive days. BAL lymphocyte numbers were also increased in these recipient mice. The transfer of lung T cells from fexofenadine-treated, fexofenadine-sensitized, and fexofenadine-challenged mice failed to induce a comparative airway eosinophilia or the increase in airway lymphocyte numbers after the 6 daily challenges. Total cell numbers and macrophage numbers were relatively unchanged.

Adoptive transfer of lung T cells from the sensitized-challenged mice induced significant increases in BAL fluid IL-4 and IL-5 levels in recipient mice (Fig 7). These changes were not observed when fexofenadine-treated mice served as donors; IL-4 levels were little different from control mice (OVA exposure alone; Fig 7, *A*), and

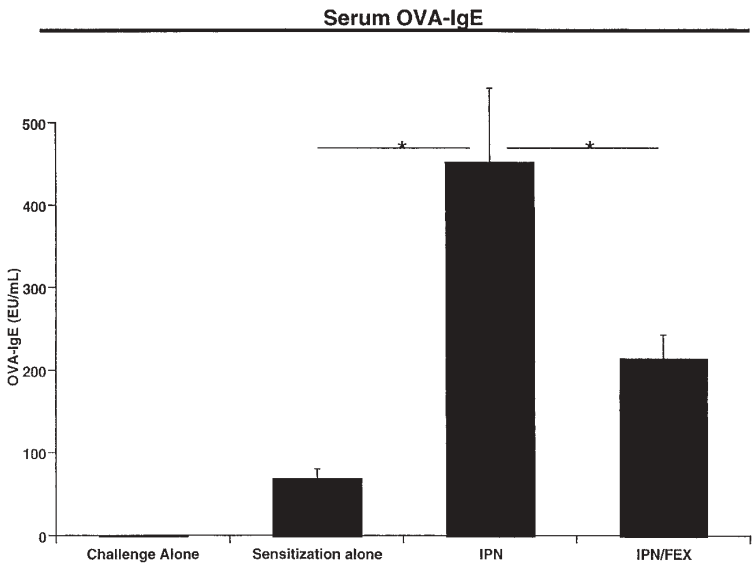

FIG 4. Effect of fexofenadine levels on serum OVA-specific IgE levels. Serum was obtained 48 hours after the last airway challenge and assayed for specific antibody levels by means of ELISA (n = 12 in each group). *IPN,* sensitization and challenge; *FEX,* fexofenadine.

