Desloratadine inhibits allergen-induced airway inflammation and bronchial hyperresponsiveness and alters T-cell responses in murine models of asthma

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Background: Histamine elicits many features of immediate hypersensitivity reactions. Recent evidence indicates that H1 receptors modulate immune responses to antigens. Desloratadine (DL), a new, long-acting, H1 receptor antagonist, has both a potent antihistaminic function and anti-inflammatory properties.
Objective: We sought to evaluate the effect of DL on allergic-airway responses in mice after inhalation of the naturally occurring aeroallergen *Aspergillus fumigatus* (*Af*) and to examine the effects of DL on specific immune responses to a defined protein antigen with the use of an ovalbumin (OVA) model of asthma.
Methods: Mice were subjected either to repeated, intranasal application of *Af* extract or to intraperitoneal immunization with OVA, followed by inhalation challenge. DL or a control fluid was given daily throughout the sensitization process. Immunoglobulin E (IgE) levels, bronchoalveolar lavage-fluid cytokines and cytology, lung histology, and physiologic responses to methacholine were assessed in the allergen-treated mice. Anti-OVA IgE responses and OVA-driven T-cell cytokine production were examined.
Results: Treatment with DL did not impair IgE production but did inhibit bronchial inflammation and bronchial hyperresponsiveness in both *Af*- and OVA-treated mice. This inhibition required that DL be administered concurrently with allergen sensitization, indicating that the attenuation of bronchial hyperresponsiveness and inflammation was not caused by anticholinergic receptor effects. OVA-responsive T cells from DL-treated mice exhibited depressed production of IL-4, IL-5, and IL-13 and normal amounts of interferon-γ. The amounts of IL-5 and IL-13 were also diminished in the bronchoalveolar lavage fluid.
Conclusion: DL, given at the time of exposure to the allergen, inhibits T_H2 responses, the induction of allergic pulmonary inflammation, and bronchial hyperresponsiveness. These results suggest that DL or similar agents given during times of antigen exposure might alter disease progression in patients with respiratory allergy. (J Allergy Clin Immunol 2003; 112:149-58.)

Key words: *Asthma, antihistamine, mouse model*

Abbreviations used
Af: *Aspergillus fumigatus*
ANOVA: Analysis of variance
BAL: Bronchoalveolar lavage
DC: Dendritic cell
IgE: Immunoglobulin E
H1R: Histamine type 1 receptor
IL: Interleukin
IFN-γ: Interferon-γ
NS: Normal saline
OVA: Ovalbumin
P_{enh}: Enhanced pause

Histamine is a well-described inflammatory mediator that elicits a number of the acute physiologic responses associated with allergic asthma.[1] Histamine is produced mainly by mast cells and basophils, where it resides in a preformed state within secretory granules. Histamine release from these cells is triggered by the interaction of an allergen with specific immunoglobulin E (IgE) bound to the high-affinity IgE receptor or by nonspecific stimuli, including exercise or cold, dry air.[2] Histamine production in the airways induces obstruction by way of smooth muscle contraction, edema, vasodilation, and mucus hypersecretion.[3,4] In addition, through its direct actions on smooth muscle and sensory nerves, as well as its indirect effects on vagal reflexes, histamine incites cough. The actions of histamine are mediated by several receptors, including the histamine type 1 receptor (H1R), a 7-transmembrane-spanning, G-protein-coupled receptor.[5] H1R is expressed in the respiratory tract, including the lung[6] and trachea,[7] and on human B cells and both $CD4^+$ and $CD8^+$ T cells.[8]

Previous studies have suggested that histamine might enhance T_H2 responses through modulation of dendritic cell (DC) function and regulation of IL-10 and IL-12 production. DCs express H1R,[9] and their exposure to histamine induces a shift toward the DC2 phenotype, which promotes T_H2 immune responses.[10,11] Histamine inhibits IL-12 production in monocytes treated with *Staphylococcus aureus*, Cowan strain, and inhibits IL-12 production while enhancing IL-10 synthesis in lipopolysaccharide-treated leukocytes.[12,13] Because IL-12 is critical for T_H1 responses, and IL-10, by inhibiting T_H1 responses,[14] might favor T_H2 expansion, these observations could suggest a T_H2-promoting influence of histamine. In contrast, studies with mice bearing a targeted deletion of the

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H1R show reduced production of IFN-γ and increased IL-4 and IL-13 secretion, results more consistent with a T_H1-polarizing function of this receptor.[15]

Although H1R blockers have not seen widespread use in the therapy of asthma, there is clinical evidence that antihistamines might affect several aspects of asthmatic pathophysiology, including acute airflow obstruction, chronic bronchial inflammation, and immune sensitization to inhaled allergens. In bronchoprovocation studies, H1 antagonists have been shown to protect against acute obstruction elicited by allergen inhalation, hyperventilation, and neuropeptides.[16-20] There is some experience with the use of H1R blockers in clinical situations as well. In subjects with allergic asthma, there is evidence for modest bronchodilation and perhaps, even a steroid-sparing effect, particularly in patients with *both* asthma and allergic rhinitis.[21-27] The H1R antagonist loratadine provides an additive benefit in asthmatic patients who are being treated with the leukotriene modifier montelukast.[28] However, antihistamines have not demonstrated significant practical utility in asthma management and have not been shown to benefit individuals with established, severe, persistent asthma.[29-31] There are indications that when given early, H1R blockers can prevent sensitization to inhaled allergens and prevent disease progression. In the Early Treatment of the Atopic Child study, regular use of cetirizine by nonasthmatic children with atopic dermatitis was found to significantly decrease the chance of progression to allergic asthma.[32,33]

