Clin Exp Allergy 2004; **34**:1124–1130 doi:10.1111/j.1365-2222.2004.01974.x

Oral administration of desloratadine prior to sensitization prevents allergen-induced airway inflammation and hyper-reactivity in mice

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Summary

Background Histamine-1-receptor (H1R)-antagonists were shown to influence various immunological functions on different cell types and may thus be employed for immune-modulating strategies for the prevention of primary immune responses.

Objective The aim of this study was to investigate the effects of an H1R-antagonist on allergen-induced sensitization, airway inflammation (AI) and airway hyper-reactivity (AHR) in a murine model.

Methods BALB/c mice were systemically sensitized with ovalbumin (OVA) (six times, days 1–14) and challenged with aerosolized allergen (days 28–30). One day prior to the first and 2 h prior to every following sensitization, mice received either 1 or 0.01 μg of desloratadine (DL) or placebo *per os*.

Results Sensitization with OVA significantly increased specific and total IgE and IgG1 serum levels, as well as *in vitro* IL-5 and IL-4 production by spleen and peribronchial lymph node (PBLN) cells. Sensitized and challenged mice showed a marked eosinophilic infiltration in broncho-alveolar lavage fluids and lung tissues, and developed *in vivo* AHR to inhaled methacholine. Oral treatment with DL prior to OVA sensitization significantly decreased production of OVA-specific IgG1, as well as *in vitro* Th2-cytokine production by spleen and PBLN cells, compared with OVA-sensitized mice. Moreover, eosinophilic inflammation and development of *in vivo* AHR were significantly reduced in DL-treated mice, compared with sensitized controls.

Conclusion Treatment with H1R-anatagonist prior to and during sensitization suppressed allergen-induced Th2 responses, as well as development of eosinophilic AI and AHR. This underscores an important immune modulating function of histamine, and implies a potential role of H1R-anatagonists in preventive strategies against allergic diseases.

Keywords airway inflammation, airway reactivity, desloratadine, H1-receptor-antagonist, mice, Th1/Th2 cytokines

Submitted 9 July 2003; revised 7 November 2003; accepted 12 March 2004

Introduction

Histamine is produced and released upon activation of mast cells and basophils following cross-linking of IgE and is the main mediator of immediate-type of hyper-sensitivity reactions [1]. IgE-dependent mast cell activation is the central mechanism of the early asthmatic reaction leading to allergen-induced, specific bronchoconstriction [2]. In contrast, the role of histamine in the development of airway inflammation (AI) and airway hyper-reactivity (AHR), two cardinal features in the pathogenesis of bronchial asthma, remains controversial (reviewed [3]). Treatment of asthmatic patients with first and second generation antihistamines turned out to be rather ineffective (reviewed in [3]), and H1-antagonists are not considered to be a standard medication for asthma therapy.

More recently, however, it has been shown in numerous *in vitro* studies that histamine demonstrates immune modulating effects on different cell types like antigen-presenting cells (APCs) or T cells via the H1 [4–6]- or the H2-receptor [7–9]. Addition of histamine or blockage of histamine by receptor antagonists altered the expression of co-stimulatory molecules and the production of various cytokines [4–10]. These intriguing data suggested that antihistamines are not only capable of preventing immediate-type of hyper-sensitivity reactions but may also influence primary allergen-induced immune responses. A few clinical studies have addressed this hypothesis and showed that early and sustained treatment of high-risk infants suffering from atopic dermatitis with histamine-1-receptor (H1R)-antagonists reduced the risk for subsequent development of asthma [11–13].

To further address the question of immune modulating capacities of H1R-antagonists, we utilized a murine model of

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allergen-induced sensitization, AI and AHR [14]. H1R-antagonist was applied systemically prior to and during the sensitization period to investigate the effects on primary systemic and secondary local allergen-induced immune responses. Here, we show that oral treatment with desloratadine (DL) – a potent third-generation H1R-antagonist – prior to and during sensitization leads to decreased allergen-induced specific Th2-cytokine and IgG1 production. Associated with this inhibition of primary sensitization were reduced development of eosinophilic AI and AHR following allergen airway challenges. These data indicate that oral treatment with DL inhibited primary and modulated secondary immune responses after allergen sensitization and airway challenges.

Materials and methods

Animals

Female BALB/c mice, 6–8 weeks old, were obtained from the BGVV, Berlin, Germany. Mice were kept under pathogen-free conditions and maintained on ovalbumin (OVA)-free diets. All experiments were approved by the animal care committee.

