

RECOMBINANT ALLERGENS PROMOTE EXPRESSION OF AMINOPEPTIDASE-N (CD13) ON BASOPHILS IN ALLERGIC PATIENTS

K. SONNECK¹, C. BAUMGARTNER¹, L. REBUZZI^{1,2}, K. MARTH³, K.W. CHEN³,
A.W. HAUSWIRTH¹, S. FLORIAN¹, S. VRTALA³, H.J. BÜHRING⁴, R. VALENTA³ and P. VALENT¹

¹Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna; ²University of Veterinary Medicine Vienna, Department of Small Animals and Horses, Clinic for Internal Medicine and Infectious Diseases, Vienna; ³Department of Pathophysiology, Division of Immunopathology, Medical University of Vienna, Austria; ⁴University of Tübingen, Department of Internal Medicine II, Division of Hematology and Immunology, Tübingen, Germany

Received June 22, 2007 - Accepted September 17, 2007

IgE-dependent activation of basophils is associated with upregulation of several surface molecules. We recently identified the surface enzyme aminopeptidase N (CD13) as a novel activation antigen on human basophils. In the present study, we asked whether CD13 can be employed as a novel marker of allergen-induced activation of basophils in allergic individuals. Patients allergic to major allergens from grass pollen (Phl p 1, Phl p 5), birch pollen (Bet v 1), or house dust mites (Der p 2), were examined. Blood basophils were exposed to various concentrations of recombinant allergens for 15 minutes, and examined for expression of CD13 by multicolor flow cytometry. The allergen-induced upregulation of CD13 was compared with allergen-dependent increases in expression of CD63 and CD203c. Exposure to recombinant allergens resulted in an increase in expression of CD13 on basophils in all sensitized individuals, whereas no increase in CD13 was seen in healthy controls. The effects of the recombinant allergens on CD13-expression were dose- and time-dependent, were not observed in the absence of extracellular calcium, and were counteracted by preincubation of basophils with the PI3-kinase-targeting drugs staurosporin and LY294002. There was a good correlation between allergen-induced upregulation of CD13, CD63, and CD203c on basophils. In aggregate, our data show that recombinant allergens promote expression of CD13 on basophils in sensitized individuals. The functional significance and diagnostic implications of this observation remain to be determined.

Immunoglobulin E (IgE)-associated allergies represent an increasing problem in industrialized countries worldwide (1-3). Notably, as much as 15-30% of the urban population suffers from symptoms of IgE-mediated reactions to one or more allergens. Allergic symptoms include rhinitis, conjunctivitis,

asthma, and atopic dermatitis, with variable clinical presentations, depending on the type of allergen, organ sites involved, activation-status of cells, and susceptibility of the host (1-2). In many instances, the symptoms require continuous medical care and treatment, and sometimes, allergic reactions are

Key words: allergy, aminopeptidase-N, basophils, CD13, CD203c

Mailing address: Peter Valent, M.D.
Department of Internal Medicine I,
Division of Hematology and Hemostaseology,
Medical University of Vienna, Währinger Gürtel 18-20,
A-1090 Vienna, Austria
Tel: ++43 1 40400 -4416, -6085 Fax: ++43 1 40400 4030
e-mail: peter.valent@meduniwien.ac.at

0394-6320 (2008)

Copyright © by BÍOLIFE, s.a.s.

This publication and/or article is for individual use only and may not be further reproduced without written permission from the copyright holder. Unauthorized reproduction may result in financial and other penalties

grave or even life-threatening (2).

Allergens are (glyco)proteins that usually are widely distributed and exhibit several (or even multiple) IgE-binding sites (3-5). Recent progress in allergology has led to the characterization and molecular cloning of multiple allergens from plant- and animal sources (3-5). More recently, recombinant allergens have also been used to develop specific diagnostic tests (3-8). However, in many cases, clinical symptoms are not predictable from biologic skin tests (Prick-test) or serology tests (9-12). Therefore, research has focused on the direct measurement of allergic reactions *in vitro* using blood basophils and recombinant allergens (5-6, 13).

However, so far, only a few effector cell-based *in vitro* allergy-tests and only a few reliable markers have become available (5-6, 19-22). In recent years, allergen-induced upregulation of CD63 on basophils has been introduced as a novel assay to quantify basophil responses to allergens (19-22). More recently, we identified the ectoenzyme ectonucleotide pyrophosphatase / phosphodiesterase 3 (ENPP-3 = CD203c) as another basophil activation antigen (23-26). Thus, recombinant allergens promote surface expression of CD63 and CD203c on basophils in sensitized individuals (26). However, apart from these two markers, little is known about allergen-induced upregulation of cell surface antigens on basophils.

Aminopeptidase N (CD13) is a cell surface antigen that is widely distributed in myeloid cells including basophils (27-28). We have recently shown that basophils exposed to an anti-IgE antibody express increased levels of CD13 (29). In the present study, we examined the effects of various purified recombinant allergen molecules on expression of CD13 on blood basophils in sensitized individuals, and compared CD13-upregulation with allergen-induced upregulation of CD63 and CD203c.

MATERIALS AND METHODS

Patients

A total number of 15 patients, allergic to grass pollen allergens (Phl p 1 and Phl p 5, $n=5$), the major birch pollen allergen Bet v 1 ($n=5$), or the major house dust mite allergen, Der p 2 ($n=5$), were examined. Allergy diagnosis was based on clinical symptoms and specific

IgE measurements. Levels of specific IgE were expressed in luminescence units (LU). The patients' characteristics are shown in Table I. The control group consisted of five healthy non-allergic donors.

