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_J Immunol_ 2004; 173:3281-3286; doi: 10.4049/jimmunol.173.5.3281

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Arthropod-Derived Histamine-Binding Protein Prevents Murine Allergic Asthma

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Because histamine receptor type 1 blockade attenuates allergic asthma, we asked whether complete neutralization of histamine by an arthropod-derived, high affinity histamine-binding protein (EV131) would prevent allergic asthma. Intranasal administration of EV131 given before Ag challenge in immunized mice prevented airway hyperreactivity by 70%, and abrogated peribronchial inflammation, pulmonary eosinophilia, mucus hypersecretion, and IL-4 and IL-5 secretion. Saturation with histamine abrogated the inhibitory effect of EV131 on bronchial hyperreactivity. The inhibitory effect of EV131 on bronchial hyperreactivity was comparable to that of glucocorticosteroids. These results demonstrate that histamine is a critical mediator of allergic asthma. Therefore, complete neutralization of histamine, rather than specific histamine receptor blockade, may have a profound effect on allergic asthma. The Journal of Immunology, 2004, 173: 3281–3286.

Allergic asthma is a chronic disease characterized by reversible obstruction of the airways, bronchial hyperresponsiveness (BHR), edema, infiltration of lungs by inflammatory cells, and mucus overproduction (1–3). Allergen-specific Th2 lymphocytes producing IL-4, IL-5, IL-13, IgE, and mucus overproduction are the hallmarks of asthma. In asthmatic individuals, a second contact with the sensitizing allergen results in early and late inflammatory responses. The early reaction is due to IgE-mediated mast cell degranulation, with the release of preformed bioactive molecules such as histamine and 5-hydroxytryptamine, which induce mucus hypersecretion, bronchoconstriction, and increased protein exudation. Although the initial phase may resolve, it is followed by a late-phase response characterized by infiltration of eosinophils and lymphocytes, accompanied by BHR. The repeated exposure to allergens promotes chronic inflammation, leading to the long-term sequellae of asthma.

Histamine, first identified as a potent vasoactive amine, is now recognized for its multiple regulatory activities in the respiratory, digestive, and immune systems, and CNS. Mast cells and basophils are the major producers of preformed histamine that they release from intracellular granules in response to Ag-mediated cross-linking of IgE receptors. Nevertheless, other cells release neo-formed histamine immediately after its synthesis, such as neutrophil, platelets (4), dendritic cells (DCs) (5), and T cells (6), pointing to the important role of this molecule. In lungs, mast cells are present in bronchial walls near vessels, in muscles, and in the bronchial lumen (7). Histamine was one of the first inflammatory mediators of allergic asthma recognized in human and guinea pig models (8).

Interestingly, histamine has been shown to modulate cytokine production in different cell types (9), Ab and T cell responses (10) maintaining a predominant Th2 response in allergic disorders (11). Histamine exerts its effects through four receptor subtypes: histamine receptor 1 (HR1) and HR2, both expressed on lymphoid and nonlymphoid cells; HR3, mainly expressed in the brain (12); and HR4, which has been described recently in leukocytes (13), but is also present in airways (14). However, the role of histamine as a critical effector molecule in a murine model of allergen-induced bronchoconstriction has been questioned, because histamine itself fails to induce direct bronchoconstriction under conditions in which 5-hydroxytryptamine and acetylcholine are effective (15). Furthermore, HR1 antagonists are not recognized as clinically effective therapy against asthma (16).

Although blood-feeding ectoparasites can cause histamine-mediated inflammation in the host, ticks have evolved to suppress inflammation and facilitate feeding by secreting histamine-binding proteins at the site of feeding (17–19). The tick Rhipicephalus appendiculatus secretes three histamine-binding proteins, which have been purified, cloned, expressed, and characterized (20). Their crystallographic structure and biological activity indicate that they sequester histamine, competing with HRs for histamine binding. Both histamine-binding proteins rEV131 and rEV504 bind histamine with high affinity. However, rEV131 has a distinctive feature because it presents a second specific binding site for histamine with lower affinity than the high affinity binding site (20, 21).

In this study, using rEV131, we demonstrate that neutralization of histamine inhibits experimental allergic asthma, suggesting that histamine is a critical mediator in its pathogenesis, and opening new perspectives for asthma therapy.