Experimental protocol

Mice (three to four mice per group per experiment) were systemically sensitized six times within 3 weeks by intraperitoneal (i.p.) injections of 10 μg OVA (Grade VI, Sigma, St Louis, MO, USA) in a total volume of 100 μL of phosphate-buffered saline (PBS). Allergic AI and AHR was induced by repeated airway allergen challenges with 1% aerosolized OVA (Grade V, Sigma, St Louis, MO, USA) for 20 min daily on days 29, 30, and 31. Negative control animals received sham sensitization (100 μL PBS i.p.) and airway challenges with OVA (referred to as PBS animals).

DL application prior to sensitization: One day prior to the first and 2 h prior to every following OVA-injection, mice received orally either 1 μg (DL1/OVA $n = 12$, 50 μg/kg bodyweight, similar to a single application of 5 mg in man) or 0.01 μg (DL0.01/OVA $n = 8$) of DL (solved in PBS/0.1% ethanol, kindly provided by Schering-Plough Research Institute, Kenliworth, NJ, USA). Controls were DL-treated, sham-sensitized animals (DL/PBS, $n = 4$). Positive controls received vehicle according to the same protocol and OVA sensitization (PBS/OVA $n = 12$).

Measurement of in vivo airway reactivity (AR)

On day 32, 24 h after the last airway challenge, *in vivo* lung function to inhaled methacholine (Mch, Sigma, St Louis, MO, USA) was measured by whole-body plethysmography (WBP, EMKA Technologies, Paris, F), as previously reported [14]. AR was expressed for each individual animal as the fold increase of the enhanced pause (Penh) of each of the increasing doses of Mch (6–50 mg/mL) compared with baseline values of PBS nebulizations ($Penh_{Mch\text{-}dose}/Penh_{PBS\text{-}Baseline}$). Additionally, the dosage of Mch required for an increase of Penh by 200% was calculated for each individual animal ($PC_{200}Penh$).

Broncho-alveolar lavage (BAL)

On days 33 and 34, mice were assessed for AI. Lungs were lavaged via a tracheal tube twice with 0.8 mL cold PBS (Seromed, Biochrom, Berlin, Germany). Cells were counted and cytospin slides were stained with Diff Quik (Dade Behring AG, Düdingen, Switzerland). BAL cells were differentiated due to morphological criteria by counting at least 200 cells by using standard haematologic procedures.

Immunohistochemistry

Distribution of eosinophils in lung tissues was determined by staining with monoclonal antibody (mab) against mouse major basic protein (MBP, kindly provided by Dr J. J. Lee, Mayo Clinic, Scottsdale, AZ, USA), as previously described [14, 15]. Slides were studied by fluorescence microscopy. One representative picture of each group is presented.

Cell preparation and culture

On days 33 and 34, individual spleen mononuclear cells (MNC) were isolated by density gradient centrifugation (Lympholyte-M, Cedarline Laboratories, Hornby, Canada). Pooled peribronchial lymph nodes cells (PBLN, two to three mice per group per experiment) were obtained by passing PBLN through a nylon mesh. Cells were washed three times in PBS, and 10^6 cells/200 μL were resuspended in RPMI 1640 (Seromed, Biochrom, Berlin, Germany), supplemented with Amphotericin B, Penicillin, Streptomycin (Seromed, Biochrom, Berlin, Germany), L-glutamine (PAA Laboratories, Linz, Austria) and 10% fetal calf serum (FCS, PAA Laboratories, Linz, Austria). Cells were cultured in 96-well round-bottom plates (Greiner GmbH, Frickenhausen, Germany) at 37 °C, 5% CO_2 [1] with RPMI 1640, [2] with concanavalin A (Con A, 2.5 μg/mL, Sigma, St Louis, MO, USA) or [3] with OVA (50 μg/mL, OVA VI, Sigma, St Louis, MO, USA). After 48 (IFN-γ) and 96 h (IL-5, IL-4), respectively, supernatants were harvested and stored at –20 °C until further analysis.

Cytokine measurements

Levels of IL-5, IL-4 and IFN-γ in cell culture supernatants were assessed by ELISA, as previously described [16]. Limits for detection were 16 pg/mL for IL-5 and IL-4, and 39 pg/mL for IFN-γ.