Desloratadine (descarboethoxyloratadine, CAS 100643-71-8) (DL) is a novel, highly selective, and potent H1R antagonist. DL has been found to exert several effects on immune functions that could influence the onset and clinical expression of allergic asthma. DL affects the expression of several proinflammatory adhesion molecules and cytokines and alters mast cell functions.[34] Exposure to DL reduces the expression of P-selectin on cultured, human endothelial cells[35] and intercellular adhesion molecule-1 surface expression and promoter activity on human respiratory epithelial cells.[36,37] DL inhibits IL-6 and IL-8,[35,38,39] as well as tumor necrosis factor α, IL-3, and granulocyte macrophage colony-stimulating factor[39] production by mast cells and basophils. Genovese et al[40] have reported that DL inhibits the release of histamine, tryptase, leukotriene C4, and prostaglandin D_2 from mast cells.

In the present study, we used murine asthma models to assess the effects of DL on both the asthmatic pathophysiology elicited by repeated allergen inhalation and the induction of specific B- and T-cell responses to a defined protein antigen. We and others have previously reported that the repeated inhalation of aqueous extracts of the naturally occurring allergen, *Aspergillus fumigatus* (*Af*) alone (without prior intraperitoneal antigen priming), evokes a potent, eosinophil-predominant inflammation of the airways, as well as bronchial hyperresponsiveness (BHR) to methacholine in mice.[41-44] We now show that intragastric administration of DL (analogous to the oral route of clinical administration) throughout the period of *Af* antigen inhalation, but not administration after sensitization, potently suppresses the induction of allergic inflammation, pulmonary IL-5 and IL-13 production, and BHR to methacholine. To determine the effects of DL on specific immune responses, a standard protocol of intraperitoneal ovalbumin (OVA) sensitization followed by inhalation challenge was used. This analysis confirmed the protective effect of DL in allergen-induced airway inflammation, goblet cell hyperplasia, and BHR and demonstrated that, although DL had no effect on OVA-specific antibody responses or IFN-γ production by OVA-stimulated T cells, it significantly reduced the production of IL-4, IL-5, and IL-13. Our results indicate that H1 antagonists, administered at the time of allergen sensitization, might alter antigen-specific T-cell responses and attenuate allergic inflammation of the airways. These findings support the hypothesis that H1R blockade in susceptible, atopic individuals might prevent the onset of asthma.

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METHODS

Animals

Female BALB/c mice, 4-8 weeks old, were obtained from Jackson Laboratories (Bar Harbor, Me) and housed in the Children's Hospital specific pathogen-free Animal Resource Facility. Children's Hospital is committed to comply with the National Research Council's *Guide for the Care and Use of Laboratory Animals* and is accredited by the Association for Assessment and the Accreditation of Laboratory Animal Care International. Animals were maintained on an OVA-free diet.

Sensitization

Acute airway inflammation to *Af* was induced by mucosal sensitization by intranasal application, as previously reported.[43] Mice were given 50 μL *Af* antigen or normal saline (NS) 3 times a week for 3 weeks and were studied 12 hours after the last dose.

Immunization to OVA was achieved by means of intraperitoneal injection of 10 μg OVA (grade V, Sigma Chemical Co, St Louis, Mo) in alum (3 mg) on days 0 and 14. These mice were then challenged for 20 minutes to aerosolized 1% OVA in saline by ultrasonic nebulization on days 28, 29, and 30 to induce antigen-driven lung inflammation and were studied 24 hours after the last dose.

Desloratadine treatment

DL (Schering-Plough Corp) was dissolved in 0.4% methyl cellulose (Sigma Chemical Co) in NS. Because DL has been shown to have antihistaminic activity lasting 24 hours in mice,[45] DL was given once daily throughout the entire experiment (days 0-20 for *Af*, days 0-30 for OVA) in a volume of 250 μL.

Airway responses to methacholine

Lung functions were determined by whole-body plethysmography of unrestrained mice (Buxco, Troy, NY) 24 hours after the last exposure to aerosolized OVA. Enhanced pause (P_{enh}) was determined as previously described.[46] Baseline readings were taken and averaged for 5 minutes. Aerosolized saline or methacholine (Mch) in increasing concentrations was nebulized through an inlet of the main chamber for 2 minutes.

Analysis of bronchoalveolar lavage and peripheral blood cells

Immediately after the mice were humanely killed, cells in the lungs were recovered by flushing 0.8 mL of bronchoalveolar lavage (BAL) fluid (1 mmol/L ethylenediaminetetraacetic acid, 10% fetal

