

IgE-mediated 15-hydroxyeicosatetraenoic acid (15-HETE) generation by peripheral blood leukocytes: its association with basophil activation

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Abstract

Introduction: Allergen-induced basophil activation has been associated with the release of several mediators and with an increased expression of CD203c molecules on basophils.

Aim: To assess the influence of specific allergens on the generation of 15-hydroxyeicosatetraenoic (15-HETE) from peripheral blood leukocytes in relation to basophil activation, on the basis of CD203c molecule expression and histamine release.

Material and methods: The study included 15 patients with clinical symptoms of birch pollen allergy confirmed by a positive skin prick test with the birch allergen, and 6 healthy controls. Leukocytes isolated from peripheral blood were incubated with 3 concentrations of the birch pollen allergen (*Bet v 1*), anti-IgE or with ionophore A23187.

Results: *In vitro* challenge of leukocytes from allergic patients with 1 ng/ml of allergen induced a significant increase in 15-HETE generation. An increase above 30% was observed in almost half the allergic patients, with mean values ranging from 40% to 46%, but not in healthy controls. Anti-IgE antibodies increased 15-HETE generation in 5 patients (termed IgE+), and the allergen induced a significant increase in 15-HETE in all patients who reacted to anti-IgE. The mean CD203c expression on basophils of the allergic patients increased after allergen challenge, but a significant increase (> 30%) was observed only in patients who demonstrated an increased expression after anti-IgE exposure. A significant correlation was seen between 15-HETE generation and histamine release induced by the highest concentration of the allergen ($r = 0.95$; $p < 0.01$).

Conclusions: Allergen-induced, IgE-mediated activation of basophils is associated with a significant increase in 15-HETE generation.

Key words: basophil activation, birch allergy, CD203c, 15-hydroxyeicosatetraenoic.

Introduction

Allergy to birch pollen, clinically manifesting as rhinitis, conjunctivitis or bronchial asthma, is associated with the immunoglobulin E (IgE)-related inflammatory process. Allergen-induced bridging of the IgE antibodies, followed by their binding to high affinity FcεRI receptors on mast cells and basophils, results in cell activation and the release of several preformed and newly-synthesized mediators which induce allergy symptoms [1]. Basophils and mast cells act not only as effector cells during the early phase of the allergic reaction, but also contribute to the development of chronic allergic inflammation [1–3].

The activation of basophils and mast cells during hypersensitivity reactions is associated with the enhancement of the arachidonic acid metabolism and the generation of cyclooxygenase (prostaglandins and thromboxanes) and 5-lipoxygenase (cysteinyl leukotrienes) metabolites [3].

15-lipoxygenase (15-LO) is an enzyme which metabolizes arachidonic acid to 15-hydroxyeicosatetraenoic acid (15-HETE), which is a chemically active metabolite characterized by a short half-life. It has been documented that unstimulated peripheral blood leukocytes produce 15-HETE, and this production may be enhanced by

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aspirin in aspirin-hypersensitive, but not in aspirin-tolerant, patients [4]. It has not been assessed whether allergen-induced basophil activation is associated with the release of 15-HETE.

Traditionally, activation of basophils by the allergen *in vitro* has been assessed by measurement of histamine release, which is a highly-specific marker of activation [5]. More recently, measurement of the expression of CD63 and CD203c molecules on the surface of leukocytes has been used to assess basophil activation [6]. The CD203c molecule is expressed on the surface of basophils, mast cells and their precursor CD34⁺ cells, and its expression is rapidly increased after the cells have been activated [7–10].

Aim

The aim of the study was to assess the influence of specific allergens on the generation of 15-hydroxyeicosatetraenoic (15-HETE) from peripheral blood leukocytes in relation to basophil activation, on the basis of CD203c molecule expression and histamine release.