Serum levels of anti-OVA and total Ig

Anti-OVA IgE, IgG1 and IgG2a serum levels were measured by ELISA as previously described [16]. Total IgE levels were calculated by comparison with known mouse IgE standards (The Binding Site Limited, Birmingham, GB), the detection limit was 3.9 ng/mL. Levels of OVA-specific IgG1 and IgG2a antibodies were related to pooled standards that were generated in our laboratory, and expressed as ELISA units per mL (EU/mL). OVA-specific IgE was presented as optical density units (OD units).

Statistical analysis

Animals of parallel groups of three independent experiments were pooled. Values for all measurements are expressed as

mean ± standard error of the mean. PBLN data are expressed as mean ± standard deviation. Pairs of groups (PBS/PBS vs. PBS/OVA or PBS/OVA vs. DL/OVA) were compared by standard unpaired two-tailed *t*-test including variance testing. Values of *P* for significance were set to 0.05.

Results

Oral treatment with DL prior to sensitization prevented the development of AHR and AI

Allergen airway challenges of sham-treated, sensitized BALB/c mice (PBS/OVA, $n = 12$) significantly increased *in vivo* AR to inhaled Mch, as indicated by a left-shift of the response curves and five times higher fold increases of Penh values, compared with sham-sensitized animals (PBS/PBS, $n = 8$, Fig. 1). In addition, PC_{200}Penh was significantly reduced in PBS/OVA mice (8.2 ± 0.9 mg/mL), in comparison with negative controls (55.3 ± 12 mg/mL, $P < 0.05$, data not shown). Oral treatment with DL prior to and during sensitization significantly inhibited the development of *in vivo* AHR. Treatment of mice with doses of either 0.01 μg (~ 0.5 μg/kg bodyweight) or 1 μg (~ 50 μg/kg bodyweight) of DL significantly reduced maximum *in vivo* AR by more than 50%, compared with OVA-sensitized and challenged animals (Fig. 1). Similarly, PC_{200}Penh was significantly increased in DL-treated sensitized and challenged animals, comparable with values of negative controls (DL 0.01/OVA: 46 ± 14 mg/mL, $P < 0.05$, DL1/OVA: 31 ± 9 mg/mL, $P < 0.05$).

Development of AHR in sensitized and challenged mice was associated with significant AI, showing a fivefold increase in numbers of leucocytes in BAL fluids, compared with negative controls (Fig. 2). Differential cell counts of BAL fluids

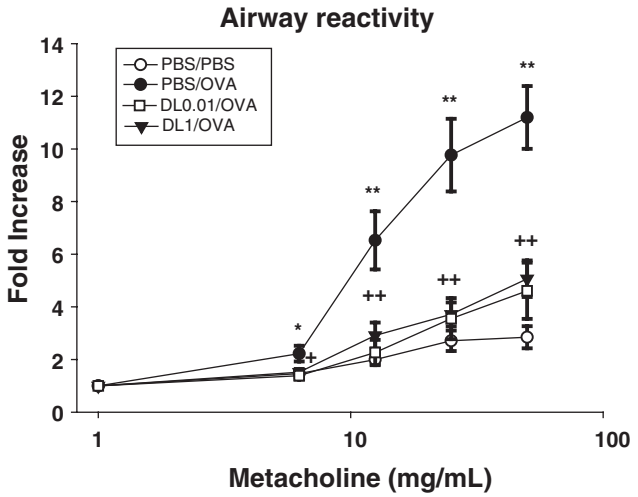

Fig. 1. DL prior to sensitization prevented the development of AHR. BALB/c mice were sensitized to OVA and challenged via the airways with allergen. Prior to sensitization, mice received either 1 μg (DL1/OVA $n = 12$) or 0.01 μg DL (DL0.01/OVA $n = 8$) or only vehicle (PBS/OVA $n = 12$) p.o. Negative controls were sham-treated and sham sensitized but allergen-challenged mice (PBS/PBS $n = 8$). AR was measured by whole-body plethysmography. Expressed are fold increases of Penh after challenge with rising concentrations of inhaled methacholine compared with baseline Penh values (**$P < 0.01$ PBS/PBS vs. PBS/OVA, $^{+}P < 0.05$, $^{++}P < 0.01$ DL-treated groups vs. PBS/OVA). DL, desloratadine; AHR, airway hyperreactivity; OVA, ovalbumin; PBS, phosphate-buffered saline; AR, airway reactivity; Penh, enhanced pause.

