

RESEARCH ARTICLE

Effects of venom immunotherapy on serum level of CCL5/RANTES in patients with *Hymenoptera* venom allergy

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Abstract

Hymenoptera venoms are known to cause life-threatening IgE-mediated anaphylactic reactions in allergic individuals. Venom immunotherapy is a recommended treatment of insect allergy with still the mechanism not being completely understood. We decided to assess the serum CCL5/RANTES level in patients who experienced severe anaphylactic reaction to *Hymenoptera* venom and to find out changes in the course of immunotherapy. Twenty patients (9 men, 11 women, mean age: 31.91 ± 7.63 years) with history of anaphylactic reaction after insect sting were included into the study. Diagnosis was made according to sIgE and skin tests. All of them were enrolled into rush venom immunotherapy with bee or wasp venom extracts (Pharmalgen, ALK-Abello, Horsholm, Denmark). Serum levels of CCL5/RANTES were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN). CCL5/RANTES serum concentration are higher in insect venom allergic patients than in healthy controls (887.5 ± 322.77 versus 387.27 ± 85.11 pg/ml). Serum concentration of CCL5/RANTES in insect venom allergic patient was significantly reduced in the course of allergen immunotherapy already after 6 days of vaccination (887.5 ± 322.77 versus 567.32 ± 92.16 pg/ml). CCL5/RANTES serum doesn't correlate with specific IgE. Chemokine CCL5/RANTES participates in allergic inflammation induced by *Hymenoptera* venom allergens. Specific immunotherapy reduces chemokine CCL5/RANTES serum level already after initial days of venom immunotherapy.

Keywords

Allergen immunotherapy, CCL5/RANTES, chemokine, *Hymenoptera* venom allergy, IgE

History

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Introduction

Hymenoptera venoms are known to cause life-threatening IgE-mediated anaphylactic reactions in allergic individuals. Up to 3% of an exposed population may give a history of immediate systemic allergic reaction to an insect sting.

Anaphylactic reactions to insect stings may range from skin symptoms only to life-threatening pulmonary or cardiovascular events¹. Symptoms usually start within 5–15 min after the sting, but they may develop within 1 min or as late as several hours after the sting. Though insect sting allergy is well-documented, the mechanisms involved in this process are not fully clear. Changes in the immune response to bee venom have been recognized in venom allergic patients.

Immunotherapy with insect venom (VIT) is an established therapy for the treatment of patients with severe IgE-mediated hypersensitivity to *Hymenoptera* insects. There is no doubt about its clinical efficacy. Immunotherapy with wasp and honey bee allergens leads to a variety of specific immunological changes, but we still do not fully understand mechanisms of successful immunotherapy. Positive result of

immunotherapy is associated with the induction of peripheral tolerance in T cells by the generation of T regulatory cells that secrete suppressive cytokines, IL-10 and TGF- β . Evidence of parallel down-regulation of allergen-specific CD4⁺ Th2 responses in favor of Th1 responses in the periphery and reactivity in target organs was highlighted².

One of the chemokines released during allergic reaction is CCL5/RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted) that attracts inflammatory cells into areas of injury and normally binds to the receptor CCR5. CCL5/RANTES is a cytokine of chemotactic activity, belonging to a C–C chemokine family of characteristic structural motif containing two adjacent cysteines. CCL5/RANTES is chemotactic for monocytes, memory T cells, eosinophils, natural killers cells and dendritic cells^{3–6}. Moreover, CCL5/RANTES activates eosinophils and basophils^{4,7} and has histamine-releasing activity^{4,5}. Chemokines play a pivotal role in cellular recruitment through interactions with both cell surface G protein-coupled receptors and glycosaminoglycans (GAGs)⁸. CCL5/RANTES and CCL11 have been pointed out as two major chemokines involved in recruiting circulating eosinophils to allergic airway tissues. Elevated serum level of CCL5/RANTES was found in atopic dermatitis, aspirin-sensitive and aspirin-tolerant asthma/rhinitis patients, atopic asthma, irritant contact dermatitis^{7,9,10,11}. Multiple molecular

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studies of non-CF polyps have established that CCL5/RANTES may play an important role in nasal polyposis¹². Data on the role of CCL5/RANTES in *Hymenoptera* venom allergy are scarce and related to kinetics of RANTES plasma levels in children in the first days of rush VIT, or in the first 2 month of VIT in nine children or expression of mRNA for RANTES and cytokine in skin biopsies of patients undergoing wasp VIT^{13–15}.