Recombinant allergens and other reagents

Recombinant allergens (rBet v 1, rPhl p 1, rPhl p 5, rDer p 2) were expressed in *E. coli* and purified as described (13, 18, 30-31). Recombinant allergens were dissolved and diluted in phosphate buffered saline (PBS). All recombinant allergens tested were found to be functionally active allergens as evidenced by their ability to induce histamine release from basophils in allergic patients (not shown). The anti-IgE (mAb) monoclonal antibody E-124-2-8 (De2), the fluorescein isothiocyanate (FITC)-labeled monoclonal antibody (mAb) CLB-gran12 (CD63), the phycoerythrin-cyanin5 (PC5)-conjugated mAb Immul03.44 (CD13), and the phycoerythrin (PE)-labeled mAb 97A6 (CD203c) were purchased from Immunotech (Marseille, France). The PE-labeled mAb WM15 (CD13) and the phycoerythrin-cyanin5 (PE-Cy5)-conjugated mAb 9F5 (CD123) were from Becton Dickinson (San Diego, CA). Recombinant human (rh) interleukin-4 (IL-4), rhIL-5, and rhIL-6 were from Strathmann Biotech (Hannover, Germany), rhIL-3 and rh granulocyte-macrophage colony-stimulating factor (GM-CSF) from Sandoz (Basel, Switzerland), and staurosporin, LY294002, PD98059, and rapamycin from Calbiochem (San Diego, CA).

Determination of specific IgE

IgE antibodies specific for a panel of 40 respiratory and food allergen extracts were quantified by chemiluminescence assay (CLA[®]) following the manufacturer's recommendations (Hitachi, Maidenhead, Berkshire, UK).

Incubation of basophils with recombinant allergens and other compounds

Peripheral blood (from 15 allergic individuals and 5 non-allergic controls) was collected in heparinized tubes. Basophils were examined in whole blood or after enrichment by Ficoll gradient centrifugation. For measuring the direct effects of the allergens, whole blood aliquots (100 μ l) were incubated with serial dilutions of recombinant allergens (10^{-5} to 10 μ g/ml), anti-IgE antibody (1 μ g/ml), or control buffer (PBS) for 15 minutes at 37°C. In time course experiments, blood cells were incubated with allergens (1 μ g/ml each) or anti-IgE (1 μ g/ml) for various time periods (range: 0.5 to 180 minutes). In select experiments, cells were exposed to allergens in the presence or absence of 20 mM EDTA (Gibco Life Technologies, Gaithersburg, MD).

To determine signal transduction pathways contributing to IgE-dependent upregulation of CD13, Ficoll-enriched basophils were incubated with pharmacologic inhibitors (LY294002, 20 μ M; staurosporine, 100 nM; PD98059, 50 μ M; rapamycin, 20 nM) for 15 minutes prior to incubation with allergens or anti-IgE. In a separate set of experiments, Ficoll-enriched basophils were incubated with rh IL-3 (100 ng/ml), IL-4 (100 U/ml), IL-5 (100 U/ml), IL-6 (100 ng/ml), or GM-CSF (100 U/ml) for 90 or 180 minutes before being examined for expression of CD13.

Evaluation of basophil activation by multicolor flow cytometry

After incubation with allergens, anti-IgE, or cytokines, cells were washed in PBS containing 20 mM EDTA. Cells were then incubated with 10 μ l CD13 mAb together with 10 μ l CD63 mAb and 10 μ l CD123 mAb (tube A) or together with 10 μ l CD63 mAb and 10 μ l CD203c mAb (tube B) for 15 minutes at room temperature (RT). Thereafter, samples were subjected to erythrocyte lysis with 2 ml FACS™ Lysing Solution (Becton Dickinson, San Diego, CA). Cells were then washed, resuspended in PBS, and analyzed by three-color flow cytometry on a FACScan (Becton Dickinson). Allergen-induced upregulation of CD13 and other CD antigens was calculated from mean fluorescence intensities (MFI) obtained with stimulated (MFI_{stim}) and unstimulated (MFI_{control}) cells, and was expressed as stimulation index = SI (MFI_{stim} : MFI_{control}). An SI of ≥ 2.0 (≥ 2 -fold upregulation) was considered to be indicative of a relevant (diagnostic) response. To determine the significance in differences in CD13 expression between unstimulated and stimulated basophils, the Student's t test for dependent samples was applied. Results were considered statistically significant when p was < 0.05 .

RESULTS

Baseline levels of CD13 on basophils in healthy individuals and allergic patients

In line with previous observations (27-28), basophils in all (healthy or allergic) donors were found to express CD13. The baseline levels i.e. mean fluorescence intensity (MFI) of CD13 expressed on basophils in allergic individuals did not differ from CD13 levels detected on basophils in healthy controls (MFI, mean $\pm$ SD and range in controls: 156 $\pm$ 63; 99-260 versus allergic donors: 143 $\pm$ 45; 63-220). Baseline CD13-levels on basophils did not change significantly when cells were kept in control buffer for 15 or 30 minutes (not shown).

Recombinant allergens promote expression of CD13 on basophils in allergic patients

In all sensitized individuals tested, recombinant allergens were found to promote the expression of CD13 on peripheral blood basophils in an allergen-specific manner (Fig. 1). No significant differences in upregulation of CD13 were seen, when the activities of the different allergens were compared (Fig. 1). The effects of the recombinant allergens were time- and dose-dependent, with ED₅₀ values ranging between 0.001 and 0.01 μ g/ml (Fig. 2A and 2B). Maximum effects of allergens and anti-IgE on expression of CD13 were observed after 10 minutes (Fig. 2A). In non-allergic individuals, the allergens tested did not promote expression of CD13 (Fig. 2C). Moreover, the allergens tested were found to act specifically in sensitized individuals, as other allergens, against which no allergen-specific IgE was detected, did not induce CD13-upregulation (Fig. 2D, Table II).

The allergen-induced upregulation of CD13 is dependent on extracellular calcium and inhibitable by targeting PI3-kinase

Recombinant allergens as well as anti-IgE were found to promote the expression of CD13 in the presence but not in the absence of extracellular calcium. In particular, in the presence of EDTA, no effects of the allergens (or anti-IgE) on CD13-expression were observed (Fig. 3A). In consecutive experiments, we found that the PI3-kinase inhibitors staurosporin and LY294002 inhibit the anti-IgE-induced upregulation of CD13 in basophils, whereas no effects were seen with the MAP kinase inhibitor PD98059 and the mTOR-targeting drug rapamycin (Fig. 3B). Similar results were obtained when basophils were incubated with recombinant allergens (not shown).