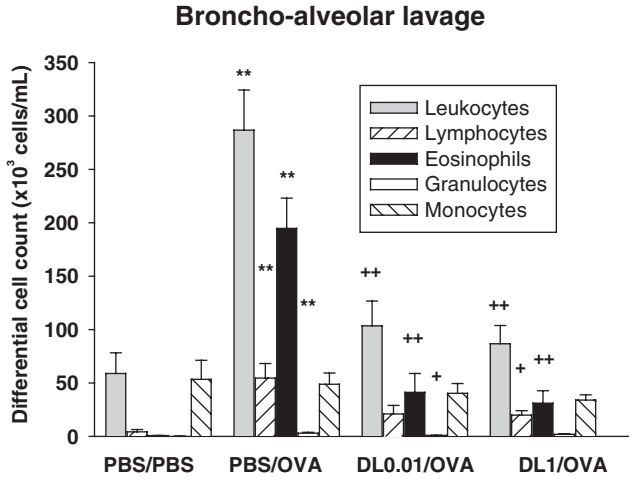

Fig. 2. DL prior to sensitization prevented the development of AI. Mice were sensitized, challenged and treated as mentioned in Fig. 1. On days 33 and 34, lungs were lavaged and differential cell counts in BAL fluids were performed. (**$P < 0.01$ PBS/PBS vs. PBS/OVA, $^{+}P < 0.05$, $^{++}P < 0.01$ DL-treated groups vs. PBS/OVA). DL, desloratadine; AI, airway inflammation; BAL, broncho-alveolar lavage; PBS, phosphate-buffered saline; OVA, ovalbumin.

revealed a dramatic increase in numbers of eosinophils (58%, 195 × 10^3 cells/mL, PBS/PBS: 2%, 0.65 × 10^3 cells/mL, $P <$ 0.005) and lymphocytes (PBS/OVA: 17%, 55 × 10^3 cells/mL, PBS/PBS: 7%, 4.2 10^3 cells/mL, $P < 0.005$). Parallel to eosinophilic infiltration in BALF, a robust peribronchial and perivascular eosinophilic inflammation was detected in lung tissues of OVA-sensitized and challenged mice by means of immunohistochemistry (Figs 3a and b).

Similar to the inhibition of *in vivo* AHR, oral treatment with DL prior to sensitization significantly suppressed eosinophilic airway, as well as tissue inflammation. The numbers of total leucocytes in BALF were significantly reduced (Fig. 2), and especially numbers of eosinophils were decreased to a fourth of cell numbers of positive controls. Concordantly, peribronchial eosinophilic inflammation of lung tissues was markedly reduced in sensitized, challenged animals treated with DL, compared with PBS/OVA mice (Fig. 3c).

Oral treatment with DL prior to sensitization reduced local Th2-type cytokine production

PBLN cells of OVA-sensitized and challenged mice produced high amounts of Th2-type cytokines after *in vitro* stimulation with allergen (IL-5: 19.1 ± 2.5 ng/mL, IL-4: 375 ± 10 pg/mL) or mitogen (IL-5: 17.9 ± 4 ng/mL) and moderate amounts of IFN-γ (359 ± 187 pg/mL) (Fig. 4).

DL treatment prior to and during sensitization significantly reduced IL-5 production by PBLN cells restimulated with OVA (by up to 50%) or mitogen, when compared with sensitized, challenged controls (PBS/OVA) (Figs 4a and b). Interestingly, the reduction in IL-5 production by PBLN was even more pronounced in mice treated with low doses of DL (0.01 μg DL/OVA). No significant differences in IFN-γ production by PBLN were detected between DL-treated and vehicle-treated sensitized and challenged mice (Fig. 4c).