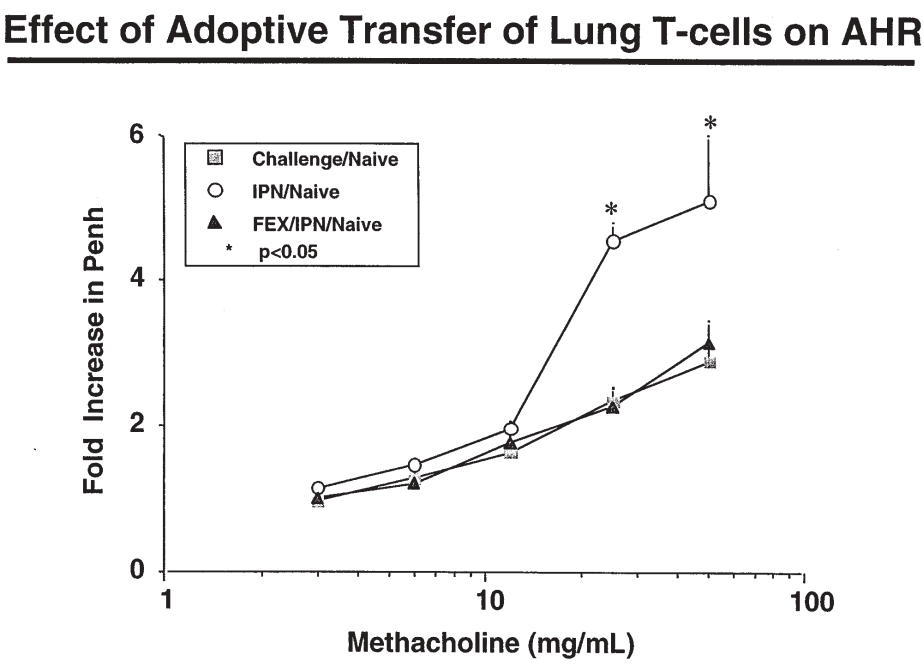

FIG 5. Effect of fexofenadine on the ability of adoptively transferred T cells to induce AHR. T cells were isolated from lungs of treated or untreated sensitized and challenged mice or mice challenged alone, as described in Fig 1, *B*, and transferred into naive recipients who were then exposed to 6 consecutive days of airway challenge. *Challenge/naive,* Mice receiving cells from donor mice that were challenged alone; *IPN/naive,* mice receiving cells from donor mice that were sensitized and challenged; *Fex/IPN/naive,* mice receiving cells from fexofenadine-treated donor mice that were sensitized and challenged (n = 12 in each group; $*P < .05$).

IL-5 levels were significantly lower than in mice receiving T cells from nontreated mice (Fig 7, *B*). IL-10 and IFN-γ levels were little changed under any of the conditions (data not shown).

Adoptive transfer of T cells from sensitized/challenged mice induced a marked increase in the number of PAS^+ cells in the tissue (Fig 8). This increase was markedly reduced in recipients of cells from fexofenadine-treated mice.

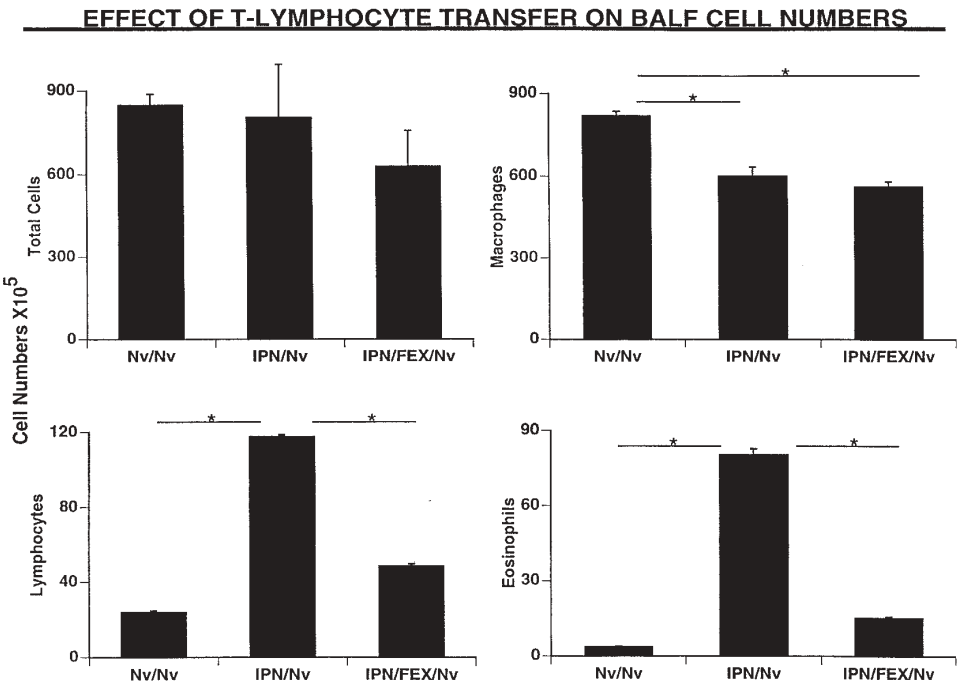

FIG 6. Cell composition in BAL fluid after adoptive transfer of T cells and airway challenge. *Challenge/naive,* Mice receiving cells from donor mice that were challenged alone; *IPN/naive,* mice receiving cells from donor mice that were sensitized and challenged; *Fex/IPN/naive,* mice receiving cells from fexofenadine-treated donor mice that were sensitized and challenged (n = 12 in each group; $*P < .05$).

There was little, if any, induction of OVA-specific IgE or IgG1 after transfer of lung T cells with this protocol (data not shown).

