

Hymenoptera-Venom-Induced Upregulation of the Basophil Activation Marker Ecto-Nucleotide Pyrophosphatase/Phosphodiesterase 3 in Sensitized Individuals

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Key Words

Basophils · Allergy · 97A6 · E-NPP3 · NPP3 · PDNP3 · CD203c

Abstract

Background: Bee and wasp venoms are potent allergens capable of inducing severe clinical reactions. To detect immediate-type hypersensitivity to these allergens, a rapid in vitro test was developed that relies on the upregulation of ecto-nucleotide pyrophosphatase/phosphodiesterase 3 (E-NPP3) on activated basophils. **Methods:** Blood basophils of 13 healthy donors and 22 patients allergic to bee or wasp venom were analyzed for E-NPP3 (CD203c) expression using monoclonal antibody 97A6. Basophils were analyzed by flow cytometry after activation with anti-IgE antibody or allergen. Venom-induced E-NPP3 upregulation on basophils was compared with diagnostic parameters, including skin tests and assessment of specific IgE. In selected samples, the increase in E-NPP3 expression on activated basophils was compared with histamine release and CD63 upregulation. **Results:** In 20/22 patients sensitized to wasp or bee venom, E-NPP3 expression on basophils was upregu-

lated in response to activation by allergen or anti-IgE. The maximum increase in E-NPP3 expression (above ten times of baseline) was achieved after 15 min of stimulation with 1 µg/ml of allergen or anti-IgE antibody. Sensitized individuals who failed to upregulate E-NPP3 in response to IgE receptor cross-linking also failed to induce histamine release and CD63 upregulation. **Conclusions:** Flow cytometric determination of hymenoptera-venom-induced upregulation of E-NPP3 is a novel in vitro test to identify sensitized individuals.

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Introduction

Hymenoptera stings usually cause local transient skin reactions without major clinical symptoms. However, in sensitized individuals, hymenoptera stings may cause severe and even life-threatening allergic reactions [1, 2]. The diagnosis of hymenoptera allergy is mainly based on case history, skin tests, and the determination of specific IgE [3]. To confirm the allergic state, allergen-induced release of histamine or leukotriene C4 is assessed in selected cases [4].

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Recently, flow cytometry-based tests have been developed that rely on the binding of fluorochrome-conjugated allergen to specific cell-bound IgE or allergen-induced cell surface expression of basophilic granule antigens [4–9]. Most of the reports describe the induced cell surface expression of the granule-associated molecule CD63 on the cell surface of activated basophils [6, 9]. In other studies, the direct binding of phospholipase A₂ to basophils of patients allergic to bee venom was reported [5]. All tests are suitable to analyze the binding of molecularly defined allergens, to detect immediate-type hypersensitivity, and possibly also to monitor the success of hyposensitization in allergic patients.

Ecto-nucleotide pyrophosphatase/phosphodiesterase 3 (E-NPP3) is a type II transmembrane protein initially detected in the uterus, prostate and glioma [10–12]. The molecule is composed of a short N-terminal cytoplasmic domain, followed by the transmembrane region, two somatomedin-like domains, the catalytic domain, and a C-terminal EF-hand or nuclease-like domain [10, 13]. In the hematopoietic system, E-NPP3 is selectively expressed on basophils, mast cells, and their CD34⁺ precursor cells [14]. Very recently, the monoclonal antibody 97A6 was shown to recognize an extracellular epitope of functional E-NPP3 [15]. During the conference on human leukocyte differentiation antigens in Harrogate, UK (June, 20–24, 2000), E-NPP3 was clustered to CD203c [16]. Little is known about the role of CD203c in basophils and the factors that regulate CD203c expression. In the present study, we analyzed whether cross-linking of the high-affinity IgE receptor by allergen results in an altered expression. We show that, after challenge with the appropriate allergen, CD203c is rapidly upregulated on the cell surface of basophils of allergic individuals sensitized to bee and/or wasp venom. In addition, we demonstrate that basophils from allergic patients or healthy individuals who fail to upregulate E-NPP3 expression in response to IgE receptor cross-linking also fail to release histamine and to induce CD63 upregulation.

Patients and Methods

Individuals

Twenty-two patients with known allergy to bee or wasp venom (12 women and 10 men, 21–70 years old, mean: 43 years) were studied. Allergy diagnosis was based on skin tests, specific IgE content in the peripheral blood, and clinical history. The peripheral blood was drawn after informed consent according to the guidelines of the local ethics committee of Tübingen, Germany. According to the severity of the reported allergic reaction after bee or wasp stings, the 22 patients were classified as grades I–IV according to Mueller [17]:

grade I (n = 1), grade II (n = 11), grade III (n = 7), grade IV (n = 3). None of the patients had received antihistamines or corticosteroids within the last 4 weeks prior to the blood donation. The control group consisted of 13 healthy donors (8 women and 5 men, 23–54 years old) without any known history of allergy. Two individuals of the control group had a recent history of wasp stings.