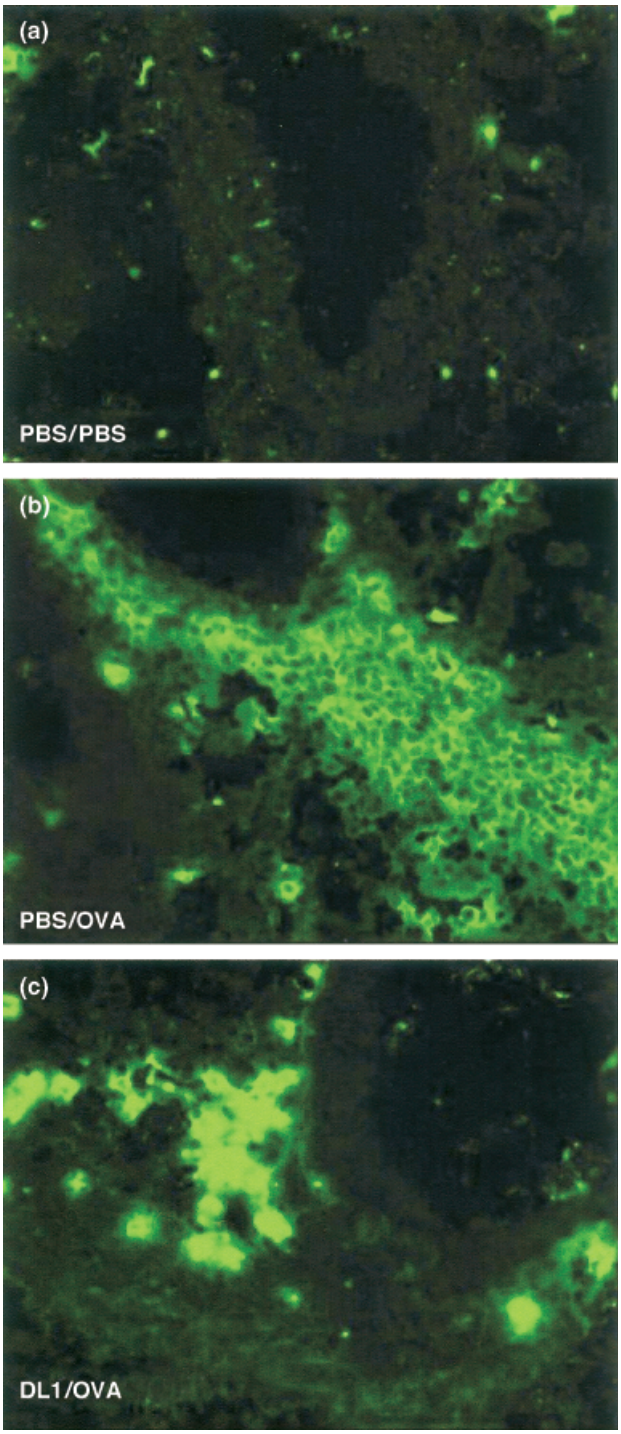

Fig. 3. DL prior to sensitization reduced the development of peribronchial tissue eosinophilia. Experimental design and study groups as indicated in Fig. 1. On days 33 and 34, 2 days after the last airway challenge, lungs were harvested. The frozen lung sections were stained by immunohistochemistry for MBP-positive cells. Each picture (× 20) is representative for one out of four animals per group tested: (a) PBS/PBS; (b) PBS/OVA; (c) DL1/OVA. DL, desloratadine; MBP, major basic protein; PBS, phosphate-buffered saline.