DISCUSSION

Histamine is an important chemical mediator of allergic inflammation. Indeed, histamine can trigger virtually all of the pathologic responses important in asthma, including vasodilation, edema, mucus hypersecretion, and smooth muscle contraction.[11-14] If there is a basis for histamine-driven asthma and potential intervention with antihistamines, then histamine must demonstrate a variety of proinflammatory effects. This does appear to be the case on a variety of cell types important in the initiation of the allergic-inflammatory cascade. Dendritic cells are potent APCs[30,31] capable of releasing numerous cytokines and chemokines.[32,33] Histamine induced the expression of an important accessory molecule, CD86, in a dose-dependent fashion.[16] Similarly, histamine induced the expression of the key adhesion molecules E-selectin, intercellular adhesion molecule 1, and leukocyte function–associated molecule 1 in a dose-dependent manner on epithelial cells, endothelial cells, and fibroblasts.[12,34] Furthermore, histamine can trigger the production of IL-6 and IL-8 by endothelial cells[35,36]; production of IL-1, IL-6, IL-18, and IFN-γ but suppression of IL-12 by PBMCs[37-40]; induction of IL-10 and IL-6 from macrophages[17,41]; and induction of IL-1β, IL-6, IL-8, and MCP-1β in dendritic cells.[16] Histamine can also stimulate CD8 T cells, inhibiting antibody synthesis, T-cell proliferation, and T cell–mediated cytotoxicity.[17-19] Overall, the immunoregulatory effects appear to deviate the immune response to a T_H2-like response.

Histamine is believed to exert these effects through interactions with H1, H2, and H3 receptors.[12,14] The proinflammatory and immunomodulatory effects were thought to be mediated through H2 receptors to a large extent.[42] However, it is now apparent that many of the effects of histamine on, for example, intercellular adhesion molecule 1 expression, CD86 expression, and chemokine and cytokine production are indeed mediated through H1 receptors and are effectively blocked by H1 receptor antagonists.[16-18] Epithelial cells from nasal biopsy specimens from patients with seasonal allergic rhinitis were cultured with fexofenadine. In these studies fexofenadine blocked eosinophil-induced changes in electrical resistance and release of proinflammatory mediators from these cells and significantly attenuated both eosinophil chemotaxis and adherence to endothelial cells induced by conditioned medium from these nasal epithelial cultures.[43]

Of interest, 2 articles were recently published on H1 receptor–deficient mice. In one article,[44] H1 receptor–deficient mice had low T- and B-cell proliferative responses and lower IFN-γ production, whereas IL-4 production was enhanced. In a second article,[45] H1 receptor–deficient mice were shown to release less IFN-γ but more IL-4 and IL-13 after T-cell stimulation, but again, the differences were small, indeed much smaller than those in H2 receptor–deficient cells. Thus although both articles suggest that histamine might stimulate T_H1 cells