Materials and Methods

Animals and immunization

The BP2 strain of mice was obtained from Janvier, France (15, 22). The local ethics committee approved all protocols used in this study. The mice, aged 6–8 wk, were immunized s.c. twice at weekly intervals with a 0.4 ml saline containing 100 μg of OVA and 1.6 mg of alum for the initial experiments, and for subsequent experiments the Ag dose was reduced to 1 μg of OVA per injection. One week after the second immunization, at day 14, intranasal challenge was performed under light i.v. ketamine anesthesia by applying 50 μl of OVA in alum-free saline solution (10 μg) or saline alone as a control.
The rEV131 protein (340 μg, m.w. 20,406; Evolutec) or budesonide (375 μg, m.w. 430.5; AstraZeneca, Uppsala, Sweden), a potent corticosteroid (positive control), was administered intratracheally (50 μl in saline buffer) under ketamine anesthesia, 1 h before the Ag challenge and/or immediately before the OVA challenge. However, a single administration of EV131, 1 h prior to challenge, proved to be equally effective. We chose the intratracheal route for the administration of the protein and budesonide to assure optimal airways deposition, although the intranasal administration was active. To ascertain that the effect is due to histamine scavenging, histamine-saturated EV131 (incubation at molar ratios 1 and 2 for 30 min of histamine and EV131) was compared with native EV131 for inhibition of BHR (histamine dihydrochloride, m.w. 184; Sigma-Aldrich, St. Louis, MO). The HR antagonists, mepyramine (340 μg, m.w. 402; Sigma-Aldrich), cimetidine (340 μg, m.w. 409; Sigma-Aldrich), and thioperamide (340 μg, m.w. 409; Sigma-Aldrich) were administered intranasally in 50 μl with saline buffer 1 h before the Ag challenge.

**Airways resistance**

The airways resistance was evaluated by whole-body plethysmography (2). BHR to aerosolized methacholine was investigated at several time points after OVA challenge. Unrestrained conscious mice were placed in whole-body plethysmography chambers (Buxco Electronics, Sharon, CO). Mice were ventilated on high oxygen conditions to avoid hypoxemia induced by methacholine administration. Methacholine at 50 mM was aerosolized for 1 min, and mean airway bronchoconstriction readings, as assessed by enhanced respiratory pause (Penh), were obtained over 15-min periods. Penh can be conceptualized as the phase shift of the thoracic flow and the nasal flow curves; increased phase shift correlates with increased respiratory system resistance.

Penh is calculated by the formula Penh = (Te/RT - 1) × PEF/PIF, where Te is expiratory time, RT is relaxation time, PEF is peak expiratory flow, and PIF is peak inspiratory flow.

The mouse is placed in barometric plethysmography with two chambers linked to suction pump, which ensure constant air flow. The intratracheal route for the administration of the protein and budesonide was stopped with sulfuric acid 2 N, and the plates were read at 450 nm with an automatic microplate reader. The lower limits of detection of these assays are ~10 pg of IL-4/ml sample and 5 pg of IL-5/ml.

**Statistical analysis**

Data are presented as means and SEM indicated by error bars. Statistical significance was determined by Student’s t test. Values of p < 0.05 were considered statistically significant.

**Results**

The histamine-binding protein EV131 prevents BHR in Ag-sensitized mice

Because histamine is produced during allergic asthma, we tested the potential interference of the arthropod-derived histamine-binding protein EV131 on BHR. Upon Ag challenge, the mice developed a robust BHR in response to aerosolized methacholine at 24 h (Fig. 1A). Administration of EV131 by the intratracheal route, 1 h and just before OVA challenge, inhibited methacholine-induced bronchoconstriction (70% of controls; p < 0.01). To test whether the inhibitory effect is related to histamine scavenging, we compared histamine-presaturated EV131 and native EV131 on BHR. As expected, the inhibitory effect of presaturated EV131 with a 2-molar excess of histamine was abrogated (Fig. 1B). EV131 presaturated at equimolar histamine showed loss of activity as compared with native EV131 on BHR of OVA-challenged mice, suggesting that the two histamine pockets are implicated in histamine scavenging.
Therefore, in situ neutralization of histamine by EV131 acting as a soluble receptor with high affinity binding for histamine had a profound effect on BHR, and we therefore asked whether the recruitment of eosinophils during the allergic asthma was influenced.

Reduction of pulmonary inflammation and of the recruitment of eosinophils by EV131

Ag challenge caused a substantial recruitment of inflammatory cells into the BAL fluid at 72 h. Administration of EV131 before OVA challenge reduced significantly the numbers of mononuclear cells in the BAL fluid ($p < 0.05$; Fig. 2A). Budesonide had a slightly more pronounced effect ($p < 0.01$; Fig. 2A). Only few eosinophils and neutrophils were found in the BAL fluid of saline-challenged mice. By contrast, the Ag challenge resulted in a significant increase of eosinophil counts in the BAL fluid ($p < 0.01$; Fig. 2B). EV131 administration before the Ag challenge prevented largely the recruitment of eosinophils ($p < 0.01$;Fig. 2B). Furthermore, we demonstrated that the prevention of eosinophil recruitment was due to histamine scavenging, as histamine-presaturated EV131 was ineffective (data not shown). Finally, budesonide had an inhibitory effect on the recruitment of all cell types ($p < 0.01$; Fig. 2B). Representative cytospin preparations of BAL obtained from saline control or OVA-challenged mice in the presence or absence of inhibitors are shown in Fig. 3.