Comparison between allergen-induced upregulation of CD13 and allergen-induced upregulation of CD63 and CD203c

As assessed by multicolor flow cytometry, the allergen-induced upregulation of CD13 was found to be accompanied by upregulation of CD63 and CD203c in all allergic patients examined (Fig. 4A-4C). Similarly, in all healthy donors tested, the anti-IgE-induced upregulation of CD13 was found to be

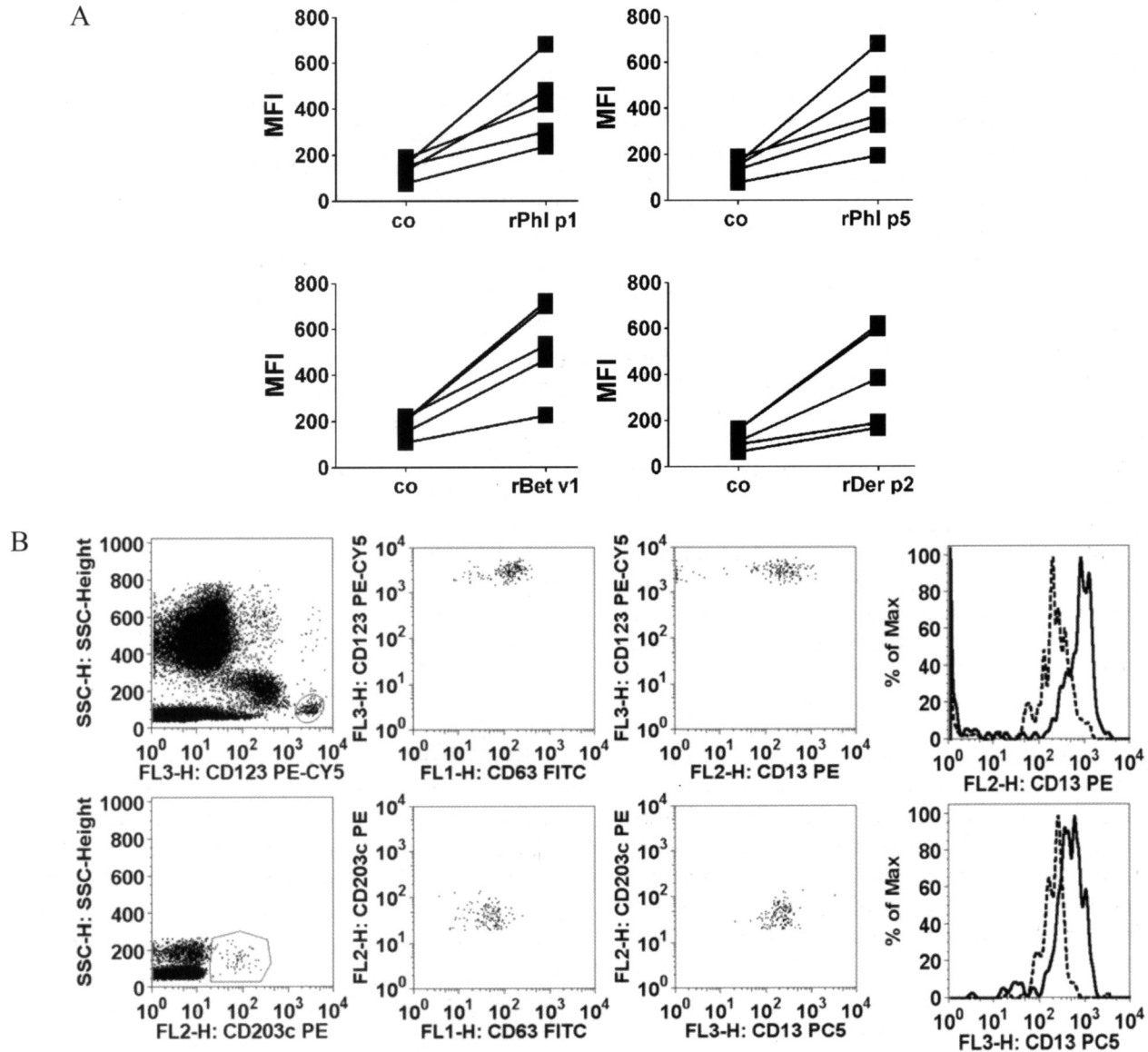


Fig. 1. Effects of recombinant allergens on expression of CD13 on basophils. **A:** Whole blood samples obtained from patients allergic to Phl p 1 (left upper image), Phl p 5 (right upper image), Bet v 1 (left lower image) or Der p 2 (right lower image) were incubated with control buffer (co) or with the respective recombinant (r) allergens (1 μ g/ml) at 37°C for 15 minutes. Results show the mean fluorescence intensities (MFI) measured in each patient (co versus allergen). The effects of the allergens on CD13 expression in all donors were found to be statistically significant in each case ($p < 0.05$): Respective MFI values (mean $\pm$ SD) for the various allergens were: control: 143 ± 42 versus Phl p 1: 424 ± 173 ($p < 0.05$); control: 143 ± 42 versus Phl p 5: 406 ± 170 ($p < 0.05$); control: 179 ± 47 versus Bet v 1: 529 ± 202 ($p < 0.05$); control: 118 ± 43 versus Der p 2: 448 ± 216 ($p < 0.05$). **B:** Representative experiment showing the gating strategy for detection and evaluation of basophils as CD123 $^{+}$ /CD63 $^{+}$ cells in whole blood samples (upper panel) or as CD203c $^{+}$ /CD63 $^{+}$ cells in mononuclear cell fractions (lower panel). Cells were examined for expression of CD13 by multicolor flow cytometry using fluorochrome-conjugated antibodies against CD13, CD123, CD63, and CD203c. The effect of stimulation of basophils with allergen (Bet v 1, 1 μ g/ml; bold graph) on CD13 expression compared to control buffer (co; dashed graph) is shown in the right histogram images. Abbreviations: PE-Cy5, phycoerythrin-cyanin5; FITC, fluorescein isothiocyanate; PC5, phycoerythrin-cyanin5; PE, phycoerythrin; SSC-H, SSC-Height; FL1-H, Fluorescence 1-Height; FL2-H, Fluorescence 2-Height; FL3-H, Fluorescence 3-Height.