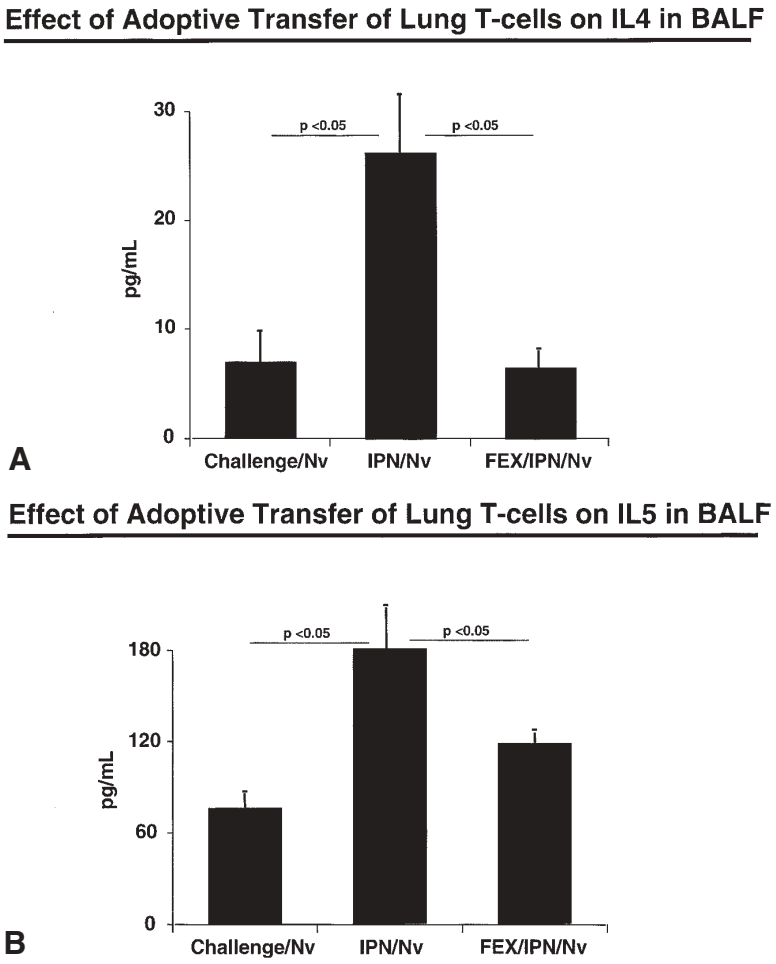

FIG 7. Effect of fexofenadine on BAL fluid cytokine levels after adoptive transfer of T cells and airway challenge: **A,** IL-4; **B,** IL-5 (n = 12 in each group). *Challenge/Nv,* Mice receiving cells from donor mice that were challenged alone; *IPN/Nv,* mice receiving cells from donor mice that were sensitized and challenged; *Fex/IPN/Nv,* mice receiving cells from fexofenadine-treated donor mice that were sensitized and challenged.

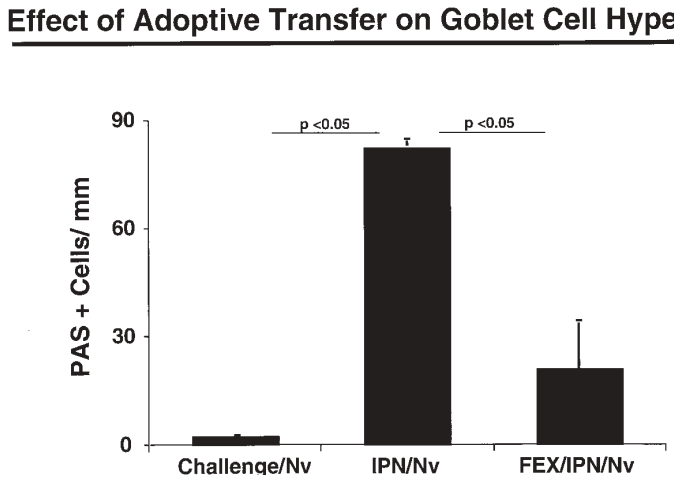

FIG 8. Fexofenadine treatment of donor mice prevents goblet cell hyperplasia. After adoptive transfer of T cells and airway challenge, lung tissues were prepared and stained with PAS. Data are expressed as number of PAS^+ cells per millimeter basement membrane (n = 12 in each group). *Challenge/Nv,* Mice receiving cells from donor mice that were challenged alone; *IPN/Nv,* mice receiving cells from donor mice that were sensitized and challenged; *Fex/IPN/Nv,* mice receiving cells from fexofenadine-treated donor mice that were sensitized and challenged.

or downregulate T_H2 cells in H1 receptor–deficient cells, at least in vitro, the results are not as clear cut as with H2 receptor–deficient cells. Furthermore, these studies were carried out in C57BL/6 mice, a strain that differs significantly from BALB/c in the response to allergen stimulation.[46] In addition, responses in H1 receptor–deficient mice might be different than interfering with or blocking the H1 receptor itself.

In the studies presented here, allergen sensitization and challenge triggered a marked alteration in airway function to inhaled methacholine and a skewing toward a T_H2 response characterized by airway and tissue eosinophilia, increases in IL-4 and IL-5 production, goblet cell hyperplasia, and increases in serum levels of OVA-specific IgE. We and others have previously shown an association between development of AHR and a dependence on eosinophilia, IL-4, and IL-5.[26,29] Virtually all of the T_H2 responses were abrogated if the sensitized mice were treated with fexofenadine before allergen challenge. Moreover, fexofenadine resulted in a normalization of BAL fluid IL-10 levels. IL-10 has been implicated in both causative and protective roles described in the regulation of allergic responses. Levels have been shown to be lower in sensitized and challenged mice, as demonstrated here and in allergic asthmatic subjects.[47] These changes in cytokine levels might result from several factors, including the prevention of histamine-mediated effects on T-cell recruitment, activation as a result of decreased adhesion molecule expression, or both[15]; chemokine release[16]; accessory molecule expression[16]; or changes in the cytokine milieu.[17] Lymphocyte numbers in BAL fluid and tissue were reduced after fexofenadine treatment. In addition, fexofenadine treatment reduced both BAL fluid and tissue eosinophil numbers. We previously showed a closer correlation between AHR and tissue eosinophil numbers than eosinophils in the BAL fluid.[26] In addition, if EPO is a marker of eosinophil activation, fexofenadine treatment also reduced EPO levels in the BAL fluid.