Material and methods

The study included 15 patients allergic to birch pollen: 9 females aged 22–60 years (mean age 34.6 ± 4.7) and 6 males aged 23–42 years (mean age 25.4 ± 3.8). All the patients were diagnosed with seasonal rhinitis and conjunctivitis. One patient was diagnosed with atopic bronchial asthma. Birch pollen allergy was confirmed with a positive skin prick test and conjunctival provocation test with *Bet v 1* allergen. The study was carried out before the birch pollen season, and at the time of the study, the patients were not taking any antihistamine drugs or nasal glucocorticosteroids. The control group consisted of 6 healthy subjects with negative skin prick tests to airborne allergens: 2 females, aged 28–58 years (mean: 43.0 ± 15.0) and 4 males, aged 22–34 years (mean: 27.6 ± 2.31 years).

Leukocytes were isolated from 9 ml of peripheral blood in anti-coagulant EDTA according to the protocol by Benveniste [11]. The blood sample was centrifuged for 5 min at 200× *g* at 4°C. The plasma was collected into a separate tube and centrifuged for 10 min at 500× *g* at 4°C. After removal of plasma, commercial reagent kit (Uti – Lyse™, Erythrocyte – Lysing Reagent, Dako Glostrup, Denmark) was used for lysis of erythrocytes from obtained sediment, cell pellet was resuspended in 900 µl of cold PBS (buffered saline with Ca²⁺ and Mg²⁺, Biomed Lublin). After isolation, the cells were incubated for 45 min at 37°C with anti-IgE (Monoclonal Antibody Anti-Human IgE (D&2), Beckman Coulter), ionophore A23187 (Calcium ionophore, Calbiochem, A23187, Merc) or with *Bet v 1* allergen at 3 concentrations: 1, 10 or 100 ng/ml. After centrifuging the samples for 5 min (200× *g*, at 4°C), the supernatant was separated from the cell precipitate and stored at –20°C. The histamine and 15-HETE concentrations were measured by ELISA

(Assay Designs, Inc. Hines Drive Ann Arbor, MI 48108). The histamine test was considered significant for values more than 60% above baseline, 15-HETE increase was considered significant at over 30% above the baseline and an increase in G-mean was also considered significant when 30% above the baseline.

The expression of CD203c molecules, expressed as the G-mean fluorescence, was evaluated with flow cytometry, using Flow Max software. In brief, cells were incubated with 100 µl of cold buffered saline with Ca²⁺ and Mg²⁺ ions (Biomed Lublin) and 20 µl of anti-CD203c monoclonal antibodies stained with phycoerythrin (PE-Rycoer-phycoerythrin) (CD203c-PE, PN IM3575, 97A6, Immunotech). Next, the samples were incubated for 60 min at 4°C and then analyzed by flow cytometry: at least 50,000 cells were analyzed. Based on reported methodology, a 30% increase in CD203c molecule count over non-stimulated controls was considered to be significant [10, 12].

Statistical analysis

The Mann-Whitney *U* test was applied to analyze the differences between groups. The Spearman's rank correlation coefficient was used to evaluate the relationship between CD203c and 15-HETE production, and the release of histamine. All statistical analyses were performed with the Statistica software package. The level of statistical significance was $p \leq 0.05$.

Results

Release of 15-HETE from leukocytes.

The stimulation of leukocytes with *Bet v 1* allergen increased the mean concentration of 15-HETE in leukocyte supernatants from all allergic patients, but the increase was significant only for the samples subjected to 1 ng/ml of allergen: from a mean value of 6.93 ± 1.55 ng/ml to 8.32 ± 1.80 ng/ml ($p = 0.03$) (Figure 1). An increase in 15-HETE generation of more than 30% was observed in almost half the patients: 7 of them (46.7%) after stimulation with 1 ng/ml of allergen, 6 (40%) after stimulation with 10 ng/ml of allergen, and 7 (46.7%) after stimulation with 100 ng/ml of allergen. After cell stimulation with anti-IgE, the mean level of 15-HETE significantly increased from a mean value of 6.93 ± 1.55 ng/ml to 9.60 ± 3.70 ng/ml ($p < 0.01$). An increase greater than 30% was observed in 5 of the 15 (33.3%) patients and these patients have been defined as the anti-IgE responders (anti-IgE(+)), while the remaining patients assigned as anti-IgE non-responders (anti-IgE(-)). Retrospective analysis revealed that all anti-IgE(+) patients released 15-HETE in response to the allergen at all three concentrations: The 15-HETE concentration increased from baseline 4.21 ± 2.87 ng/ml to 11.18