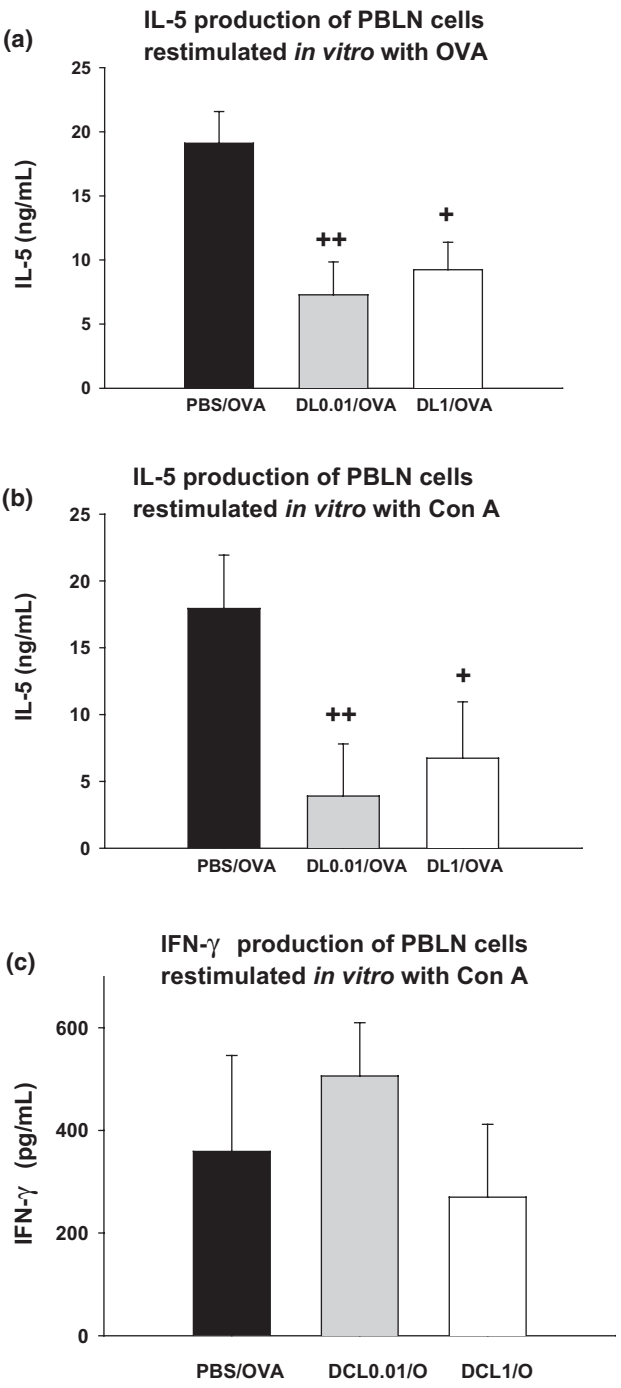

Fig. 4. DL treatment prior to sensitization reduced local Th2-type cytokine production by PBLN cells. Experimental design and study groups as indicated in Fig. 1. On days 33 and 34, 2 days after the last airway challenge, PBLN cells of two to three mice per group and experiment were pooled and cultured *in vitro* with OVA (a) or Con A (b and c). IL-5 (a and b) and IFN-γ (c) production in culture supernatants were measured by ELISA. Presented are the data from three independent experiments ($^{+}P<0.05$, $^{++}P<0.01$ vs. PBS/OVA). DL, desloratadine; PBLN, peribrochial lymph node; OVA, ovalbumin; PBS, phosphate-buffered saline.

Oral DL treatment prior to sensitization reduced systemic production of Th2-type cytokines and allergen-specific IgG1

Repeated OVA sensitization and airway challenges (PBS/OVA) induced increased systemic Th2-type cytokine production when compared with sham-sensitized animals (PBS/PBS, Table 1). IFN-γ production after allergen stimulation was not detectable in culture supernatants of any group (data not shown). In contrast, high *in vitro* IFN-γ production by spleen MNCs stimulated with ConA was observed in PBS/PBS

Table 1. Systemic effects of DL treatment prior to allergen-sensitization

Treatment/sensitization	Immunoglobulin levels				Cytokine levels		
	Total IgE (ng/mL)	OVA-IgE (OD units)	OVA-IgG1 (EU/mL)	OVA-IgG2a (EU/mL)	IL-5 (ng/mL)	IL-4 (pg/mL)	IFN-γ (pg/mL)
PBS/PBS	603.5 (± 87)	ND	ND	ND	ND	ND	1829 (± 319)
PBS/OVA	7796 (± 2159)*	0.244 (± 0.03)*	8313 (± 1135)*	33 (± 7)*	8.0 (± 2.1)*	133 (± 38)*	673 (± 152)*
DL0.01/OVA	5710 (± 2091)	0.185 (± 0.03)	2944 (± 868)‡	32 (± 7)	4.2 (± 1.6)	70 (± 15)	848 (± 251)
DL1/OVA	5918 (± 1121)	0.234 (± 0.03)	3509 (± 953)‡	22 (± 3)	2.4 (± 0.7)†	45 (± 9)†	641 (± 108)

Experimental design and study groups as indicated in Fig. 1. Mice were bled on day 33 and serum levels of total and OVA-specific Ig were determined by ELISA. Splenic MNCs were obtained on day 33 and 34 and restimulated *in vitro* with OVA (IL-5 and IL-4) or Con A (IFN-γ). Cytokine production in supernatants was measured by ELISA. OVA, ovalbumin; PBS, phosphate-buffered saline; MNC, mononuclear cell; DL desloratadine; OD, optical density; ND, not detected.
*$P<0.01$ vs. PBS/PBS. †$P<0.05$. ‡$P<0.01$ vs. PBS/OVA.