We further investigated the recruitment of eosinophils and other inflammatory cells in lung tissue sections from OVA-sensitized and challenged mice. OVA challenge in immunized mice caused peribronchial cell recruitment and hyperplasia of the bronchial smooth muscle and of goblet cells containing mucus (Fig. 4). Administration of EV131 reduced significantly the peribronchial eosinophilia, mucus hypersecretion, and hyperplasia of bronchial smooth muscles (Fig. 4). Therefore, complete in vivo neutralization of histamine with the high affinity histamine-binding protein EV131 inhibited the inflammatory cell recruitment and suppressed the characteristic allergic inflammation of the airways.

EV131 reduced IL-4 and IL-5 levels in BAL fluid

Allergic asthma involves the recruitment of Th2-biased T cells, resulting in increased production of Th2 cytokines. Ag challenge of immunized mice resulted in increased levels of IL-4 and levels of IL-5 (Fig. 5; $p < 0.01$) in the BAL fluid. EV131 given before Ag challenge inhibited significantly IL-4 and IL-5 (Fig. 5; $p < 0.05$) levels. The steroid control budesonide was slightly more effective than EV131 in inhibiting the production of IL-4 and of IL-5 ($p < 0.01$). Therefore, neutralization of histamine with EV131 appears to inhibit the allergen-induced Th2 cytokine response.

Duration of the inhibitory effect of EV131 on BHR and comparison with HR blockade

We further investigated the duration of the effect of EV131 on BHR. The inhibitory effect of EV131 was maintained for 48 h after OVA challenge in all mice (Fig. 6A) and, although reduced, was still present at 72 h, when animals received two intratracheal administrations of EV131 (Fig. 6B). Thus, EV131 administered before Ag challenge has a persistent effect.

We finally studied the efficacy of classical HR antagonists in the murine model of allergic asthma. Mepyramine, a selective HR1 antagonist and thioperamide, with mixed HR3 and HR4 antagonistic activity, reduced BHR (Fig. 7). Cimetidine, a classical HR2 antagonist, had no effect on BHR. Therefore, HR1, HR3, and possibly HR4 antagonists have inhibitory effects on BHR, but full inhibition requires on a molar basis larger amounts. Using EV131, which sequesters histamine, may be an interesting and effective alternative approach to inhibit BHR.

Discussion

We show in this study that the high affinity histamine-binding protein EV131 prevents experimental allergic asthma, supporting a critical role for histamine in Ag-induced BHR and bronchial inflammation. It is reasonable to assume that EV131 by locally sequestering histamine prevents the access to its receptors.
observations, we confirm in our system that a high dose of HR1 mice has already been reported (24). In agreement with previous HR1 and HR4 (12, 23). Modulation of BHR by HR antagonists in mucosal secretion, plasma exudation, and vasodilatation, essentially via decreases contraction and hyperplasia of airway smooth muscles, muscular eosinophil infiltration (C). H&E staining. EV131 inhibits mucosal secretion in mice challenged with OVA (arrows mark peribronchial infiltrate by eosinophils). EV131 inhibits peribronchial and perivascular eosinophil infiltration in mice challenged with OVA (arrows mark peribronchial infiltration by eosinophils). EV131 inhibits peribronchial and perivascular eosinophil infiltration (C). H&E staining. EV131 inhibits mucus secretion in lungs of OVA-challenged mice. Mucus secretion changes in lungs of saline (D)- and OVA (E)-challenged mice: significant mucus secretion in mice challenged with OVA (arrows mark mucus secreted by epithelial cells). EV131 inhibits mucus secretion in mice challenged with OVA (F). Periodic acid Schiff mucus staining. Intermediate power micrographs are shown on A, C, D, E, and F (magnification ×200), and a higher magnification shows eosinophil infiltration on B (magnification ×400). These micrographs are representatives of three independent experiments, with four mice per group each.