Thus, we decided to assess the serum CCL5/RANTES level in patients who experienced severe anaphylactic reaction to *Hymenoptera* venom and to find out if the serum CCL5/RANTES level is changed in patients before the ultrarush immunotherapy was introduced and after the first 6 days of the treatment.

Material and methods

Patients included in the study had a history of a systemic anaphylactic reaction after a bee or wasp sting. Twenty patients (9 men, 11 women; mean age: 31.91 ± 7.63 year, aged 23–54 years) with clinically relevant allergy to *Hymenoptera* were included into the study (a group HYM) on the basis of a history of systemic anaphylactic reaction after *Hymenoptera* stings, positive intradermal skin tests to wasp or honey bee venom and allergen-specific IgE. There were seven subjects sensitive to bee venom (a BEE group) and 13 sensitive to wasp venom (a WASP group).

The severity of the anaphylactic reactions was graded according to Mueller¹ as follows: (I) skin symptoms (generalized urticaria, itching, erythema) or anxiety; (II) angioedema, nausea or vomiting, diarrhea, abdominal pain; (III) respiratory symptoms (shortness of breath, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, feeling of impending disaster, difficulty in swallowing); and (IV) hypotension, collapse, loss of consciousness, incontinence and cyanosis. Sixteen patients was after grade IV systemic reaction and four patient was graded III.

All of insect venom patients were enrolled into rush VIT with bee or wasp venom extracts (Pharmalgen, ALK-Abello, Horsholm, Denmark). All patients had undergone the same initial ultrarush protocol and a monthly maintenance dose of 100 µg. The ultrarush schedule consists of nine injection in the period of up to 12 h.

Twenty-two healthy subjects (12 men, 10 women, mean age: 28.64 ± 6.85 years; range: 19–42 years) with no signs of atopy and without any other relevant medical conditions were enrolled as control group (CON group). The both groups were comparable in terms of age.

Informed consent was obtained from all patients. The Ethics Committee of Medical University of Silesia, Katowice, Poland, has approved the study.

Skin tests

The diagnosis of venom allergy was established with the help of the anamnesis, confirmed by intradermal skin tests with serial 10-fold dilutions of wasp and honey bee venom (Pharmalgen, ALK-Abello, Horsholm, Denmark) beginning with the lowest concentration of 10^{-6} g/ml. Skin tests were considered positive if reaction occurred at a concentration of 10^{-3} g/ml or less. Reactions were evaluated after 15–20 min

and interpreted as positive if there was a wheal ≥ 5 mm with no reaction to negative control and positive reaction to histamine control¹. In both investigated groups we performed skin prick tests with important inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, grasses, rye, birch, hazel, *Artemisia vulgaris*, *Plantago lanceolata*, cat and dog dander, *Alternaria alternata*) (Allergopharma, Reinbek, Germany). The results were scored positive by wheal diameter ≥ 3 mm).

Laboratory methods

Ten milliliters of peripheral blood were collected from all studied subjects and then centrifuged at 2500 rpm for 20 min at room temperature. The serum was divided into aliquots, frozen and stored at -70°C until testing. Serum levels of CCL5/RANTES were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN). Sensitivity of the method was 2.5 pg/ml. Serum total levels of IgE (tIgE) and specific IgE against bee and/or wasp allergen were measured with commercially available fluoroimmunoassay kits (Pharmacia CAP System, Uppsala, Sweden) according to the manufacturers' instructions. All insect venom allergic patients were treated with specific immunotherapy according to ultrarush schedule. Second blood samples were drawn on 6th day after completion of the initial phase of VIT in all patients.