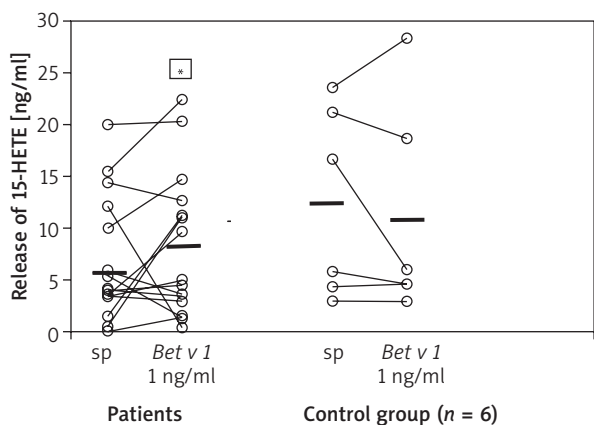


Figure 1. The release of 15-HETE acid in patients after stimulation with birch allergen *Bet v 1* ($*p = 0.03$)

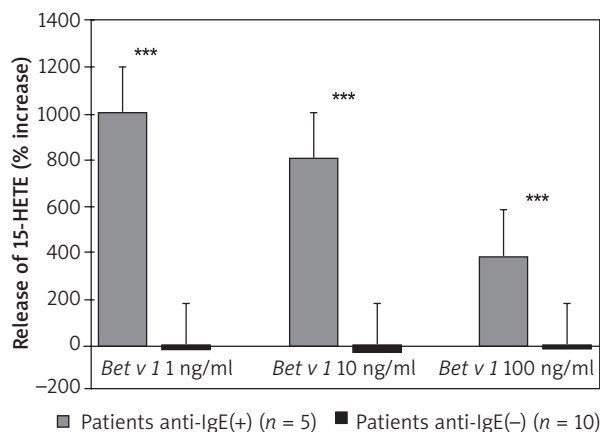


Figure 2. Allergen-induced increase in 15-HETE generation in anti-IgE(+) and anti-IgE(-) patients ($***p < 0.01$)

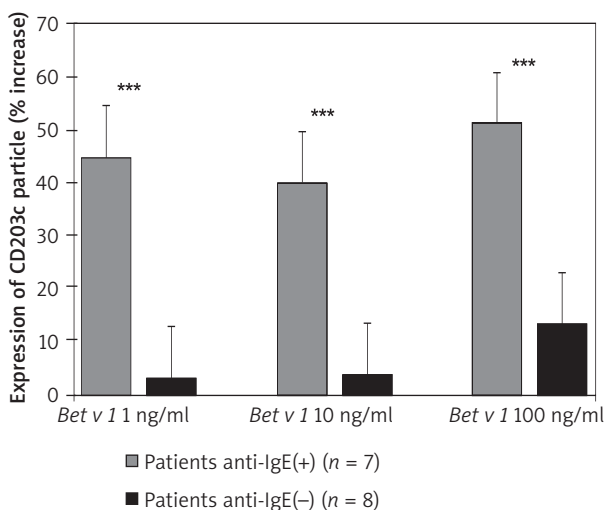


Figure 3. Allergen-induced increase in CD203c molecule expression in anti-IgE(+) and anti-IgE(-) patients ($***p < 0.01$)

± 3.35 ng/ml after 1 ng of allergen, 10.55 ± 3.70 ng/ml after 10 ng of allergen and 7.77 ± 4.09 ng/ml after 100 ng of allergen (Figure 2). No change in mean 15-HETE generation was noticed in anti-IgE(-) patients after allergen challenge. In the group of healthy subjects, neither the allergen at any of the three different concentrations, nor the anti-IgE induced any significant increase in mean 15-HETE generation.