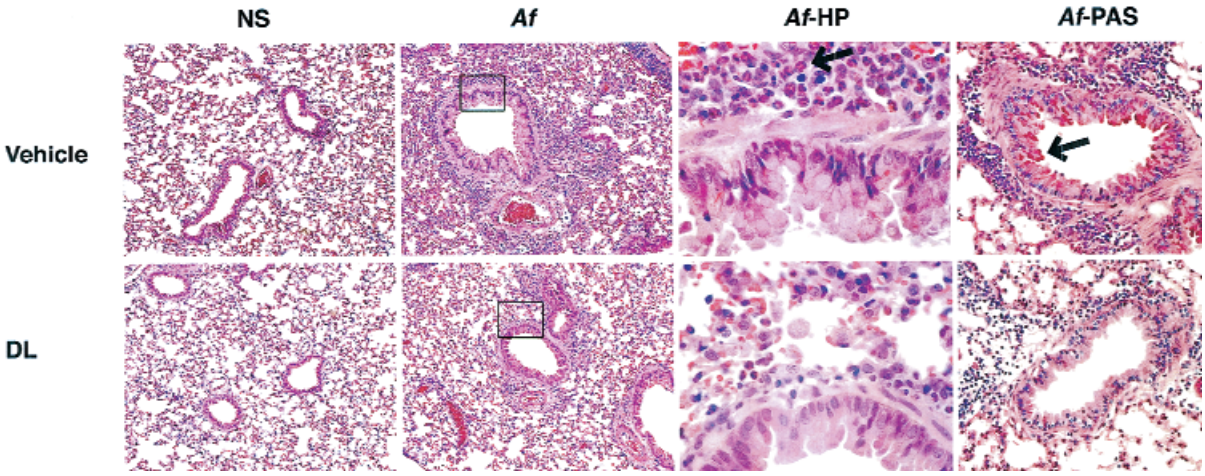

FIG 1. DL inhibits *Af*-induced allergic inflammation in the lungs. Mice were sensitized to *Af* by intranasal administrations and received intragastric vehicle *(upper panels)* or 10 mg/kg DL *(lower panels)* daily throughout sensitization. Representative hematoxylin-and-eosin-stained lung sections at 100× are shown for each group of animals analyzed (n = 10-14) as well as 600× views of the indicated areas of bronchial epithelium. *Arrow* indicates an eosinophil. Periodic acid-Schiff-stained sections are shown on the *right*; mucus-secreting cells are marked by an *arrow.*

calf serum, and 1× phosphate-buffered saline) into the lungs through the trachea. Total cell counts were determined, and differential cell counts were performed after being stained (Diff-Quik stain set, Baxter Healthcare, Miami, Fla). Cytokine levels in the remaining fluid were determined by enzyme-linked immunosorbent assay (ELISA).

Blood was collected after airway-function measurements were taken. The absolute eosinophil count was determined by counting the cells, in a hemocytometer, from a heparinized blood sample stained with Discombe's fluid.[47]

Histology

For histological examination, lung tissue was taken, fixed in 10% buffered-formalin under negative pressure overnight, and embedded in paraffin. Multiple 4-μm sections were stained with hematoxylin and eosin or periodic acid-Schiff reagent.

In vitro IL-4, IL-5, IL-13, and IFN-γ

Single-cell suspensions of spleen cells were prepared in complete RPMI 1640 (Sigma Chemical Co) with 10% fetal calf serum, 1 mmol/L sodium pyruvate, 2 mmol/L-glutamine, 0.05 mmol/L 2-mercaptoethanol, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured at 2 × 10^6/mL in 24-well plates in the presence of OVA (50 μg/mL). Supernatants were collected after 96 hours of culture, and cytokine levels were determined by sandwich ELISA with capture and biotinylated detection antibodies (BD Pharmingen, San Diego, Calif) and avidin-horseradish peroxidase and ABTS substrate development (Zymed Laboratories Inc, San Francisco, Calif).

Serum IgE

A sandwich ELISA was used to quantify total and OVA-specific IgE antibodies.[48] OVA-specific IgE was determined by comparison with a standard curve constructed with the use of purified, mouse, OVA-specific IgE secreted by the hybridoma TOε, a kind gift of Dr Mamoru Kiniwa.[49]

RESULTS

DL prevents the development of lung inflammation and airway hyperresponsiveness after repeated allergen inhalation

Af inhalation elicits asthma-like responses without a requirement for prior intraperitoneal antigen-priming (unlike OVA-based protocols), rendering this model useful for analysis of primary immune responses after bronchial mucosal exposure to inhaled allergen.[41-44] To assess the effect of DL on pulmonary inflammation and BHR driven by allergen inhalation, we subjected mice to *Af* sensitization with or without concurrent intragastric administration of DL. *Af* treatment evoked intense inflammation in the lung (Fig 1). This inflammation was primarily peribronchial and examination under high power (see HP panels) revealed large numbers of eosinophils admixed with mononuclear cells and disrupted epithelium. PAS staining revealed marked allergen-induced goblet cell hyperplasia. Daily treatment with DL dramatically reduced the extent of the inflammation and goblet cell expansion, and under high-power examination significantly fewer eosinophils and epithelial damage were evident.

Repeated *Af* inhalation induced a significant influx of leukocytes in the BAL (668.9 ± 41.5 × 10^3 cells/mL). DL significantly reduced this cell count to 440.0 ± 32.1 × 10^3 cells/mL (P = .05) (Table I) . Eosinophils and lymphocytes were most significantly affected by DL treatment, with statistically significant reductions of 50% and 60%, respectively. IL-5 and IL-13 levels in the BAL were also