Statistics

Results are reported as median and range. The non-parametric Mann–Whitney *U*-test was used to detect the differences between patient and control groups. Correlations between data were assessed by the non-parametric Spearman's correlation test. Differences were considered significant at $p < 0.05$. The data were analyzed using Statistica PL package, version 5.01.

Results

CCL5/RANTES serum levels

Serum levels of CCL5/RANTES were significantly higher in the insect venom allergic group than in the control group (both before and after immunotherapy) (887.5 ± 322.77 pg/ml, and 570.45 ± 252.34 pg/ml, versus 387.27 ± 85.11 pg/ml, $p = 0.0001$). The serum CCL5/RANTES levels in the BEE group and WASP group did not differ significantly (871.82 ± 311.57 pg/ml versus 893.07 ± 330.06 pg/ml).

Serum concentration of CCL5/RANTES in insect venom allergic patient significantly reduced in the course of allergen immunotherapy already after 6 days of desensitization ($p = 0.00022$). Detailed data are shown in Figure 1.

Serum concentration of CCL5/RANTES in insect venom allergic patient didn't significantly differ between patients without ($n = 17$) and with ($n = 3$) concomitant inhalant allergies, respectively (879.41 versus 930.00 pg/ml ($p > 0.05$)).

Total IgE level

Total IgE serum levels were significantly higher in the insect venom allergic group than in the control group. Values of tIgE and allergen-specific IgE against bee and venom are shown in Table 1.

Figure 1. CCL5 (RANTES) levels in *Hymenoptera* sensitive patients before and after immunotherapy and in the controls.

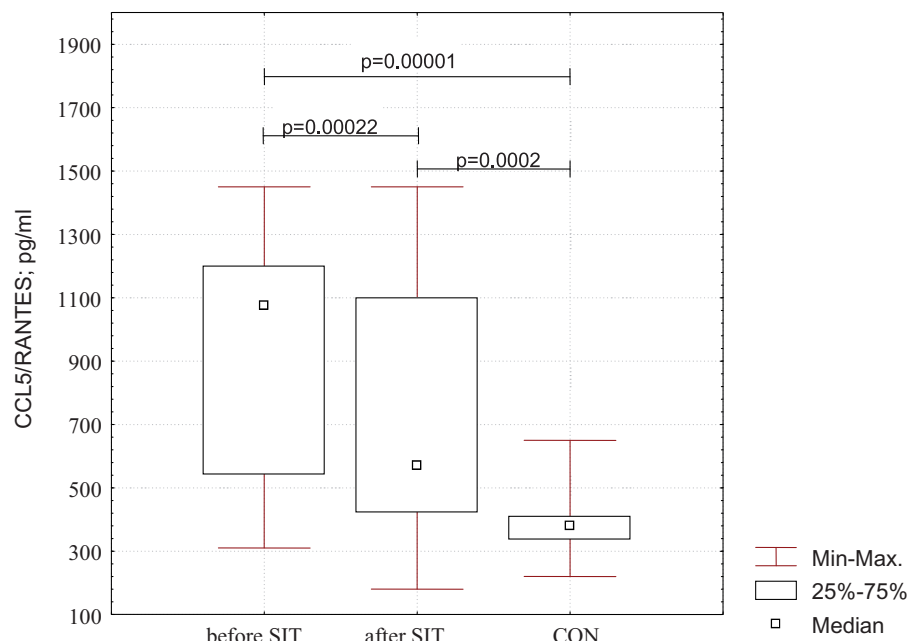


Table 1. Serum level of RANTES, total IgE, specific IgE against wasp and bee allergens in *Hymenoptera* venom sensitive and control groups.