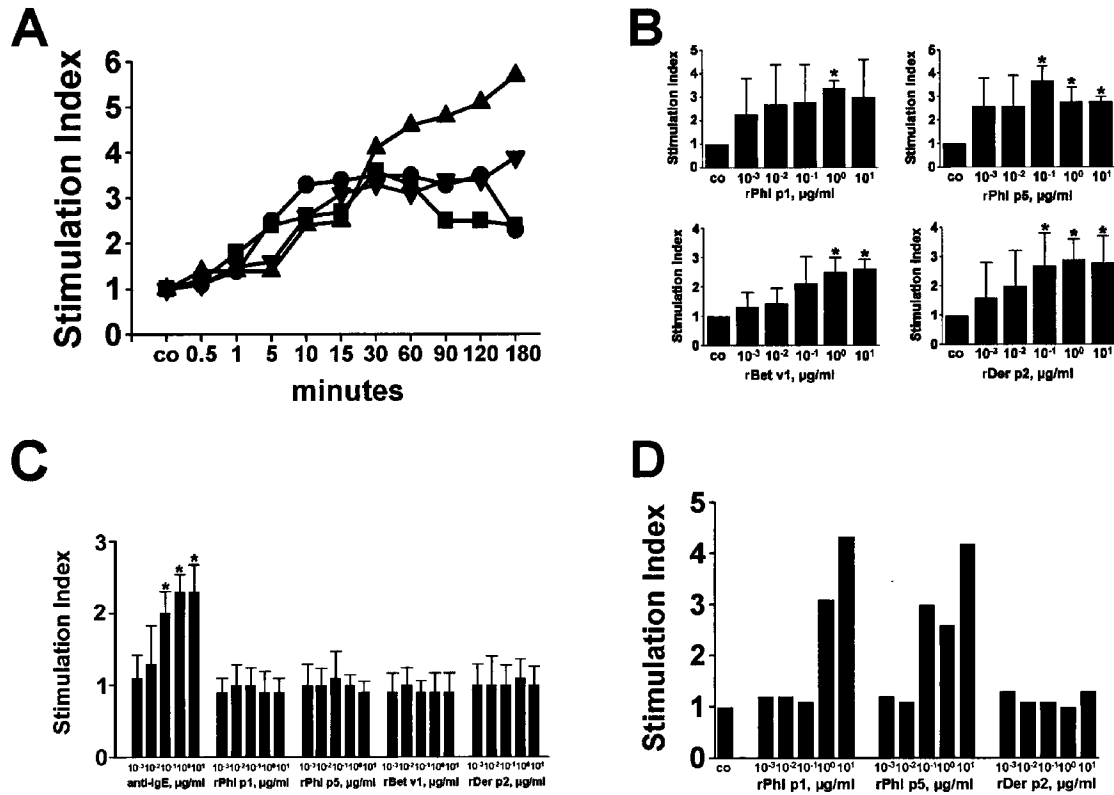


Fig. 2. Time- and dose-dependent effects of recombinant allergens on expression of CD13 on basophils. **A:** Time-dependent effects of the allergens Phl p 1 (■-■), Phl p 5 (●-●), Bet v 1 (▲-▲) or Der p 2 (▼-▼) compared to control buffer (co) on expression of CD13 on basophils in sensitized individuals. Whole blood samples were incubated with allergens for various time periods at 37°C as indicated. **B:** Dose-dependent effects of allergens on expression of CD13 on basophils. Whole blood obtained from patients allergic to Phl p 1 (left upper image), Phl p 5 (right upper image), Bet v 1 (left lower image) or Der p 2 (right lower image) were incubated with various concentrations of the respective recombinant allergens or control buffer (co) as indicated at 37°C for 15 minutes. Results show the SI values and represent the mean±S.D. of at least three independent experiments (three different patients). Asterisk (*): $p < 0.05$. **C:** Effects of anti-IgE (10^{-3} to 10^1 μg/ml) and recombinant allergens (each 10^{-3} to 10^1 μg/ml) on expression of CD13 on basophils in non-allergic donors. Results represent the mean±S.D. of at least three independent experiments. Asterisk (*): $p < 0.05$. **D:** Effects of recombinant allergens or control buffer (co) on expression of CD13 on basophils in a patient allergic to Phl p 1 and Phl p 5, but not to Der p 2. Expression of CD13 on basophils was determined by multicolor flow cytometry. Results are expressed as stimulation index (SI) defined by the mean fluorescence intensities obtained with stimulated (MFI_{stim}) and unstimulated ($MFI_{control}$) cells ($SI = MFI_{stim} : MFI_{control}$).

accompanied by upregulation of CD63 and CD203c on basophils (Fig. 4D).

Effects of recombinant cytokines on expression of CD13 in basophils

To determine the specificity of allergen-induced upregulation of CD13 in basophils, we examined the effects of several recombinant cytokines on

expression of CD13. In these experiments, no significant effects of IL-3 or of other cytokines on CD13 expression on basophils were found in controls or allergic individuals after 15 minutes. After 90 and 180 minutes, IL-3 slightly augmented the expression of CD13 on basophils in controls and in allergic patients, although statistical significance was not reached ($p > 0.05$). No effects were observed