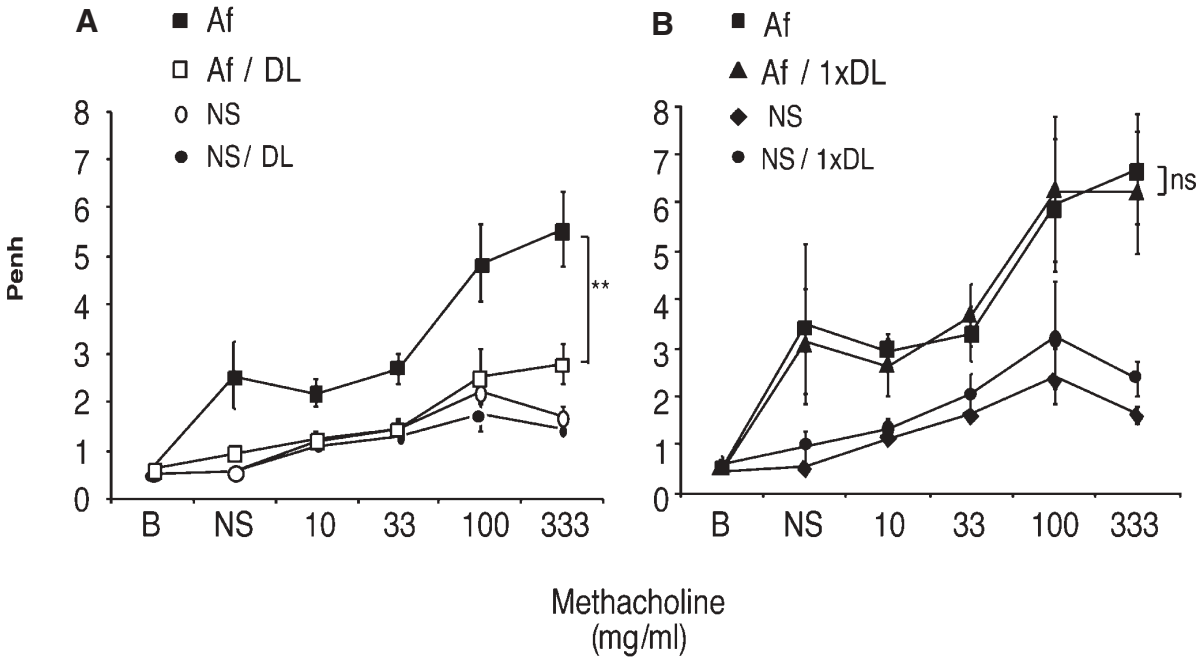

FIG 2. DL inhibits *Af*-induced BHR. **A,** Mch responsiveness was tested on the mice from Fig 1 by whole-body plethysmography (BUXCO). Mean P_{enh} values ± SEM are presented as a function of dose of Mch (B = pretreatment baseline, NS=normal saline). $^{**}P < .0001$ by 2-way ANOVA compared with the *Af* group. **B**, Mice (n = 5) subjected to *Af* sensitization (*Af*) or normal saline control inhalations (*NS*) were evaluated for Mch responsiveness with or without a single dose of DL (1 × DL) given 24 hours before study. *Af*-treated mice (mean ± SEM) receiving a single dose of DL did not differ significantly (ns; $P = .8874$ by 2-way ANOVA) from untreated, *Af* animals.

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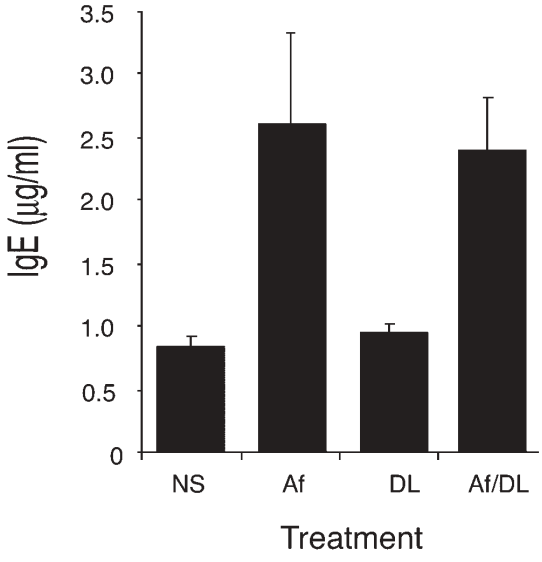

FIG 3. The induction of IgE production by *Af* is unaffected by DL treatment. Serum IgE levels (µg/mL) were determined by ELISA in mice (n = 6 per group) subjected to 3 inhalations per week of either NS or *Af* extract (*Af*) with or without DL.

significantly affected by DL treatment, with reductions of 74% and 94%, respectively, in the allergen-exposed mice given DL (Table I).

Af inhalation induced significantly enhanced sensitivity to Mch, with P_{enh} values in allergen-exposed animals versus NS-treated controls at all doses tested (Fig 2, *A*). DL treatment throughout the period of *Af* sensitization potently suppressed this allergen-induced hyperresponsiveness. *Af*-sensitized mice treated with DL displayed Mch sensitivity similar to that of NS-exposed, control mice, indicating an almost complete suppression of allergen-induce BHR.

DL has been reported to possess anticholinergic properties.[50] In order to rule out the possibility that anticholinergic effects of DL were interfering with the actions of Mch by way of muscarinic receptors, a single dose of DL was given 12 hours before the Mch challenge. A single exposure to DL had no detectable effect on bronchial responsiveness to Mch in *Af*-treated mice (Fig 2, *B*). These data demonstrate that DL treatment reduces the characteristic asthmatic pathophysiology of lung inflammation, eosinophil-dominated cell infiltration, and airway hyperresponsiveness after repeated allergen inhalation, but not through its short-term inhibitory effect on cholinergic receptors.