Histamine plays a critical role in allergic asthma because it induces contraction and hyperplasia of airway smooth muscles, mucus secretion, plasma exudation, and vasodilatation, essentially via HR1 and HR4 (12, 23). Modulation of BHR by HR antagonists in mice has already been reported (24). In agreement with previous observations, we confirm in our system that a high dose of HR1 antagonist mepyramine blocks BHR, whereas HR2 antagonist cimetidine does not share this property. High dose of thioperamide, which has HR3 and HR4 antagonistic properties, also inhibited BHR in the murine model. Despite sequence homology and structural similarities, HR3 and HR4 have distinct pattern of expression. Indeed, HR4 subtype is essentially expressed on eosinophils, DCs, basophils, Th cells, mast and B cells, and in human lung cells, including fibroblasts, smooth muscle, epithelial, and endothelial cells (13, 25–27), whereas HR3 subtype is mainly located in the brain (28). Prevention of BHR by thioperamide and the specific expression pattern of HR4 subtype suggest that stimulation of this receptor is involved in allergic disorders. It can be postulated that the profound effects induced by the histamine-binding protein EV131 are in part due to the fact that HR4 is not activated, in addition to suppression of the other HR. The presently available HR antagonists are of limited clinical use, because the high doses required to inhibit allergic asthma lead to unacceptable adverse effects (29).

EV131 has a high affinity binding for histamine, as no competition was observed with other related compounds such as the HR1 antagonist mepyramine and the HR2 antagonist cimetidine in vitro assays (20). EV131 binds histamine in a competitive manner toward the HR1 on the smooth muscle cells of guinea pig ileum (20). Furthermore, EV131 inhibits histamine, but not serotonin- or bradykinin-induced contraction of guinea pig ileum. The effect of EV131 is dose dependent, and saturating concentrations of histamine or preincubation of EV131 with histamine abrogate the inhibitory effect of EV131 (G. Paesen, unpublished observations).

To ascertain that the effect on the allergic lung model is due to

FIGURE 4. EV131 inhibits inflammation and mucus hypersecretion in OVA-challenged mice. Inflammatory changes of lungs of saline (A)- and OVA (B)-challenged mice: significant peribronchial and perivascular eosinophil infiltration in mice challenged with OVA (arrows mark peribronchial infiltration by eosinophils). EV131 inhibits peribronchial and perivascular eosinophil infiltration (C). H&E staining. EV131 inhibits mucus secretion of lungs in OVA-challenged mice. Mucus secretion changes in lungs of saline (D)- and OVA (E)-challenged mice: significant mucus secretion in mice challenged with OVA (arrows mark mucus secreted by epithelial cells). EV131 inhibits mucus secretion in mice challenged with OVA (F). Periodic acid Schiff mucus staining. Intermediate power micrographs are shown on A, C, D, E, and F (magnification ×200), and a higher magnification shows eosinophil infiltration on B (magnification ×400). These micrographs are representatives of three independent experiments, with four mice per group each.

FIGURE 5. Reduced IL-4 and IL-5 in BAL fluid by EV131 administration. IL-4 (A) and IL-5 (B) levels were measured in the BAL fluid by ELISA 24 h after NaCl or OVA challenge. Significant increased IL-4 and IL-5 levels were found in mice challenged with OVA vs NaCl. EV131 inhibits IL-4 and IL-5 secretions in mice challenged with OVA. Mean values and SEM are given from two independent experiments (eight mice per group), $p < 0.05$ (*) and $p < 0.01$ (**) 

FIGURE 6. The inhibitory effect of EV131 on BHR is effective for 48 and 72 h after OVA challenge. OVA- compared with NaCl-challenged mice still exhibit BHR to methacholine at 48 h (A) and 72 h (B) postchallenge. EV131 (340 μg) administration still inhibits this BHR at 48 h (A) and at 72 h (B). Mean values with SEM are given, and the results are representative of two independent experiments (n = 4 mice per group). $*, p < 0.05$. Inhibition by EV131 at 48 h: $p < 0.01$ (**) for time 2 min, and thereafter $p < 0.05$ (*) (A). Inhibition by EV131 at 72 h: $p < 0.05$ (*) from 5 min onward (B).
histamine scavenging, we tested presaturated EV131. We showed that the histamine-saturated EV131 lost its inhibitory activity on BHR and eosinophil recruitment.

These findings account for the ability of EV131 to sequester histamine. Indeed, the crystal structure of EV131 reveals two high affinity sites for histamine binding (20). Allergen challenge enhances histamine release upon OVA-specific IgE cross-linking on mast cell and subsequent degranulation with release of histamine in sensitized mice, leading to bronchoconstriction, eosinophilia, and mucus hypersecretion (30). Histamine may also induce maturation of DCs, which polarizes naïve CD4+ T cells toward a Th2 phenotype, altering the repertoire of cytokines and chemokines secreted by mature DCs, as compared with DCs that have matured before challenged with OVA. Penh values were measured 24 h after OVA challenge (n = 8 mice per group). Inhibition by mepyramine: p < 0.01 between 3 and 6 min, and thereafter p < 0.05 (*). Inhibition by thioperamide: p < 0.01 between 2 and 6 min, and thereafter p < 0.05 (*).

Acknowledgments

We gratefully acknowledge Drs. Valerie Quesniaux and Marie-Noëlle Soler for corrections and suggestions, and Thomas Roberts for excellent technical assistance.

References