Thus it appeared that fexofenadine prevented the series of T_H2 responses that follow allergen sensitization and challenge. This was further confirmed in the adoptive transfer experiments. Here T cells isolated from the lungs of sensitized and challenged mice induced a T_H2 response and AHR in recipient mice exposed to limited allergen through the airways. After transfer of T cells, recipient mice had AHR, eosinophilia, increased IL-4 and IL-5 levels, and mucin hyperproduction–goblet cell hyperplasia. If cells were transferred from fexofenadine-treated sensitized and challenged mice, these changes were either not observed or markedly decreased. Lymphocyte numbers in the lungs of recipient mice were also lower when donor mice were treated. These results suggest that fexofenadine treatment modified T-cell function in the donor mice and possibly recruitment in recipient mice, preventing their ability to transfer T_H2 responsiveness. When cultured with OVA, the lung T cells from fexofenadine-treated mice produced lower amounts of IL-4 and IL-5 than T cells from nontreated animals. Whether this was the result of a direct effect of the drug on T cells or the modulation-prevention of induction of T_H2 responsiveness by another means or intermediates is unclear at present.

Given the specificity of fexofenadine for the H1 receptor,[48] these data add to the growing body of evidence that H1 receptor–mediated events can play a significant role in proinflammatory and allergic responses. What also emerges is that to be effective in vitro or in vivo, in animal models or in human asthma,[8-10,15-17,43] higher concentrations of the H1 receptor antagonists appear to be required than current guidelines recommend. These antagonists have been shown to demonstrate specificity for the H1 receptor. In the context of specificity, the results could be related to specific properties of the H1 receptors on certain immune-inflammatory cells, which could be modified to require higher concentrations of the antagonist for effect. Nonetheless, because of the high concentrations of the antagonist used, direct specificity of the effects does remain to be demonstrated. Moreover, it is unclear whether all drugs in this class are equally effective. One possibility currently being explored is that fexofenadine interacts with the newly identified H4 receptor, a receptor that adds new potential for the role of histamine in the immune-inflammatory system.[49]

Cumulatively, these and other results identify potential avenues for investigation of the role of H1 receptor antagonists in several allergic diseases, including asthma. As suggested by the in vivo and in vitro data, if higher concentrations of the drug than are currently used will be required for these benefits, then those drugs with wide therapeutic windows and absence of dose-limiting sedation should be aggressively investigated.

We thank Diana Nabighian for assistance in preparation of the manuscript.

REFERENCES

1. Town GI, Holgate ST. Comparison of the effect of loratadine on the airway and skin responses to histamine, methacholine, and allergen in subjects with asthma. J Allergy Clin Immunol 1990;86:886-93.
2. Eiser N. The effect of a beta 2-adrenergic agonist and a histamine H1-receptor antagonist on the late asthmatic response to inhaled antigen. Respir Med 1991;85:393-9.
3. Chan TB, Shelton DM, Eiser NM. Effect of an oral H1-receptor antagonist, terfenadine, on antigen-induced asthma. Br J Dis Chest 1986;80:375-84.
4. Bentley AM, Walker S, Hanotte F, De Vos C, Durham SR. A comparison of the effects of oral cetirizine and inhaled beclomethasone on early and late asthmatic responses to allergen and the associated increase in airways hyperresponsiveness. Clin Exp Allergy 1996;26:909-17.
5. Hamid M, Rafferty P, Holgate ST. The inhibitory effect of terfenadine and flurbiprofen on early and late-phase bronchoconstriction following allergen challenge in atopic asthma. Clin Exp Allergy 1990;20:261-7.
6. Ghosh SK, De Vos C, McIlroy I, Patel KR. Effect of cetirizine on exercise induced asthma. Thorax 1991;46:242-4.
7. Clee MD, Ingram CG, Reid PC, Robertson AS. The effect of astemizole on exercise-induced asthma. Br J Dis Chest 1984;78:180-3.
8. Taytard A, Beaumont D, Pujet PC, Sapene M, Lewis PJ. Treatment of bronchial asthma with terfenadine; a randomized controlled trial. Br J Clin Pharmacol 1987;24:743-6.
9. Rafferty P, Jackson L, Smith R, Holgate ST. Terfenadine, a potent histamine H1-receptor antagonist in the treatment of grass pollen sensitive asthma. Br J Clin Pharmacol 1990;30:229-35.
10. Wood-Baker R, Smith R, Holgate ST. A double-blind, placebo controlled