DL does not affect *Af*-induced rises in IgE

Af sensitization induced a significant rise in serum IgE levels (to 2.6 ± 0.7 µg/mL) that was unaffected by treatment with DL (2.4 ± 0.4 µg/mL) (Fig 3). These observations indicate that although inflammatory responses to *Af* were attenuated by DL, antibody production was unaffect-

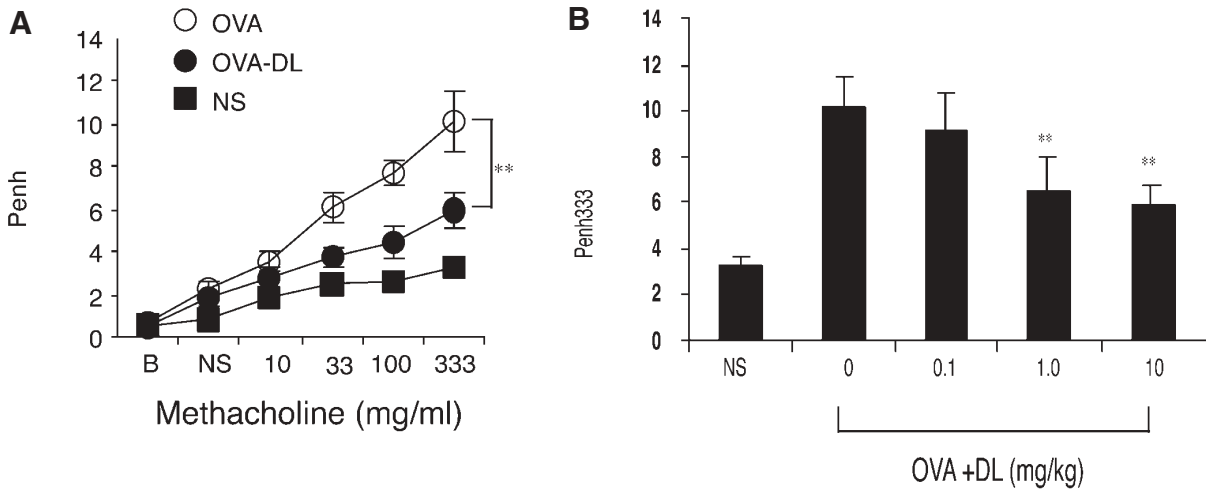

FIG 4. DL inhibits Mch responsiveness of mice sensitized by intraperitoneal OVA and challenged by inhalation of nebulized OVA. **A,** Mice (n = 13 per group) were sensitized to OVA or NS, followed by nebulized challenge with OVA, with or without intragastric administration of DL (10 mg/kg). Mch dose responses were assessed by whole-body plethysmography (values shown ± SEM). $^{**}P < .001$ by 2-way ANOVA. **B,** Dose dependence of DL-induced protection from BHR was examined in OVA-sensitized mice treated with 0.1-1.0 mg/kg DL. The mean P_{enh} ± SEM after maximal Mch dose (333 mg/mL) is shown for each group (n = 5-13). $^{**}P < .05$ by 2-way ANOVA compared with untreated group.

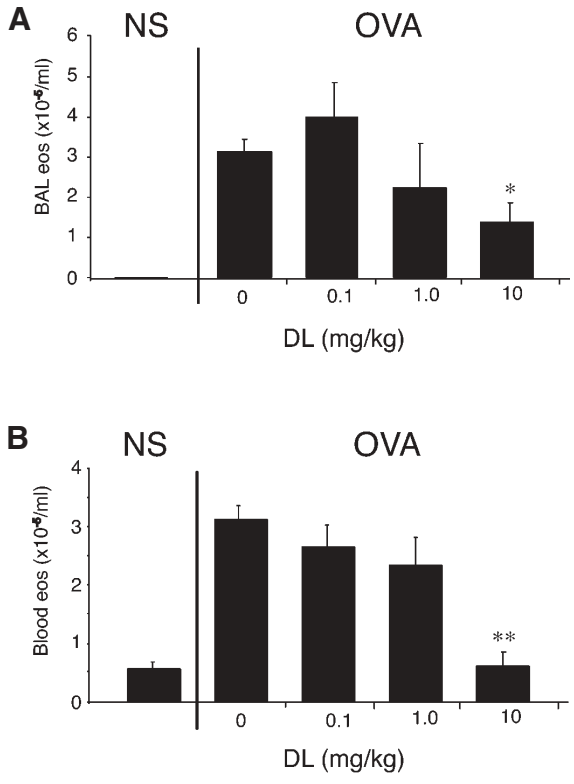

FIG 5. DL blocks OVA-induced increases in eosinophil counts in both the blood and BAL fluid. Eosinophil counts were determined in **(A)** the BAL fluid and **(B)** blood of OVA- and OVA/DL-treated mice. Significant decreases (compared with receiving no DL) were observed in the blood and BAL of animals treated with 10 mg/kg DL ($^{*}P = .013$, $^{**}P = .000003$). Values represent mean ± SEM.

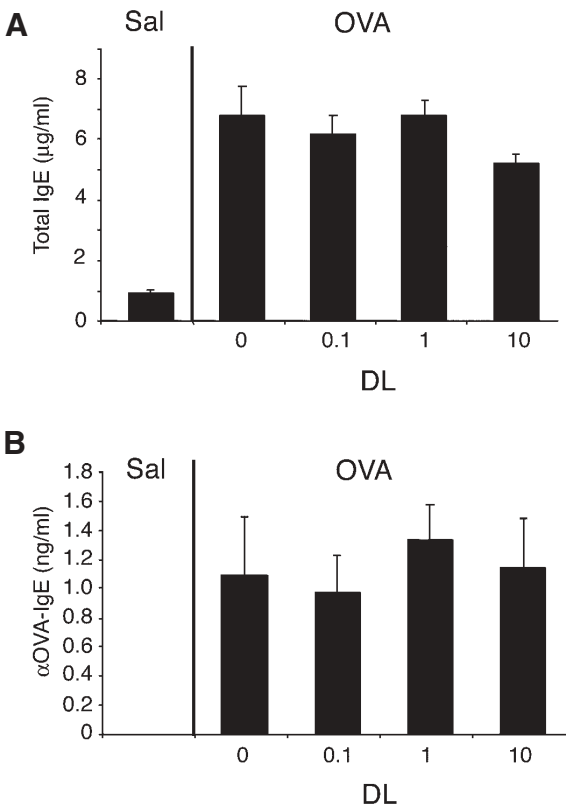

FIG 6. DL does not affect total IgE levels or the production of OVA-specific IgE in OVA-challenged mice. **A,** Total IgE levels (μg/mL) in OVA- or NS-sensitized mice (n = 5 per group) with or without concomitant daily intragastric administration of DL. **B,** OVA-specific IgE levels (μg/mL) were measured in sera from the same animals. Values represent mean ± SEM.