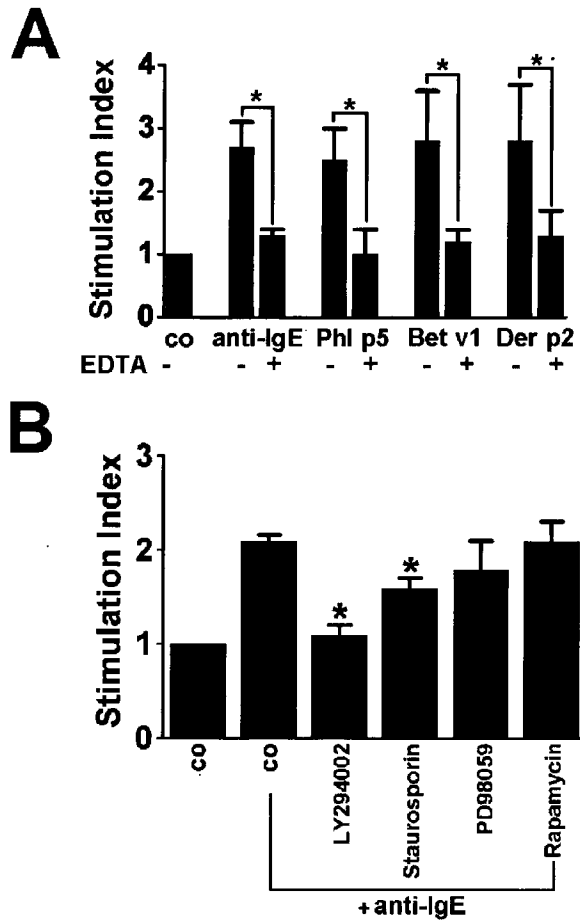


Fig. 3. Role of extracellular calcium and PI3-kinase in allergen-induced upregulation of CD13 on basophils. **A:** Basophils from allergic individuals were incubated with anti-IgE (1 μ g/ml) or recombinant allergens as indicated (each 1 μ g/ml) in the absence (-) or presence (+) of EDTA (20 mM) at 37°C for 15 minutes. The buffer control (co) is also included. Results show the stimulation index (SI) compared to buffer-control (co = 1) and represent the mean $\pm$ SD from at least 3 independent experiments (three different patients). Asterisk (*): $p < 0.05$. **B:** Basophils from 3 non-allergic individuals were preincubated with various signal transduction inhibitors (LY294002, 20 μ M; staurosporine, 100 nM; rapamycin, 20 nM; PD98059, 50 μ M) or control medium at 37°C for 15 minutes. Then, basophils were incubated with an anti-IgE antibody (1 μ g/ml) for another 15 minutes. The medium control (co) is also shown. Expression of CD13 on basophils was measured by multicolor flow cytometry (see text). Results are expressed as stimulation index (SI) and represent the mean $\pm$ SD of 3 independent experiments. Asterisk (*): $p < 0.05$.

with IL-4, IL-5, IL-6, or GM-CSF (Fig. 5).

DISCUSSION

Aminopeptidase N (CD13) is a cell surface membrane antigen that is expressed abundantly on various myeloid cells including basophils (27-28). We have recently identified CD13 as a novel basophil activation antigen that is upregulated in response to cross-linking of the IgE receptor by anti-IgE (29). In the present study, we show that various recombinant allergens promote expression of CD13 on basophils in sensitized individuals in an IgE-specific manner, but do not promote expression of CD13 on basophils in non-allergic controls. These data suggest that CD13 is a novel marker of allergen-dependent activation of basophils.

The effects of the recombinant allergens tested on expression of CD13 on basophils were found to be dose- and time-dependent and were seen in all donors examined. Furthermore, allergen-induced upregulation of CD13 on basophils was found to be a specific reaction confined to sensitized cells and the respective allergen, whereas no reactivity was seen in non-allergic controls or patients sensitized to other (unrelated) allergens. Therefore, allergen-induced upregulation of CD13 may be utilized as a novel approach to quantify the allergic reaction at the effector cell level *in vitro*.

During the past few years, a number of recombinant allergens have been generated and are used increasingly to define the allergic response at the effector cell level in sensitized individuals (9-10, 13). In fact, today, the consensus is that recombinant allergens are a useful tool for allergy diagnosis, and thus should be employed in all diagnostic assays (9-10, 13). It was therefore of importance to show that various recombinant allergens including major grass pollen allergens and the major birch pollen allergen Bet v 1, can induce upregulation of CD13 on blood basophils in sensitized individuals in the current study. An interesting observation was that the responses to allergens varied from patient to patient without a clear correlation to IgE levels or clinical parameters. The reason for this discrepancy remains unknown. The most likely explanation is that apart from CD13, many other (IgE-dependent) factors and basophil-derived mediators contribute to clinical

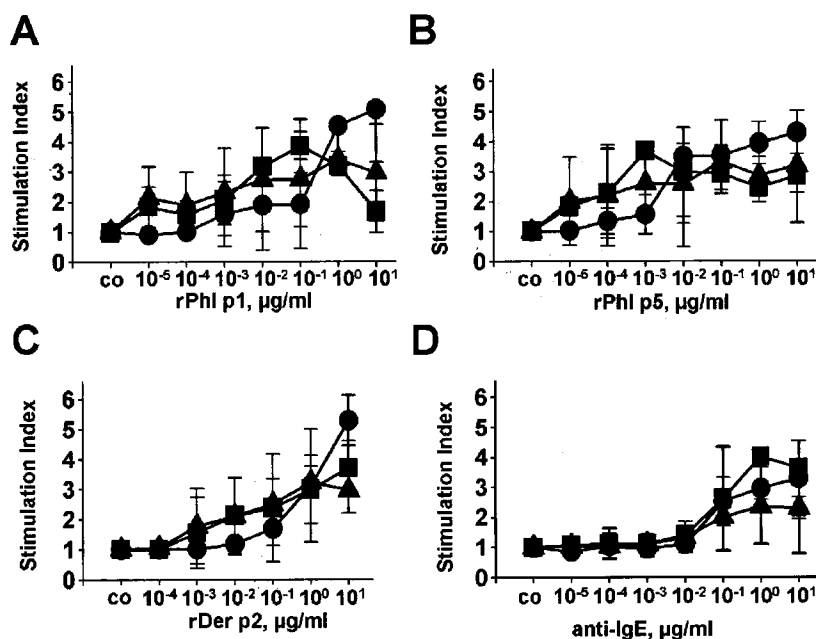


Fig. 4. Comparison between upregulation of CD13, CD63, and CD203c on basophils after exposure to recombinant allergens or anti-IgE. Basophils from 3 patients allergic to Phl p 1 (A), 3 patients allergic to Phl p 5 (B), 3 patients allergic to Der p 2 (C), and from 3 non-allergic donors (D) were analyzed. Basophils were incubated with various concentrations of the respective allergen or anti-IgE or control buffer (co) as indicated at 37°C for 15 minutes. Thereafter, expression of CD13 ($\blacktriangle$ - $\blacktriangle$), CD63 ($\blacksquare$ - $\blacksquare$), and CD203c ($\bullet$ - $\bullet$) on basophils was analyzed by multicolor flow cytometry as described in the text. Results are expressed as stimulation index (SI) values and represent the mean $\pm$ S.D. of 3 independent experiments (3 donors).

symptoms and IgE production in allergic individuals. On the other hand, the CD13 assay was found to be a sensitive test indicating an 'allergic' *in vitro* response of basophils in all patients examined.