animals, that was decreased in spleen cells of OVA-sensitized, challenged mice (Table 1). Mice receiving DL prior to and during sensitization showed significant lower systemic Th2-type cytokine production when compared with vehicle-treated sensitized and challenged mice. Especially, the higher dose of DL treatment (1 μg/mouse) resulted in a significant reduction of *in vitro* IL-4 and IL-5 production by spleen MNCs cultured with OVA. Similar to local cytokine patterns of PBLN cells, no significant up-regulation of IFN-γ production by splenic MNC after mitogenic restimulation was observed in DL-treated animals when compared with positive controls (PBS/OVA, Table 1). Application of DL prior to sham-sensitization (DL/PBS) did not lead to significant differences in IL-5 or IFN-γ production by splenic MNCs stimulated *in vitro* with ConA, compared with PBS-treated, sham-sensitized animals (PBS/PBS) (data not shown). This indicates that DL showed no unspecific immune suppression, but rather immune modulating effects dependent on simultaneous antigen/allergen sensitization. In conclusion, the data illustrate that DL treatment prior to and during sensitization reduced allergen-specific Th2-type cytokine production, but did not result in a general shift towards a predominant systemic Th1-cytokine profile.

This observation is also mirrored in the pattern of Ig production (Table 1): OVA-sensitized and challenged mice showed a dramatic increase in total IgE production, high OVA-specific IgE and IgG1 and low, but detectable allergen-specific IgG2a serum levels, when compared with negative controls (PBS/PBS). DL treatment prior to and during sensitization significantly reduced OVA-specific IgG1 production when compared with vehicle-treated controls (PBS/OVA). However, OVA-specific IgE and IgG2a, as well as total IgE production was not significantly altered by DL treatment. In summary, DL treatment did not generally suppress Th2-mediated Ig production as indicated by high total IgE levels in all sensitized, challenged mice. However, parallel to a reduction in systemic OVA-specific IL-4 and IL-5 production, allergen-specific IgG1 production was down-regulated in sensitized, challenged mice after treatment with DL prior to and during sensitization.

Discussion

In the present paper, we show that the oral treatment of mice with DL – a third generation H1R-antagonist – prior to and during allergen sensitization significantly reduced eosinophilic AI and the development of AHR following allergen airway challenges. Associated with these phenotypical changes after DL treatment were alterations in allergen-induced immune responses both on the local and the systemic level resulting in decreased Th2-cytokine production.

Anti-inflammatory effects of H1R-antagonists with regard to allergen sensitization have recently been reported in two *in vivo* studies [17, 18]. Treatment of mice with ceterizine, a second-generation H1R-antagonist, after completion of sensitization but prior to nasal allergen challenges [18] showed a down-regulation of eosinophilic infiltration into nasal mucosal tissues accompanied by reduced IL-4, IL-5 and IFN-γ mRNA expression [18]. Similarly, Gelfand et al. treated mice with fexofenadine, a third-generation H1R-antagonist, after completion of allergen sensitization but prior to and during airway allergen challenges [17]. These mice showed decreased eosinophilc lung infiltration and inhibition of AHR, which was associated by reduced Th2-type cytokine production by lung T cells. The present paper greatly differs from these two above-mentioned studies in terms of the experimental protocol. Most importantly, in the two latter studies, mice were treated only after sensitization was completed and with very high doses (2×2.500 μg fexofenadine per mouse daily) [17]. Applying a similar protocol and treating already sensitized animals prior to airway challenges with lower doses of DL (up to 100 μg DL per mouse/dose) did not inhibit development of AI and AHR in our hands (data not shown). In contrast, oral treatment with DL during the sensitization period effectively down-regulated allergen-induced AHR and AI after application of very low doses of DL (0.01 μg/mouse/dose), possibly reflecting the very potent affinity of DL to the H1R [19, 20].

Recently published data demonstrated that DL exhibits a variety of immunological effects on various cell types [21–23]. *In vitro* studies showed that DL significantly reduced the expression of adhesion and co-stimulatory molecules like ICAM-1 and HLA-DR by nasal epithelial cells [21], suppressed IL-6 and IL-8 release by endothelial cells [22] and inhibited production of RANTES by epithelial cells [23]. Here, we report that oral treatment with DL prior to and during sensitization significantly suppressed local Th2-type cytokine production by PBLN cells after subsequent allergen airway challenges. Especially, IL-5 production by PBLN cells was considerably reduced when compared with sensitized and challenged controls, most likely explaining the reduction in

allergen-induced eosinophilic AI observed in DL-treated mice [24, 25]. Similar, systemic Th2 cytokine production by spleen cells and OVA-specific IgG1 serum levels were decreased in DL-treated mice, suggesting that DL may influence primary immune responses towards allergen on the local, as well as on the systemic level.