TABLE I. Cell content in bronchial alveolar lavage fluid of saline or *Af*-sensitized mice receiving 10 mg/kg DL (or vehicle control) daily throughout sensitization

	Treatment group (inhalant/drug)			
	Saline/vehicle	***Af*/vehicle**	**Saline/DL**	***Af*/DL**
Total cells	29.0 ± 5.3	668.9 ± 41.5	43.0 ± 8.9	440.0 ± 32.1
Macrophages	28.6 ± 5.2	86.4 ± 19.8	42.0 ± 8.9	140.0 ± 26.9
Eosinophils	0.3 ± 0.2	535.3 ± 36.0	0.01 ± 0.01	270.2 ± 42.0**
Neutrophils	0.06 ± 0.04	37.0 ± 11.7	0.4 ± 0.2	26.0 ± 6.7
Lymphocytes	0.04 ± 0.04	10.2 ± 3.7	0.2 ± 0.2	4.1 ± 1.4**
IL-5	< 31.25	802.4 ± 160.4	< 31.25	210.4 ± 124.2 **
IL-4	< 31.25	< 31.25	< 31.25	< 31.25
IL-13	65.6 ± 31.2	807.2 ± 304.1	< 31.25	44.8 ± 44.8**

BAL fluid (0.8 mL) was obtained, and total cell counts (cells/mL × 10^{-3}) were determined. One hundred microliters of BAL fluid was stained to differentiate cell types. Cell numbers were calculated as a product of the percentage of each cell and the total number of cells per milliliter; n = 10 per group. Samples were centrifuged, and supernatants were analyzed for cytokine products by ELISA (pg/mL); n = 4 or 5 per group.
**$P < .05$ (by Student t test) compared with *Af*/vehicle.

TABLE II. Cell content in bronchial alveolar lavage fluid of saline or OVA-sensitized mice receiving 10 mg/kg DL (or vehicle control) daily throughout sensitization

	Treatment group (immunization/drug)		
	Sham/vehicle	**OVA/vehicle**	**OVA/DL**
Total cells	25.0 ± 2.9	452.5 ± 54.8	317.5 ± 54.1
Macrophages	24.0 ± 2.8	113.2 ± 48.1	154.2 ± 78.7
Eosinophils	0 ± 0	311.7 ± 34.6	139.2 ± 47.6**
Neutrophils	0.2 ± 0.1	9.2 ± 8.0	8.2 ± 4.5
Lymphocytes	0.75 ± 0.57	18.4 ± 4.6	15.9 ± 3.6

NS- or OVA-sensitized mice received 10 mg/kg DL (or vehicle control) daily throughout sensitization. BAL fluid (0.8 mL) was obtained, and total cell counts (cells/mL × 10^{-3}) were determined with a hemocytometer. BAL fluid (100 μL) was cytospun onto slides and stained to differentiate cell types. Cell numbers were calculated as a product of the percentage of each cell and the total number of cells per milliliter; n = 10 per group.
**$P < .05$ (by Student t test) compared with OVA/vehicle.

ed. To further explore the effect of DL on specific immune responses in a system with a defined protein antigen, we performed additional studies with an OVA model of asthma.

DL administration during OVA sensitization induces dose-dependent suppression of inflammation and BHR

OVA sensitization and challenge evoked a significant hyperresponsiveness to Mch challenge in sensitized mice compared with NS-treated animals (Fig 4, *A*). DL treatment during OVA sensitization conferred marked protection against induction of BHR; the dose of Mch required to increase the P_{enh} to 5 was ~10-fold less in animals treated with DL than in those treated with vehicle, and the maximal P_{enh} (at 333 mg/mL Mch) of DL-treated mice was 58% of that observed in control animals. DL induced dose-dependent decreases in the level of airway hyperresponsiveness, with 10 mg/kg being the most effective dose tested (Fig 4, *B*).

DL also dose-dependently reduced the numbers of eosinophils in the BAL from 311.7 ± 34.6 × 10^3/mL in untreated mice to 139.2 ± 47.6 × 10^3/mL in mice that received 10 mg/kg (Fig 5, *A* and Table II; $P = .01$). Peripheral blood eosinophil counts were also significantly suppressed by DL at the maximal dose of 10 mg/kg. At this dose of DL, peripheral blood eosinophil numbers were indistinguishable between DL-treated, OVA-exposed mice and unsensitized mice (Fig 5, *B*).

Treatment with DL does not affect the increases in total and antigen-specific IgE elicited by OVA immunization

In confirmation of our observations with the *Af* sensitization protocol (see Fig 3), we observed that DL administration throughout the range of doses tested (0.1-10 mg/kg) had no effect on the rise in total IgE levels or on antigen-specific IgE responses (Fig 6) induced by intraperitoneal immunization with OVA, followed by inhalation challenge. Taken together with the data on IgE responses in *Af*-sensitized animals, these findings establish that the administration of DL during sensitization does not affect IgE production.