A number of surface antigens have been described to be upregulated on exposure to anti-IgE or exposure to an allergen. These antigens include CD63 (original baso-test) and the ecto-enzyme CD203c (19-26). In the present study, we compared allergen-induced upregulation of CD13 with upregulation of CD63 and CD203c. The results of our study show that allergen-induced upregulation of CD13 is accompanied by upregulation of CD63 and CD203c in all donors tested with comparable ED_{50} values and similar maximum levels of upregulation.

A number of downstream signal transduction molecules and pathways have been implicated in IgE-dependent activation and consecutive degranulation of basophils (32-34). In the present study, we asked whether allergen/anti-IgE-induced upregulation of

CD13 is associated with specific signal transduction events in basophils. In a first step, we were able to show that allergen- and anti-IgE-induced upregulation of CD13 requires the presence of extracellular calcium. Then, we examined the effects of various signal transduction inhibitors and found that allergen- and anti-IgE-induced upregulation of CD13 on basophils is inhibitable by two PI3-kinase-targeting compounds, but not by a MAP kinase inhibitor or an mTOR inhibitor. These data suggest that allergen-induced upregulation of CD13 requires activation of the PI3-kinase. Similar data have recently been described for anti-IgE-dependent upregulation of CD63, CD164, and CD203c (29, 35). All in all, our previous and current data suggest that upregulation of distinct CD antigens is associated with basophil activation that leads to mediator release. However, the relevance of CD antigen expression for mediator release remains unknown. An interesting aspect in this regard is that several

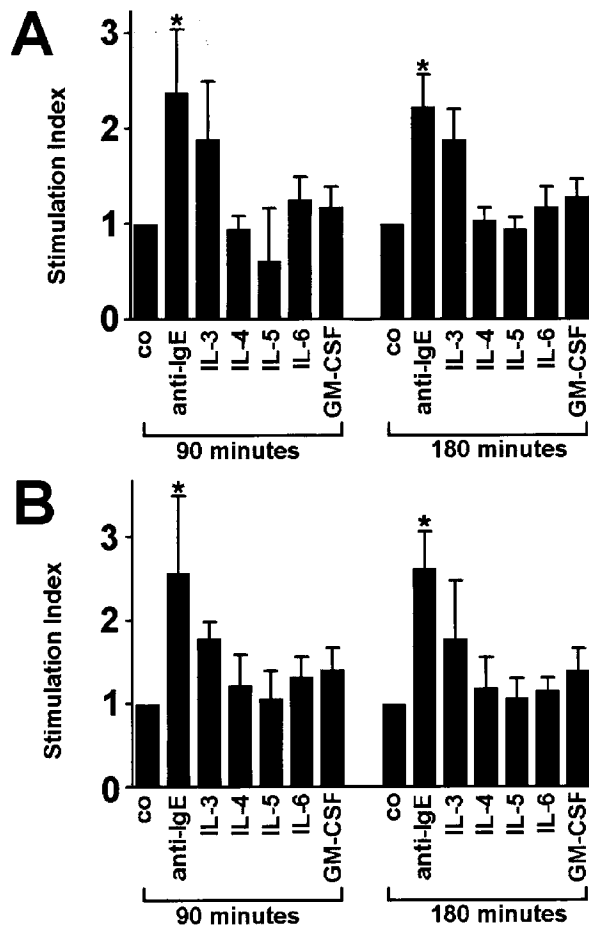


Fig. 5. Effects of cytokines on expression of CD13 on basophils. Basophils obtained from non-allergic donors (A) and allergic patients (B) were incubated with anti-IgE antibody (1 μ g/ml), with recombinant cytokines at receptor-saturating concentrations (IL-3, 100 ng/ml; rIL-4, 100 U/ml; IL-5, 100 U/ml; IL-6, 100 ng/ml; or GM-CSF, 100 U/ml) or control medium (co) at 37°C for 90 or 180 minutes. Thereafter, cells were examined for expression of CD13 by multicolor flow cytometry. Results are expressed as stimulation index (SI) values and represent the mean $\pm$ SD of 3 independent experiments (3 donors). Asterisk (*): $p < 0.05$.

of these upregulated CD antigens represent integral lysosomal membrane components (36), and possibly also functional components of basophil granules. Whether these CD antigens are actively involved in the granule transport to the surface or even in the process of membrane fusion or degranulation and exocytosis, remains at present unknown.

A number of recent data suggest that specific

upregulation of CD antigens on basophils by recombinant allergens may be utilized for the development of specific *in vitro* allergy tests. Apart from the CD63-baso-test, this has recently been proposed for the ectoenzyme CD203c (25-26, 35). The current attitude towards the optimization of specific allergy tests is to combine such markers. Likewise, the advantage of CD203c over CD63 and CD13 may be that CD203c is specific for basophils (25-26, 35), whereas, on the other hand, CD203c is not only upregulated in response to anti-IgE or allergen, but also after incubation with other stimuli such as IL-3 (37). Therefore, it was of particular importance to test whether basophil-targeting cytokines like IL-3, IL-4, IL-5, or GM-CSF, would upregulate expression of CD13. The results of our study show that none of these cytokines upregulate expression of CD13 on basophils after 15 minutes, the time after which allergen-induced upregulation of CD13 is measured. This observation would be in favor of CD13 as a possible diagnostic marker. However, it was also found that IL-3 can induce a slight increase in CD13 on basophils after 90 and 180 minutes of incubation. Interestingly, no effects of the other cytokines tested on expression of CD13 on basophils were seen after 90 or 180 minutes of incubation. With regard to a diagnostic allergy test, CD13 alone may not be an optimal reagent, as many other cells also display CD13. Therefore, as suggested above, a more suitable approach may be to combine CD13 with CD203c (or CD63) to develop an allergy test. This consideration is supported by the notion that a respective antibody-combination can easily be applied and showed reliable and highly reproducible results in this study. Such assay may be useful for monitoring the disease status and the response to various anti-allergic drugs (such as glucocorticosteroids) or to specific immunotherapy.

In summary, CD13 is a novel marker of allergen-induced activation of human blood basophils. Further studies are now required to validate this new assay in clinical practice and clinical trials.

ACKNOWLEDGEMENTS

We wish to thank Pierre Paolini (Immunotech Company) for providing CD203c antibody. This

Table I. Patients' characteristics.