	HYM group Median (interquartile range)	CON group (n = 22) Median (interquartile range)	p Value
RANTES (n = 20)	920 (580–1125)	380 (335–410)	0.00001
RANTES after VIT (n = 14)	570 (420–1100)	380 (335–410)	0.0002
tIgE	39 (31.5–48.5)	12.5 (7.25–29.8)	0.0004
Bee-IgE	1.035 (0.37–4.88)	0.3	n.a.
Wasp-IgE	2.34 (1.09–3.79)	0.3	n.a.

n.d., not done; n.a., non-applicable; VIT, immunotherapy; bee-IgE, specific IgE against bee allergens; wasp-IgE, IgE against wasp allergens; tIgE, total serum level of IgE; HYM, a group of patients sensitive to *Hymenoptera* venom; CON, a control group.

Correlations

Correlations between CCL5/RANTES serum level and tIgE levels and between CCL5/RANTES serum level and as-IgE levels are shown in Table 2.

Discussion

The present study shows that the serum levels of CCL5/RANTES in patients with *Hymenoptera* venom allergy were significantly higher both before and shortly after initial VIT than in healthy controls. We observed significant decrease in serum CCL5/RANTES level after initial phase of ultrarush VIT. Data concerning the role of CCL5/RANTES in *Hymenoptera* venom allergy are scarce.

Allergy to *Hymenoptera* venom allergens is a distinct nosological entity; however, clinical manifestation of this disease includes all anaphylactic symptoms, such as urticaria, angioedema, rhinitis, bronchial obturation, hypotension or cardiac arrest. Thus, different organs may be involved in the pathogenesis of the hypersensitivity reaction to *Hymenoptera* venom. In our study, serum levels of CCL5/RANTES were high despite the fact the serum sample was taken in the symptoms-free period that may reflect the readiness of the allergic subject to develop an anaphylactic reaction. VIT has

Table 2. Correlations between serum CCL5 (RANTES) level and age, total and allergen-specific IgE levels in *Hymenoptera* and control groups.

	HYM		CON	
	Coefficient (r_s)	p Value	Coefficient (r_s)	p Value
RANTES versus age	0.49	0.06	0.45	0.034
RANTES versus tIgE	−0.36	0.1	0.01	0.9
RANTES versus bee-IgE	−0.1	0.6	n.d.	
RANTES versus wasp-IgE	−0.2	0.3	n.d.	

n.d., not done; tIgE, total serum level of IgE.

been proved to be efficacious in reducing the severity of anaphylactic reactions following stings in patients with *Hymenoptera* venom allergy and seems to be the most efficacious form of specific immunotherapy. The mechanism by which specific immunotherapy exerts its beneficial effect still remains not completely clear.

CCL5/RANTES is thought to play particular role in immune system. Its receptor has been found on a variety of immunocompetent cells and has been shown to play an important role in the host response to pathogens. CCL5/RANTES regulates protective immunity to viral infections and intercellular interactions.

CCL5/RANTES is one of the most extensively studied C–C chemokines in allergic inflammation. A growing body of evidence suggests that many cell types present in asthmatic airways, allergic rhinitis and atopic dermatitis have the capacity to generate CCL5/RANTES, which directly supported the potential role of CCL5/RANTES in these diseases^{7,9,10,11,16}. Increased expression of CCL5/RANTES was observed in the lesional skin of patients with atopic eczema, and cDNA for CCL5 was upregulated in allergic rhinitis^{16,17}. It was also demonstrated that the level of CCL5/RANTES in exhaled breath condensate was significantly correlated with parameters of airflow limitation¹⁸. Castro et al.¹⁹ further observed that CCL5 significantly increased in asthmatic airway tissues along with worsening of the disease evoked by stopping steroid treatment. The recent published meta-analysis suggests that the RANTES promoter gene –28C/G polymorphism is a risk factor for asthma²⁰. RANTES along with other chemokines was found in nasal tissue of allergic rhinitis patients presenting higher concentration in severe compare to mild severity of the diseases and controls²¹. There are only few studies investigating CCL5/RANTES in serum of allergic patients under immunotherapy especially with insect vaccines^{13–15}.