OVA-stimulated splenocytes from DL-treated animals display reduced antigen-induced production of IL-4, IL-5, and IL-13 but normal IFN-γ responses

Histamine has variable effects on cytokine production, by both its direct effects on established T-cell clones (in which it favors T_H1 responses) and its indirect, polarizing

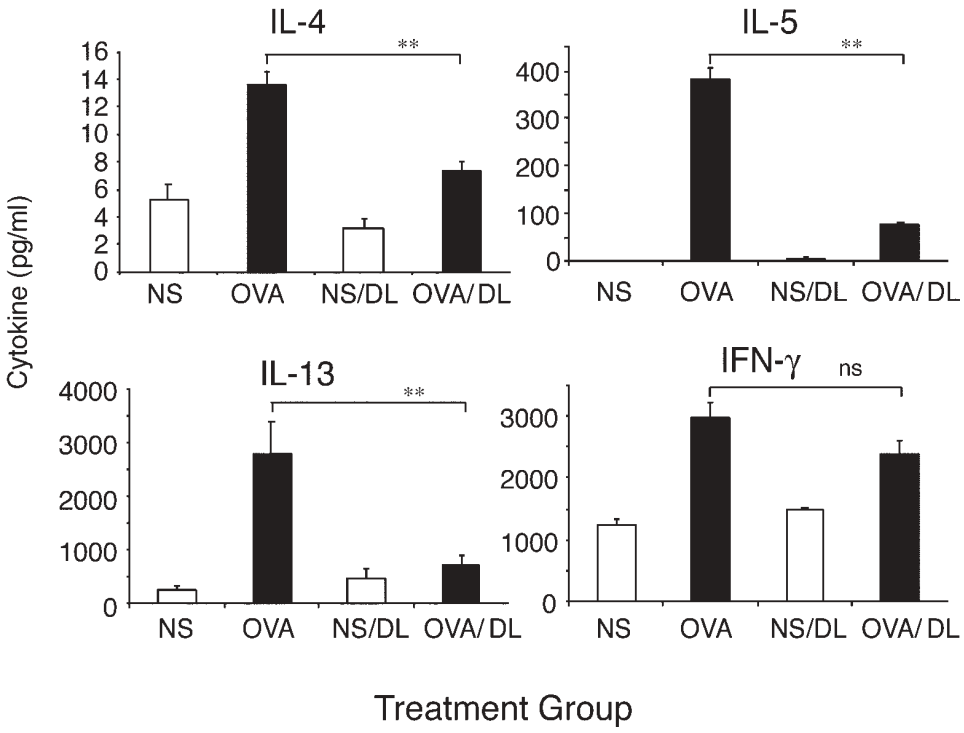

FIG 7. OVA-stimulated T cells from DL-treated, OVA-challenged mice produce decreased levels of IL-4, IL-5, and IL-13 but normal amounts of IFN-γ. Splenocytes from OVA-exposed mice (OVA) or from nonimmune, NS-treated controls (NS) were stimulated in culture with OVA, and 72 hours later, supernatants were analyzed for cytokine content. T cells from mice treated with DL at 10 mg/kg produced significantly lower levels of IL-4 ($P = .0004$), IL-5 ($P = .0001$), and IL-13 ($P = .005$) than did cells from vehicle-treated, control animals. IFN-γ production, however, was not significantly altered ($P = .053$). $^{**}P \leq .05$ by Student *t* test; *ns,* not significant. Values represent mean ± SEM.

effect on DCs (in which histamine promotes T_H2 responses). We also investigated whether the presence of the H1R antagonist DL during antigen sensitization might alter the cytokine profile of the ensuing T-cell response.

Splenocytes from OVA-sensitized animals that were treated with DL (or vehicle-treated control animals) were stimulated with antigen. IL-4, IL-5, and IL-13 produced by splenocytes from OVA-sensitized mice were all significantly reduced in animals that received DL at the time of sensitization (Fig 7). In contrast, IFN-γ responses were not significantly affected. These data indicate that DL administration during allergen sensitization alters the polarity of the evolving T-cell response to inhibit the production of T_H2 cytokines.

DISCUSSION

The pathogenesis of asthma involves the interaction of a complex array of recruited inflammatory cells and their mediators with resident cells of the airway mucosa, vasculature, neuronal network, and smooth muscle. Given the expression of H1R on so many of these tissues and cells and on the DCs and lymphocytes responsible for generating antigen-specific, adaptive, immune responses, we anticipated that a potent H1R antagonist could exert significant effects on allergen sensitization and the induction of inflammation and BHR. In this study, we used *Af*, a naturally occurring, inhaled aeroallergen that elicits allergic inflammation and BHR in mice after mucosal antigen exposure, to analyze the effects of H1R blockade during inhalation sensitization. Exposure to *Af* resulted in the induction of airway hyperresponsiveness accompanied by pulmonary inflammation, increased BAL IL-5 and IL-13, and rises in serum IgE levels. DL, delivered intragastrically throughout sensitization, attenuated pulmonary T_H2 cytokines, BHR (Fig 2, Table I), and cellular infiltration (Fig 1, Table I). This protective effect was not mediated by short-acting muscarinic-blocking activity.

We extended our analysis of how H1R blockade during allergen sensitization inhibits the ensuing allergic airway inflammation and BHR by using a defined antigen system. The induction of pulmonary inflammation and BHR after intraperitoneal OVA sensitization and inhalation OVA challenge were inhibited by DL in a dose-dependent manner (Table II and Figs 4 and 5). Allergen-specific T cells arising in the presence of DL showed significantly diminished production of IL-4, IL-5, and IL-13, with no significant effect on IFN-γ responses (Fig 7).