study of the effect of the specific histamine H1-receptor antagonist, terfenadine, in chronic severe asthma. Br J Clin Pharmacol 1995;39:371-5.
11. White M. Mediators of inflammation and the inflammatory process. J Allergy Clin Immunol 1999;103:378-81.
12. Bachert C. Histamine: a major role in allergy? Clin Exp Allergy 1998;28:15-9.
13. Naclerio R. Clinical manifestations of the release of histamine and other inflammatory mediators. J Allergy Clin Immunol 1999;103:382-5.
14. Novak I, Falus A. Molecular biology and role of histamine in physiological and pathological reactions: a review. Acta Biol Hung 1997;48:385-94.
15. Paolieri F, Battifora M, Riccio AM, Bertolini C, Cutolo M, Bloom M, et al. Terfenadine and fexofenadine reduce in vitro ICAM-1 expression on human continuous cell lines. Ann Allergy Asthma Immunol 1998;81:601-7.
16. Caron G, Delneste Y, Roelandts E, Duez C, Herbault N, Magistrelli G, et al. Histamine induces CD86 expression and chemokine production by human immature dendritic cells. J Immunol 2001;166:6000-6.
17. Triggiani M, Gentile M, Secondo A, Granata F, Oriente A, Taglialatela M, et al. Histamine induces exocytosis and IL-6 production from human lung macrophages through interaction with H1 receptors. J Immunol 2001;166:4083-91.
18. Simons REF, Simons KJ. Antihistamines. In: Middleton F Jr, Reed CE, Ellis EF, et al, editors. Allergy: principles and practice. 4th ed. St. Louis: Mosby Year Book; 1993. p. 856-78.
19. Lichtenstein LM, Henney CS. Adenylate cyclase-linked hormone receptors: an important mechanism for the immunoregulation of leukocytes. Prog Immunol 1976;2:73-83.
20. Melmon KL, Weinstein Y, Bourne HR, Poon T, Shearer G, Castagnoli N. The pharmacological effects of conjugates of pharmacologically active amines to complex or simple carriers: a new class of drug. Mol Pharmacol 1976;12:701-10.
21. Beer DJ, Matloff SM, Rocklin RE. The influence of histamine on immune and inflammatory responses. Adv Immunol 1984;35:209-68.
22. Makela MJ, Kanehiro A, Borish L, Dakhama A, Loader J, Joetham A, et al. Interleukin-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation following allergic sensitization. Proc Natl Acad Sci U S A 2000;97:6007-12.
23. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. Am J Respir Crit Care Med 1997;156:766-75.
24. Kanehiro A, Ikemura T, Makela MJ, Joetham A, Dakhama A, Gelfand EW. Inhibition of phosphodiesterase 4 attenuates airway hyperresponsiveness and airway inflammation in a model of secondary allergen challenge. Am J Respir Crit Care Med 2001;163:173-84.
25. Tomkinson A, Kanehiro A, Rabinovitch N, Joetham A, Cieslewicz G, Gelfand EW. The failure of STAT6 deficient mice to develop airway eosinophilia and airway hyperresponsiveness is overcome by IL-5. Am J Respir Crit Care Med 1999;160:1283-91.
26. Tomkinson A, Cieslewicz G, Duez C, Larson KA, Lee JJ, Gelfand EW. Temporal association between airway hyperresponsiveness and airways eosinophilia in ovalbumin sensitized mice. Am J Respir Crit Care Med 2001;163:721-30.
27. Hamelmann E, Takeda K, Haczku A, Cieslewicz G, Shultz L, Hamid Q, et al. IL-5 but not IgE reconstitutes airway inflammation and airway hyperresponsiveness in IL-4 deficient mice. Am J Respir Cell Mol Biol 2000;23:327-34.
28. Takeda K, Hamelmann E, Joetham A, Shultz L, Larsen GL, Irvin CG, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell deficient mice. J Exp Med 1997;186:449-54.