Skin Tests

Quantitative skin tests were performed by intradermal application of serial dilutions of bee and wasp venoms (Reless, Scherax, Hamburg, Germany) in skin test diluent. A wheal of at least 5 mm in diameter was regarded as a positive reaction. Skin test diluent and histamine were the negative and positive controls, respectively. No skin tests were performed in 12/13 individuals of the control group.

Measurement of Specific Serum IgE

The concentrations of specific IgE in serum samples obtained from patients and controls were determined by fluorescence immunoassay, following the manufacturer's instructions (UniCAP®, Pharmacia & Upjohn, Freiburg, Germany).

Basophil Activation and Flow Cytometry

Heparinized blood cells (100 µl) from the control group and allergic patients were stimulated with serial dilutions of either anti-IgE antibody (Immunotech, Marseille, France), bee or wasp venom (Reless, Scherax), or phosphate-buffered saline (PBS) for 15 min at 37°C. In selected experiments, stimulation times were modified (range: from 30 s to 120 min). To stop the Ca²⁺-dependent reaction, the cells were washed in PBS/20 mM EDTA and resuspended in 50 µl FACS buffer (0.1% NaN₃ + 0.1% BSA in Hanks' balanced salt solution). The cells were then incubated with 10 µl MoAb 97A6-PE (1 µg/ml final concentration) for 15 min at room temperature. In some experiments, the cells were double-stained either with 97A6-PE and anti-IgE-FITC (Immunotech), or with CD63-PE (Becton Dickinson) and anti-IgE-FITC. After red blood cell lysis with 2 ml IOTest lysing reagent (Immunotech), the cells were washed, resuspended in FACS buffer, and analyzed on a FACSCalibur flow cytometer. Modulation of E-NPP3 expression was evaluated by calculating the stimulation index (SI) and the percentage of stimulated basophils. The SI is calculated as the median fluorescence intensity of allergen- or anti-IgE-stimulated 97A6⁺ cells/median fluorescence intensity of PBS-stimulated 97A6⁺ cells. The percentage of activated basophils is calculated as the percentage of allergen- or anti-IgE-stimulated 97A6^{bright} cells (97A6⁺ cells in the 'activation gate' R2) minus the percentage of PBS-stimulated 97A6^{bright} cells (fig. 2). Total basophils (100% reference) are defined as cells residing in R1 and R2.

Histamine Release Assay

To determine the functional response of basophils, histamine release experiments were performed. In parallel, leukocyte and basophil counts from whole blood samples were determined on a Sysmex SE-9000 cell counter (Sysmex, Norderstedt, Germany). Two hundred microliters of whole blood were incubated with 1 µg/ml anti-IgE antibody (Immunotech) or PBS for 15 min at 37°C. After blocking the reaction with 200 µl of 20 mM EDTA, cells were centrifuged and the supernatant collected to determine the content of released histamine by radioimmunoassay (Immunotech). Total histamine in cell suspensions was quantified after cell lysis in distilled water and freeze-thawing. Histamine release was expressed as the percentage of total histamine.

Table 1. Summary of clinical and experimental data of 22 patients allergic to Hymenoptera venom

Patient No.	Skin test 10 ^{-x} µg/ml	Specific IgE RAST class	Mueller grade	Stimulated basophils venom/a-IgE, %	SI venom/a-IgE
<i>Bee allergy</i>					
7	neg.	3	IV	88/88	9.6/7.9
8	3	5	II	77/59	6.4/4.7
12	2	4	II	86/82	6.6/5.1
15	1	2	I–II	82/90	9.8/10.8
18	1	3	II–III	1/1	1.2/0.8
22	2	3	II	60/69	9.1/9.9
<i>Wasp allergy</i>					
1	1	0	III	64/100	5.0/7.8
2	2	3	II	77/77	4.2/4.7
3	1	3	III	82/80	5.5/4.9
4	2	2	I	77/64	6.1/5.6
5	3	4	II	74/88	7.5/9.1
6	3	3	II	85/80	12.6/10.8
9	3	3	II–III	85/71	8.9/6.5
10	3	n.d.	I–II	69/62	5.2/5.5
11	3	2	II–III	87/85	11.6/10.3
13	neg.	0	III	91/94	9.1/9.7
14	4	5	II	91/86	9.1/8.1
16	neg.	5	II	79/75	9.9/8.5
17	2	0	II	59/81	7.3/10.8
19	2	2	III	1/0	1.0/0.9
20	1	1	III–IV	71/89	7.1/11.9
21	2	4	III–IV	92/92	13.5/13.0

Specific IgE concentration (CAP-RAST class 0–6), skin test diagnosis (expressed for positive reactions as dilution of venom in 10^{-x} µg/ml), classification according to Mueller [17], and E-NPP3 upregulation (SI and the percentage of activated basophils) are shown. The calculations of SI (increase of E-NPP3 expression on basophils) and the percentage of activated basophils are described in the Methods. n.d. = Not determined; neg. = negative reaction at 10⁻¹ µg/ml of venom.