The down-regulation of Th2-type immune responses by DL may be due to a modulation of histamine-induced effects either on APCs or on T cells. *In vitro* studies with human APCs revealed that histamine up-regulated the expression of co-stimulatory molecules on immature DCs (CD86, CD40, CD49d, CD54, CD80 [5]), and modulated the production of several cytokines and chemokines [8]. DCs co-stimulated with histamine and lipopolysaccharide were polarized towards a DC2 subpopulation with decreased IL-12 [4] and enhanced IL-10 production [8], preferentially priming Th2 responses [8]. Accordingly, blocking of histamine with an H1R-antagonist, mepyramine [4], or an H2R-antagonist, cimetidine [4, 8], suppressed this polarization. It is, therefore, likely that the reduction of systemic allergen-specific Th2-type immune responses in our study was due to direct modulation of APC function by systemically applied DL.

The second possible mechanism for the results observed in our study is the direct interaction with histamine-mediated effects on T cells by DL treatment. This assumption is supported by data showing that histamine inhibited *in vitro* IFN-γ production by Th1-, and IL-4 production by Th0-like T cell clones [9], an effect that was abolished by an Histamine R-antagonist [9, 26]. In contrast to this observation and our own present results, recently published data demonstrated that stimulated splenic T cells from H1R-knockout (KO) mice showed decreased IFN-γ and enhanced IL-4 production *in vitro*, compared with wild-type animals [6, 27]. Furthermore, specific IgG1 and IgE production was enhanced in the H1R-KO mice [6]. These seemingly controversial data may be explained by differences in the experimental models. *In vivo* blockage of the H1R prior to and during the initiation of a primary immune response in normal wild-type mice may not only block the histamine effects but may also induce additional transductional signals in the T cell which will not be incited in H1R-KO animals. Additionally, the KO mice were backcrossed to the C56/BL6 (H2-b) background which differs from BALB/c mice (H2-d) used in our protocol not only in its MHC class II expression but also in the quality of allergen-induced immune responses [28].

The role of IgE-mediated mast cell degranulation and histamine for the development of bronchial asthma remains controversial. Several groups demonstrated in mouse models that the induction of eosinophilic AI and the development of AHR following allergen sensitization and airway challenges was independent of mast cells [29, 30], IgE production [31] or B cells [32]. In all of these studies, the experimental protocols included aluminium hydroxide as an adjuvant for sensitization, resulting in very strong inflammatory airway responses. In contrast, mice sensitized without adjuvant showed a milder degree of AI and AHR, that was dependent on the presence of B cells [15], IgE [33] and mast cells [30, 34, 35]. This shows that the development of AHR in models eliciting mild AI is rather IgE/mast cell/histamine dependent, whereas in protocols leading to strong inflammatory responses, it is more likely to be due to pure IL-5-mediated eosinophilia and rather histamine independent [30, 34, 35]. Accordingly, in the present study utilizing an experimental protocol without adjuvant, development of AI and AHR was inhibited by blockage of histamine-dependent functions. This notion is supported by data from clinical studies in which infants and small children with high risk to develop asthma were treated with H1R-antagonist leading to decreased incidence rates for asthma compared with the placebo group [12].

The higher dose of DL (1 μg/mouse) used in our protocol resembles the normal dose in man (70 μg/kg bodyweight). However, we also observed immune regulatory effects of DL after application of a much smaller dose of DL (0.01 μg/mouse = 0.5 μg/kg bodyweight). This may be explained by two mechanisms. First, the effect of DL may be transmitted primarily by blocking of the H1R, since the binding affinity of DL to this receptor is 50–200 times higher than that of other H1-blockers, like ceterizine or fexofenadine [20]. Second, the observed anti-inflammatory effects in our experiments may depend on other factors beside pure inhibition of histamine-induced reactions, like down-regulation of activation or co-stimulatory antigens on APCs or modulation of chemokine or adhesion molecule expression.

In conclusion, we hereby show for the first time that oral treatment of mice prior to and during allergen sensitization with an H1R-antagonist is effective in down-regulating allergen-mediated immune and airway inflammatory responses. This underscores an important immune modulating role of histamine, and suggests a possible role of H1R-antagonists such as DL as new preventive means for the treatment of allergic diseases.

Acknowledgements

We thank Kathrin Borgwald, Petra Ellensohn, Margret Oberreit-Menesis for their technical assistance. This work was supported by Schering-Plough and a grant of the German Research Foundation (DFG Ha 2162/2-1).

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