29. Wills-Karp M. IL-12/IL-13 axis is allergic asthma. J Allergy Clin Immunol 2001;107:9-18.
30. Cella M, Sallusto F, Lanzavecchia A. Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol 1997;9:10-6.
31. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245-52.
32. de Saint-Vis B, Fugier-Vivier I, Massacrier C, Gaillard C, Vanbervielt B, Ait-Yahia S, et al. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol 1998;160:1666-76.
33. Sallusto F, Palermo B, Lenig D, Miettinen M, Matikainen S, Julkunen I, et al. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur J Immunol 1999;29:1617-25.
34. Miki I, Kusano S, Ohta N, Hanai M, Otoshi S, Masaki S, et al. Histamine enhanced the TNF-α-induced expression of E-selectin and ICAM-1 on vascular endothelial cells. Cell Immunol 1996;171:285-8.
35. Jeannin P, Delneste Y, Gosset P, Molet S, Lassalle P, Hamid Q, et al. Histamine induces interleukin-8 secretion by endothelial cells. Blood 1994;84:2229-33.
36. Delneste Y, Lassalle P, Jeannin P, Joseph M, Tonnel AB, Gosset P. Histamine induces IL-6 production by human endothelial cells. Clin Exp Immunol 1994;98:344-9.
37. Vannier E, Dinarello CA. Histamine enhances interleukin (IL)-1-induced IL-1 gene expression and protein synthesis via H2 receptors in peripheral blood mononuclear cells: comparison with IL-1 receptor antagonist. J Clin Invest 1993;92:281-7.
38. Mor S, Nagler A, Barak V, Handzel ZT, Geller-Gernstein C, Fabian I. Histamine enhances granulocyte-macrophage colony-stimulating factor and interleukin-6 production by human peripheral blood mononuclear cells. J Leukoc Biol 1995;58:445-50.
39. Kohka H, Nishibori M, Iwagaki H, Nakaya N, Yoshino T, Kobashi K, et al. Histamine is a potent inducer of IL-18 and IFN-γ in human peripheral blood mononuclear cells. J Immunol 2000;164:6640-6.
40. van der Pouw Kraan T, Snijders CA, Beoije LC, de Groot ER, Alewijnse AE, Leurs R, et al. Histamine inhibits the production of interleukin-12 through interaction with H2 receptors. J Clin Invest 1998;102:1866-73.
41. Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP, Wilder RL. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. J Immunol 1998;161:2586-93.
42. Hill SJ. Distribution, properties and functional characteristics of three classes of histamine receptor. Pharmacol Rev 1990;42:45-83.
43. Abedelaziz MM, Devalia JL, Khair OA, Bayram H, Prior AJ, Davies RJ. Effect of fexofenadine on eosinophil-induced changes in epithelial permeability and cytokine release from nasal epithelial cells of patients with seasonal allergic rhinitis. J Allergy Clin Immunol 1998;101:410-20.
44. Banu Y, Watanabe T. Augmentation of antigen receptor-mediated responses by histamine H1 receptor signaling. J Exp Med 1999;189:673-82.
45. Jutel M, Wantanabe T, Klunker S, Akdis M, Thomet OAR, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature 2001;413:420-425.
46. Takeda K, Haczku A, Lee JJ, Irvin CG, Gelfand EW. Strain dependence of allergen driven airway hyperresponsiveness may reflect differences in eosinophil localization in the lung. Am J Physiol 2001;281:L394-402.
47. Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal and asthmatic subjects. J Allergy Clin Immunol 1996;97:1288-96.
48. Slater JW, Zechnich AD, Haxby DG. Second generation antihistamines. A comparative review. Drugs 1999;57:31-47.
49. Liu C, Wilson SJ, Kuei C, Lovenberg TW. Comparison of human, mouse, rat, and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. J Pharmacol 2001;299:121-30.