# (no)	age	f/m	symptoms	therapy	allergen-specific IgE - CLA class		
					HDM	BP	TGP
#01	16	f	AA	CS	4	0	0
#02	30	f	RC, AA	-	4	2	4
#03	30	f	RC, AA	CS	4	4	4
#04	35	m	RC	AH	4	0	1
#05	27	m	RC	-	4	0	2
#06	44	m	RC	AH	0	4	2
#07	26	m	RC	-	4	4	1
#08	64	m	RC	AH	0	4	0
#09	26	m	RC, AA	AH	4	4	4
#10	25	m	RC, AA	AH	1	4	4
#11	28	m	RC	AH	1	1	4
#12	33	f	RC	AH	0	0	4
#13	29	f	RC	-	0	0	4
#14	22	m	RC	-	4	0	4
#15	24	m	RC	AH	3	0	4

CLA, chemiluminescence; m, male; f, female; HDM, house dust mite; BP, birch pollen; TGP, timothy grass pollen, RC: rhinoconjunctivitis; AA: allergic asthma; AH: antihistamines, CS: corticosteroids. The CLA class was defined by the following score: 0, 0-26 luminescence units (LU); 1, 27-65 LU, 2, 66-142 LU, 3, 143-242 LU, 4, >242

Table II. Comparison between upregulation of CD13 and detection of specific IgE by CLA.

Donor	anti-IgE CD13 SI	Phl p 1 CD13 SI	Phl p 5 CD13 SI	grass CLA (LU)	Bet v 1 CD13 SI	birch CLA (LU)	Der p2 CD13 SI	mite CLA (LU)
#1	2.6	n.t.	n.t.	2	n.t.	2	3.5	>300
#2	2.5	n.t.	2.2	>300	n.t.	79	2.9	>300
#3	3.1	n.t.	n.t.	>300	n.t.	>300	2.0	>300
#4	2.3	n.t.	n.t.	30	n.t.	20	2.7	>300
#5	2.7	n.t.	n.t.	126	n.t.	0	3.7	>300
#6	2.1	n.t.	n.t.	98	2.4	>300	n.t.	22
#7	3.2	n.t.	n.t.	30	2.1	>300	n.t.	264
#8	2.6	1.0	1.1	16	3.5	>300	n.t.	0
#9	3.1	4.9	2.4	>300	3.0	>300	3.8	>300
#10	2.2	n.t.	n.t.	>300	2.2	>300	n.t.	35
#11	2.0	n.t.	2.3	>300	n.t.	46	n.t.	33
#12	3.2	3.1	2.6	>300	n.t.	1	n.t.	0
#13	2.7	3.3	2.3	>300	n.t.	4	1	0
#14	2.4	2.2	2.0	293	n.t.	26	n.t.	>300
#15	3.8	2.0	3.3	>300	n.t.	20	n.t.	215

Upregulation of CD13 on basophils by anti-IgE (1 µg/ml) or allergen (each 1 µg/ml) was determined by multicolor flow cytometry. A stimulation index (SI) of greater than 2 was considered as a diagnostic increase in expression of CD13. Specific IgE was determined in sera by a chemiluminescence assay (CLA) and the data are expressed in luminescence units (LU). n.t., not tested

study was supported by: Fonds zur Förderung der Wissenschaftlichen Forschung in Österreich (FWF) grants # 01801, # 01803, # 01809, 01815, and Y078GEN, and by the Christian Doppler Research Association, Vienna, Austria.

REFERENCES

1. **Kay A.B.** 1997. *Allergy and Allergic Diseases*. A.B. Kay, ed. Blackwell Science, Oxford, U.K. p. 1.
2. **Dattwyler R., A.P. Kaplan and K.F. Austen.** 1985. Human anaphylaxis. In: *Allergy 1st ed.* Kaplan AP, ed. Churchill Livingstone. New York, p. 599.
3. **Wuthrich B., C. Schindler, P. Leuenberger and U. Ackermann-Lieblich.** 1995. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. *Int. Arch. Allergy Immunol.* 106:149.
4. **Valenta R. and D. Kraft.** 2002. From allergen structure to new forms of allergen-specific immunotherapy. *Curr. Opin. Immunol.* 14:718.
5. **Purohit A., S. Laffer, C. Metz-Favre, A. Verot, F. Kricek, R. Valenta and G. Pauli.** 2005. Poor association between allergen-specific serum immunoglobulin E levels, skin sensitivity and basophil degranulation: a study with recombinant birch pollen allergen Bet v 1 and an immunoglobulin E detection system measuring immunoglobulin E capable of binding to Fc epsilon RI. *Clin. Exp. Allergy* 35:186.
6. **Kaul S., D. Luttkopf, B. Kastner, L. Vogel, G. Holtz, S. Vieths and A. Hoffmann.** 2007. Mediator release assays based on human or murine immunoglobulin E in allergen standardization. *Clin. Exp. Allergy* 37:141.
7. **Kleine-Tebbe J., S. Erdmann, E.F. Knol, D.W. MacGlashan Jr, L.K. Poulsen and B.F. Gibbs.** 2006. Diagnostic tests based on human basophils: potentials, pitfalls and perspectives. *Int. Arch. Allergy Immunol.* 141:79.
8. **Valenta R., S. Vrtala, C. Ebner, D. Kraft and O. Scheiner.** 1992. Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. *Int. Arch. Allergy Immunol.* 97:287.
9. **Valenta R.** 2002. The future of antigen-specific immunotherapy of allergy. *Nat. Rev. Immunol.* 2:446.
10. **Valenta R. and D. Kraft.** 2001. Recombinant allergen molecules: tools to study effector cell activation. *Immunol. Rev.* 179:119.
11. **Valenta R., W.R. Sperr, F. Ferreira et al.** 1993. Induction of specific histamine release from basophils with purified natural and recombinant birch pollen allergens. *J. Allergy Clin. Immunol.* 91:88.
12. **Hiller R., S. Laffer, C. Harwanegg et al.** 2002. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. *FASEB J.* 16:414.
13. **Valenta R., J. Lidholm, V. Niederberger, B. Hayek, D. Kraft and H. Grönlund.** 1999. The recombinant allergen-based concept of component-resolved diagnosis and immunotherapy (CRD and CRIT). *Clin. Exp. Allergy* 29:896.
14. **Bousquet J., B. Lebel, H. Dhivert, Y. Bataille, B. Martinot and F.B. Michel.** 1987. Nasal challenge with pollen grains, skin prick tests, and specific IgE in patients with grass pollen allergy. *Clin. Allergy* 17:529.
15. **Pelikan Z.** 1983. The diagnostic approach to immediate type hypersensitivity in patients with allergic rhinitis; a comparison of nasal challenges and serum RAST. *Ann. Allergy* 51:395.
16. **Rasaneh L., P. Kuusisto, M. Penttilä, M. Nieminen, J. Savolainen and M. Lehto.** 1994. Comparison of immunologic tests in the diagnosis of occupational asthma and rhinitis. *Allergy* 49:342.
17. **Niederberger V., P. Stübner, S. Spitzauer, D. Kraft, R. Valenta, K. Ehrenberger and F. Horak.** 2001. Skin test results but not serology reflect immediate type respiratory sensitivity: a study performed with recombinant allergen molecules. *J. Invest. Dermatol.* 117:848.
18. **Vrtala S., M. Susani, W.R. Sperr, P. Valent, S. Laffer, C. Dolecek, D. Kraft and R. Valenta.** 1996. Immunologic characterization of purified recombinant grass pollen (*Phleum pratense*) allergens (Phl p 1, Phl p 2, Phl p 5). *J. Allergy Clin. Immunol.* 97:781.
19. **Knol E.F., F.P. Mul, H. Jansen, J. Calafat and D. Roos.** 1991. Monitoring human basophil activation