Expression of CD 203c molecule on basophils

As compared to unstimulated cells (G-mean value: 15.33 ± 0.87) all three concentrations of the allergen induced significant increases in CD203c expression: 18.25 ± 1.09 ($p < 0.01$) for 1 ng/ml; 17.73 ± 1.10 ($p = 0.02$) for 10 ng/ml and 19.29 ± 0.97 ($p < 0.01$) for 100 ng/ml. An

increase greater than 30%, which was considered significant, was observed in 7 (46.7%) of 15 patients after the stimulation of cells with 1 ng/ml of allergen, in 6 of 15 (40%) patients after 10 ng/ml of allergen and in 8 of 15 (53.3%) patients after 100 ng/ml of allergen. However, in samples from the control group of non-allergic subjects, the incubation of leukocytes with allergen did not affect the mean expression of CD203c marker (Figure 3).

Cell stimulation with anti-IgE resulted in a significant increase in G-mean, above 30% from baseline, in 7 of 15 (44.7%) subjects. All anti-IgE(+) patients demonstrated an increase in expression of G = mean greater than 30% in response to all three allergen concentrations: in anti-IgE(-) patients, an increase in CD203 G-mean was observed only after the highest allergen concentration (Figure 3).

An increase in G-mean by more than 30% was also observed in 2 of 6 (33%) healthy respondents after stimulation with anti-IgE. These subjects failed to show any increase in CD203c after allergen exposure.

Histamine release from basophils

In allergic patients, the release of histamine significantly increased from baseline, 4.75 ± 1.11 ng/ml, to 30.77 ± 8.38 ($p < 0.01$) after exposure to 1 ng/ml of allergen, to 25.06 ± 8.04 ($p < 0.01$) after exposure to 10 ng/ml of allergen and to 35.94 ± 10.66 ($p < 0.01$) after exposure to 100 ng/ml of allergen. A significant increase in histamine release, over 60%, was observed in 12 of the 15 (75%) patients after application of 1 ng/ml of allergen, in 11 (73.3%) patients after 10 ng/ml of allergen and in 13 (86.6%) patients after 100 ng/ml of allergen. All but 1 patient demonstrated a significant release of histamine in response to anti-IgE.

All anti-IgE(+) patients demonstrated a release of histamine 60% greater than the mean baseline value, 4.75

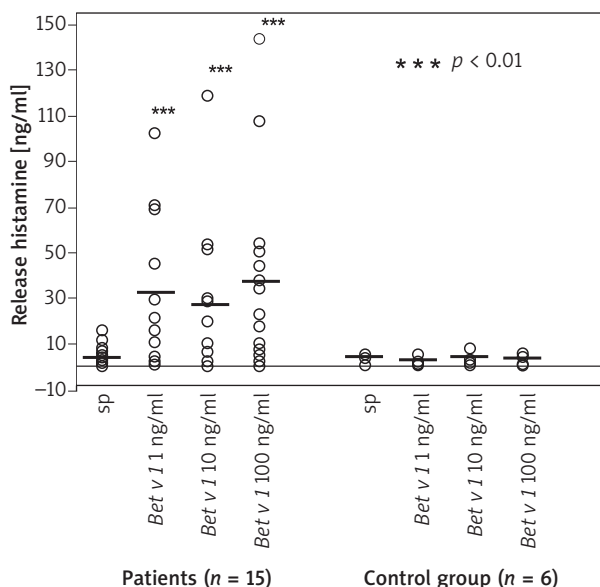


Figure 4. Allergen-induced histamine release in allergic patients and healthy controls

± 1.11 ng/ml, in response to the allergen at all three concentrations: to 30.77 ± 8.62 ng/ml after 1 ng/ml of allergen, to 25.06 ± 8.04 ng/ml after 10 ng/ml of allergen and to 35.94 ± 10.66 ng/ml after 100 ng/ml of allergen. The anti-IgE(-) patient did not demonstrate any histamine release after allergen challenge. Stimulation of leukocytes from the healthy subjects with the birch allergen did not affect histamine release (Figure 4).