The role of CCL5/RANTES has been studied in immunotherapy of other IgE-mediated diseases, i.e. in allergic rhinitis and bronchial asthma^{22–25}. However, the data are divergent, though in two studies immunotherapy reduced production of CCL5/RANTES and in another one the production increased^{22,25}. In children with bronchial asthma sensitive to house dust mites successful immunotherapy resulted in decreased spontaneous and mite-stimulated production of CCL5/RANTES by PBMC to a level that was comparable to that of the healthy group but the same parameters in the group of poor responders to immunotherapy was statistically significantly higher²². On the contrary, the increased level of CCL5/RANTES after 8 month of immunotherapy in children with asthma was found in PBMC supernatants in Chiang et al.²⁵ study.

Expression of CCL5/RANTES in nasal mucosa during pollen season was lower in patients sensitive to birch pollen who used to be treated with immunotherapy 3–5 years ago than in untreated controls²³. There are no data on serum level of CCL5/RANTES in patients with aforementioned diseases treated with immunotherapy so direct comparisons with our study are not possible.

Our results correspond with results of Ponvert et al.¹³ study. The authors examined plasma level of CCL5/RANTES in children who were treated with rush immunotherapy. They found transiently elevated plasma level at Day 2 and decreased at the Day 4. The authors suggested that plasma levels of CCL5/RANTES could reflect the reactivity of the children to wasp venom, and could be even predictive of the efficiency of VIT¹³.

The earlier study by Akoum et al.¹⁴ showed transient increase in production and expression of mRNA for CCL5/RANTES of peripheral blood mononuclear cells immediate after 3.5 h ultrarush venom immunotherapy followed by decrease in these parameters progressively up to Day 45¹⁴. The most potent histamine-releasing chemokine MCP-1 didn't change during the first 15 days after rush VIT,

therefore RANTES in the first days of VIT become important modulator of histamine release from basophils or mast cells. This study was performed in nine patients with history of anaphylactic reaction to *Vespula germanica* sting¹⁴.

Insect venom allergic patients usually present only mono-valent allergy. In our study, only three subjects were found to have concurrently positive results of skin prick tests with inhalant allergens what excludes the hypothetical impact of other, long lasting allergen sensitization on obtained results. The RANTES levels in this atopic patients were comparable to other patients but because of the small size of group our conclusions are not clear, and should be extended by future studies.

CCL5/RANTES as a key chemoattractant regulator of airway eosinophils and lymphocytes, has been demonstrated in our study in higher concentration in serum from *Hymenoptera* venom allergic patients in relation to healthy controls. Our results suggest that VIT is associated with a general decrease in CCL5/RANTES which may explain, in part, the pathomechanism of specific immunotherapy. The early reduction of RANTES serum level suggests that this chemokine is involved in the initial events underlying VIT. Measurements of CCL5/RANTES concentration in serum might be a promising approach to investigate the pathophysiology of allergy and to assess the efficacy of immunotherapy to *Hymenoptera* venom. Further studies are warranted to assess if CCL5/RANTES level may be used as a parameter of the severity of reactions and efficacy of immunotherapy. However we did not observe any relation of RANTES levels to clinical manifestation. According to current knowledge, induction of T and B cells tolerance in course of VIT is achieved through several immunologic mechanisms². Decrease in proinflammatory cytokine production of mast cells and basophils also contribute to development of tolerance in venom allergic individuals undergoing immunotherapy². Modulation of chemokine release interfere with early steps of immunologic reaction²⁶. This may occur in a few hours or days, particularly if rush and ultrarush type of AIT is used²⁷. May be it is the explanation of mechanism of observed in our study RANTES decrease, nonetheless this issue was not examined in the present work.

As a conclusion, it is impossible that a single cytokine plays crucial role in the pathogenesis of the disease. However, chemokine CCL5/RANTES participates in allergic reaction induced by *Hymenoptera* venom allergens. Specific immunotherapy reduces chemokine CCL5/RANTES serum level already after initial days of VIT.

Declaration of interest

The authors declare that they have no conflict of interest.

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