- via CD63 monoclonal antibody 435. *J. Allergy Clin. Immunol.* 88:328.
20. **Gane P., C. Pequet, H. Crespeau, P. Lambin, F. Leynadier and P. Rouger.** 1995. Flow cytometric monitoring of allergen induced basophil activation. *Cytometry* 19:361.
 21. **Sainte-Laudy J., A. Sabbah, C. Vallon and J.C. Guerin.** 1998. Analysis of anti-IgE and allergen induced human basophil activation by flow cytometry. Comparison with histamine release. *Inflamm. Res.* 47:401.
 22. **Pâris-Köhler A., P. Demoly, L. Persi, B. Lebel, J. Bousquet and B. Arnoux.** 2000. *In vitro* diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). *J. Allergy Clin. Immunol.* 105:339.
 23. **Bühring H.J., P.J. Simmons, M. Pudney, et al.** 1999. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multi- and unipotent progenitors. *Blood* 94: 2343.
 24. **Bühring H.J., M. Seiffert, C. Giesert, A. Marxer, L. Kanz, P. Valent and K. Sano.** 2001. The basophil activation marker defined by antibody 97A6 is identical with ecto-nucleotide pyrophosphatase/phosphodiesterase 3 (E-NPP3; CD203c). *Blood* 97: 3303.
 25. **Platz I.J., M. Binder, A. Marxer, G. Lischka, P. Valent and H.J. Bühring.** 2001. Hymenoptera-venom-induced upregulation of the basophil activation marker ecto-nucleotide pyrophosphatase/phosphodiesterase 3 in sensitized individuals. *Int. Arch. Allergy Immunol.* 126:335.
 26. **Hauswirth A.W., S. Natter, M. Ghannadan, Y. Majlesi, G.H. Schernthaner, W.R. Sperr, H.J. Bühring, R. Valenta and P. Valent.** 2002. Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J. Allergy Clin. Immunol.* 110:102.
 27. **Valent P., L.K. Ashman, W. Hinterberger, F. Eckersberger, O. Majdic, K. Lechner and P. Bettelheim.** 1989. Mast cell typing: demonstration of a distinct hematopoietic cell type and evidence for immunophenotypic relationship to mononuclear phagocytes. *Blood* 73:1778.
 28. **Valent P., O. Majdic, D. Maurer, M. Bodger, M. Muhm, and P. Bettelheim.** 1990. Further characterization of surface membrane structures expressed on human basophils and mast cells. *Int. Arch. Allergy Appl. Immunol.* 91:198.
 29. **Hennessdorf F., S. Florian, A. Jakob, K. Baumgartner, K. Sonneck, A. Nordheim, T. Biedermann, P. Valent and H.J. Bühring.** 2005. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res.* 15:325.
 30. **Hoffmann-Sommergruber K., M. Susani, F. Ferreira, P. Jertschin, H. Ahorn, R. Steiner, D. Kraft, O. Scheiner and H. Breiteneder.** 1997. High-level expression and purification of the major birch pollen allergen, Betv 1. *Protein Expr. Purif.* 9:33.
 31. **Pittner G., S. Vrtala, W.R. Thomas, M. Weghofer, M. Kundi, F. Horak, D. Kraft and R. Valenta.** 2004. Component-resolved diagnosis of house-dust mite allergy with purified natural and recombinant mite allergens. *Clin. Exp. Allergy* 34:597.
 32. **Falcone F.H., H. Haas and B.F. Gibbs.** 2000. The human basophil: a new appreciation of its role in immune responses. *Blood* 96:4028.
 33. **Turner H. and J.P. Kinet.** 1999. Signalling through the high-affinity IgE receptor Fc epsilonRI. *Nature* 402:24-30.
 34. **Nadler M.J., S.A. Matthews, H. Turner and J.P. Kinet.** 2000. Signal transduction by the high-affinity immunoglobulin E receptor Fc epsilon RI: coupling form to function. *Adv. Immunol.* 76:325.
 35. **Bühring H.J., A. Streble and P. Valent.** 2004. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int. Arch. Allergy Immunol.* 133:317.
 36. **Amano T., T. Furuno, N. Hirashima, N. Ohyama and M. Nakanishi.** 2001. Dynamics of intracellular granules with CD63-GFP in rat basophilic leukemia cells. *J. Biochem. (Tokyo).* 129:739.
 37. **Hauswirth A.W., K. Sonneck, S. Florian et al.** 2007. Interleukin-3 promotes the expression of E-NPP3/CD203c on human blood basophils in healthy subjects and in patients with birch pollen allergy. *Int. J. Immunopathol. Pharmacol.* 20:267.