Correlation between 15-HETE generation and basophil activation markers

A significant correlation was observed between 15-HETE production and histamine release induced by the highest concentration of the allergen ($r = 0.95$; $p < 0.01$) (Figure 5). There was a modest correlation between CD203C expression and histamine release after stimulation with the allergen ($r = 0.22$; $p = 0.03$). No correlation was seen between allergen-induced 15-HETE production and CD203C expression ($r = -0.004$; $p = 0.09$).

Discussion

This is the first study to demonstrate that peripheral blood leukocytes, presumably basophils, from allergic patients generate 15-HETE when activated with a specific allergen. It is assumed that in this model, the allergen activates basophils by bridging specific IgE molecules on the surface of the cells, which triggers IgE-mediated cell activation typical of the allergic reaction. Accordingly, no increase was observed in the production of 15-HETE after allergen exposure in the control group of non-allergic subjects. As the study was performed on a hetero-

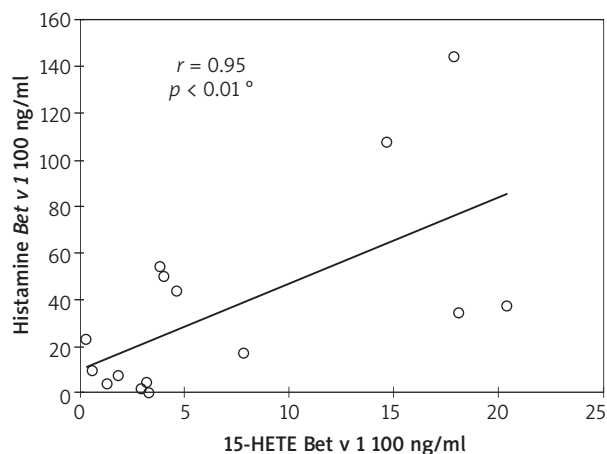


Figure 5. Correlation between allergen-induced increase in 15-HETE generation and release of histamine (100 ng/ml allergen concentration)

geneous population of cells, it cannot be excluded that other inflammatory cells were activated by the allergen: 15-HETE can be generated both by mononuclear cells and granulocytes. However, the strong correlation observed between the production of 15-HETE and increased release of histamine ($r = 0.95$), which is the most specific activation marker for basophils, implied that 15-HETE originated from the IgE-activated basophils. The increased synthesis of 15-HETE seen after cell incubation with anti-IgE, which activates basophils by linking IgE molecules bound with Fcε receptors, regardless of the specificity of the IgE molecules, further evidences that the mechanism of 15-HETE generation is IgE dependent.

The peripheral blood leukocytes from the subjects allergic to birch pollen demonstrated significant individual differences regarding the release of 15-HETE, and a specific allergen induced a significant increase in 15-HETE production only in approximately half the patients. Such variability might result from the individual “releasability” of basophils. This releasability of basophils depends primarily on the number of IgE molecules bound to the Fc receptors on the basophils, but also on intrinsic intercellular biochemical mechanisms related to Fc receptor signaling pathways which allow “releasers” to be distinguished from “non-releasers” [12].

Both inpatients from “responders” and “non-responders” groups, blood samples were collected from September to December, between the tree pollen seasons in Poland, when the birch pollen allergen *Bet v 1* is absent in the atmosphere.

Significant generation of 15-HETE was detected in only half the patients after *in vitro* challenge of leukocytes with anti-IgE. These patients were termed *algE(+)*. Retrospective analysis revealed that all anti-IgE(+) patients released 15-HETE in response to all three allergen concentrations, while anti-IgE(-) patients did not

show any increase in 15-HETE. This observation further confirms that the mechanisms of allergen-induced 15-HETE generation from basophils in allergic patients are dependent on IgE. There was a full pool of leukocytes varying in terms of quality isolated in our study, which was a potential source of 15-HETE in the tested samples. In the course of an allergic reaction, mediators are released from granules. At the same time cellular enzymes may be released. They can potentially modulate the activation of the cells, similarly to histamine itself, which if released as a feedback path, may affect the arachidonic acid cascade. Histamine may also modulate the expression of COX cyclooxygenase pathway which is associated with the release of 15-HETE. This mechanism has not been elucidated and require further research related to the use of COX inhibitor (i.e. Celecoxib).