The reduction in cytokine levels observed in OVA-stimulated T cells sensitized in the setting of H1R blockade might be fundamental to the inhibitory effects of DL on the generation of airway hyperresponsiveness. IL-5 is a key mediator in the development of eosinophilic inflammation in asthma,[51-53] and IL-5-deficient animals fail to develop airway hyperresponsiveness, whereas treatment with anti-IL-5 antibody prevents airway hyperresponsiveness.[54,55] Although a number of studies show links between eosinophil recruitment to the airways and the induction of BHR, T_H2 cytokine-driven asthmatic physiology can arise even in the absence of eosinophils.[56,57] Thus, the protective effects of DL might derive from reductions in both eosinophil-mediated and eosinophil-independent effects of IL-4, IL-5, and IL-13. Recent studies have demonstrated the requirement for IL-4/IL-13-driven responses in airway hyperresponsiveness[58,59] and the link between IL-5-driven eosinophilia and the IL-4/IL-13 cytokine axis.[60] IL-4 and IL-13 have rapid, direct, BHR-enhancing effects on airway cells,[58] and IL-4 is also critical in the expansion and maintenance of the pool of allergen-specific T_H2 cells that drive the allergic response. IL-13, although not directly supportive of T_H2 differentiation, parallels the activity of IL-4 in inducing allergic inflammation, goblet cell hyperplasia, and BHR.[61-63] The decreases in airway hyperresponsiveness observed in DL-treated mice are likely due to the combined effects of lowering IL-5, IL-4, and IL-13 cytokine production.

Interestingly, the elevation of eosinophil numbers in peripheral blood elicited by OVA sensitization was significantly inhibited by higher DL doses, suggesting that H1R might regulate eosinophilopoiesis, eosinophil emigration from bone marrow, or eosinophil survival. Reduced eosinophil production could also be explained by the attenuation of IL-5 responses that we observed in these animals. H1R might also enhance eosinophil survival, because IL-5 has been shown to be antiapoptotic to eosinophils through inhibition of Bax expression and caspase activation.[64] Thus, the attenuation in IL-5 responses in DL-treated mice could result in a reduction in survival. Histamine has been reported to inhibit the maturation of a subclone of the eosinophil cell line, HL-60.[65,66]

Whereas DL administration during allergen sensitization resulted in a significant attenuation of allergic airway pathology, immune sensitization, as reflected by the induction of IgE responses, was unaffected. Either total or antigen-specific IgE was elevated to the same extent in *Af*- and OVA-exposed mice treated with DL as in those that received vehicle control (Figs 3 and 6). Studies with H1R-deficient mice have also suggested that antibody responses are unimpaired.[15,67] The lack of inhibition of antigen-driven IgE production in DL-treated mice, together with our observations on impaired T_H2 cytokine production, suggests that the major effects of DL in these murine asthma models are on cytokine-driven, allergic inflammation of the airways and BHR. IL-5 and IL-13 production was the most dramatically suppressed by DL treatment, consistent with the critical role of these cytokines in driving asthmatic responses. Interestingly, despite the fact that IL-4 was significantly inhibited by DL treatment, sufficient IL-4 might have been available to drive class switching to IgE and facilitate the rises in serum IgE levels observed.

It was recently reported that another H1R antagonist, fexofenadine, inhibits BHR and pulmonary eosinophil infiltration in OVA-immunized mice.[68] Transfer of T cells from fexofenadine-treated mice did not confer sensitivity to naive recipients after OVA inhalation, and levels of IL-4 and IL-5 were reduced in the BAL. Our findings now suggest that the inability of T cells from fexofenadine-treated, allergen-sensitized mice to transfer allergic sensitivity to naive recipients resulted not from a failure in sensitization but, more likely, from an alteration in the cytokine production profile of the transferred cells. The findings with fexofenadine indicate that the effects of DL on T_H cell effector profiles might be shared by other H1R antagonists.

The effect of H1R blockade on cytokine production during allergen sensitization reported here contrasts with published studies on the consequences of targeted deletion of H1R. H1R-deficient mice actually display the opposite phenotype—markedly suppressed production of IFN-γ with increases in IL-4 and IL-13.[69] Several possible explanations exist for this apparent paradox. First, it is possible that the absence of H1R function throughout T-cell ontogeny (rather than only briefly during initial antigen encounter by mature T cells) exerts fixed effects on the subsequent competence of T cells to produce T_H1 cytokines. Second, it is possible that the cell population most sensitive to H1R blockers at therapeutic doses is the DC. Because histamine treatment of DCs is associated with DC2-skewing and consequent T_H2-dominant T-cell responses,[10,11] the effect of H1R blockade on this cell population would be to dampen T_H2 responses, as observed in our study of the effect of DL on the development of T_H2 cells in vivo.

In summary, we have shown that the H1R antagonist DL blocks the induction of airway inflammation and BHR in 2 separate models of allergic asthma when given intragastrically throughout the period of antigen sensitization. The antiallergic efficacy of DL in the *Af* model establishes that H1R blockade during inhalation sensitization with a naturally occurring aeroallergen attenuates subsequent development of allergic inflammation. Our parallel studies with intraperitoneal sensitization with OVA, followed by inhalation challenge, show that the effects of DL might be caused by the inhibition of T_H2 cytokine production by way of the H1R. The effects of DL on the evolution of T-cell cytokine profiles after allergen encounter at the respiratory mucosa suggest that H1 blockers might actually alter disease progression in susceptible individuals exposed to allergen. This effect might account for the clinical observation in the Early Treatment of the Atopic Child study that H1R antagonism can prevent the onset of asthma in susceptible individuals.[33]

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