Similar heterogeneity was observed in response to allergen exposure when flow cytometry was used to measure basophil activation according to membrane expression of CD203c [13, 14]. In this study, CD203c expression, expressed as the geometric mean of fluorescence intensity (G-mean), was shown to correlate with the percentage of cells demonstrating CD203c expression [15–17]. Again, CD203c expression was found to be enhanced by allergen stimulation in only about half the patients, and anti-IgE(+) patients demonstrated a significantly stronger response to the allergen than anti-IgE(–) patients. Histamine release appeared to be more sensitive than the first two cell activation markers (15-HETE and CD 203c): at the maximum concentration of the allergen, almost 90% of the patients demonstrated a significant increase in histamine release. A strong correlation was seen between increased 15-HETE generation and histamine release from basophils, strongly implicating basophils as a cellular source of 15-HETE.

Lipid mediators, specifically cysteinyl leukotrienes and prostaglandins, are important mediators of IgE mediated allergic reactions and have been also associated with allergen-induced basophil activation [18–21]. 15-HETE, which is generated from arachidonic acid, predominantly via the 15-lipoxygenase pathway, is a potent mediator of inflammation with several pro-allergic activities, which include smooth muscle constriction, increased microvascular permeability, mucus secretion, and leukocyte chemotaxis [22]. However, very little information is available on the role of 15-lipoxygenase metabolites in allergic reactions and basophil activation. IgE dependent release of 15-HETE was demonstrated originally in dog mastocytoma cells, but no other study has addressed this phenomenon since then Phillips *et al.* [23]. Expression of 15-LO may be increased in the BAL of patients with atopic asthma and in the airway mucosa of patients with severe asthma [24, 25]. A more recent study demonstrates that 15-HETE is released in the tear fluid of pollen-allergic patients, but no consistent increase in generation of 15-HETE was observed following local chal-

lenge with allergen [26]. Our current study indicates that 15-HETE may be released from basophils, and possibly mast cells, following stimulation with a specific allergen, and should be reconsidered as a potential mediator of IgE-mediated allergic reactions.

In conclusion, our study demonstrates that a significant increase in the release of 15-HETE occurs from leukocytes enriched with a basophil fraction after allergen stimulation. A highly significant correlation found between the release of histamine and the generation of 15-HETE after allergen stimulation suggests that 15-HETE plays a possible role in the immediate allergenic reaction. The mechanism of acid release 15-HETE from peripheral blood leukocytes after induction of a specific allergen has not been elucidated so far. Also, there are no research data on the real source of the release of this mediator after induction with a specific allergen. The 15-HETE probably is released *de novo* from phospholipid membranes after activation of specific enzymes in the cell [27, 28].

The release of 15-HETE in high concentrations from leukocytes after induction of a specific allergen *Bet v 1 in vivo* is responsible for the dynamics of the inflammatory reaction. A high concentration of this mediator is responsible for the inhibition of 5-LOX and the 5-lipoxygenase participates in the formation of leukotrienes LTC₄ and LTB₄ from arachidonic acid. The strongest pro-inflammatory effect is characteristic of leukotrienes derived from arachidonic acid. Leukotriene B₄ is the most active factor which modulates the chemotaxis of neutrophils. Leukotrienes C₄, D₄, E₄ are mediators of inflammation and are involved in allergic process. If these mechanisms are suppressed the consequence is the increased development of inflammation in the upper respiratory tract.

Other researchers [29] argue that mediators synthesized by the cyclooxygenase pathway can potentially affect the course and severity of the inflammatory response in the airways. They confirm that the long-term mediator stimulation may lead to structural changes in the epithelial cells, which is reflected in the airway remodeling.

Conclusions

These results suggest that 15-HETE, elicited by the IgE-dependent pathway and generated by basophils, may play a role as a mediator of allergic reactions. This observation should prompt further studies on the role of 15-lipoxygenase pathways in IgE-mediated allergic reactions.

Conflict of interest

The authors declare no conflicts of interest.

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