Results

Clinical and Serologic Characterization of Patients and Control Individuals

In the present study, 13 control donors and 22 patients with known allergy to bee or wasp venom were examined. Diagnostic tests included assessment of specific IgE, a skin test, and disease grading according to Mueller [17]. Table 1 shows that 6 patients were allergic to bee venom and 16 patients to wasp venom. Five patients were negative (No. 7, 16) or showed a nonspecific reaction (No. 3, 15, 18) in the skin test, but were positive for specific IgE (CAP-RAST class: 2 and 3) and were classified as Mueller grade II–III. Three patients allergic to wasp venom (No. 1, 13, 20) were negative or nonspecific for specific IgE and skin test, but suffered from Mueller grade III. Surprising-

ly, one of the nonallergic control individuals with a recent history of a wasp sting was positive for wasp IgE (table 2).

Allergen-Induced Upregulation of E-NPP3/CD203c

Time and Dose Dependency of E-NPP3 Upregulation.

In previous studies, the potential regulation of E-NPP3 expression on basophils was analyzed by stimulating KU-812 cells or peripheral blood cells from healthy individuals for 2 h with either interleukin (IL)-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, stem cell factor, antibody 97A6, or anti-IgE antibody, prior to analysis by flow cytometry [14]. None of the reagents modulated E-NPP3 expression except anti-IgE antibody that induced a significant upregulation of E-NPP3 (four- to six-fold compared

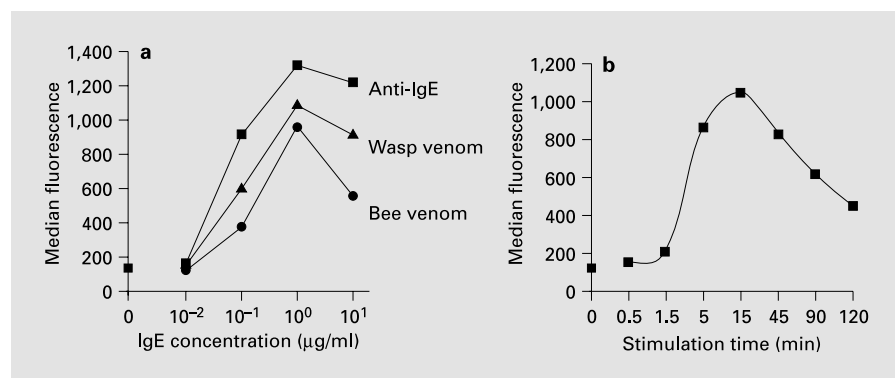


Fig. 1. a Dose-dependent upregulation of E-NPP3 expression. Whole blood cells of a patient allergic to wasp venom and cross-sensitized to bee venom (No. 5) were stimulated with serial dilutions of anti-IgE antibody, wasp allergen, or bee allergen. After 15 min of incubation, the reaction was terminated with 100 µl of 20 mM EDTA solution. Cells were stained with antibody 97A6-PE for 15 min. After red blood cell lysis and washing, the cells were analyzed on a FACS-

Calibur flow cytometer. The median values of the 97A6+ populations were plotted against venom or antibody dilutions. **b** Time-dependent upregulation of E-NPP3 expression. Whole blood cells of control individual No. 1 were stimulated with 1 µg/ml anti-IgE antibody. After defined time intervals (0.5–120 min), the reaction was stopped with EDTA. For control, cells were stimulated with PBS for 15 min.

Table 2. Summary of experimental data of 15 nonallergic control individuals

Control individuals	Specific IgE RAST class	SI venom/a-IgE
1	0	1.0/9.1
2	0	1.1/10.6
3	0	1.1/12.7
4	2	2.3/3.0
5	0	4.3/1.9
6	0	0.9/1.2
7	0	1.1/5.6
8	0	0.8/3.2
9	0	1.1/11.0
10	0	0.8/7.1
11	0	0.9/1.6
12	0	0.7/2.9
13	0	0.8/6.7

Specific IgE concentration (CAP-RAST class 0–6) and E-NPP3 upregulation (SI) of wasp venom and anti-IgE are shown. All bee venom SI ranged from 0.7 to 1.0. The calculations of SI (increase of E-NPP3 expression on basophils) are described in the Methods.

to control) [14]. This prompted us to analyze the IgE-receptor-mediated activation of basophils in allergic patients. To determine the optimal dosage of anti-IgE antibody or allergen, heparinized blood cells of individuals allergic to bee and wasp venom were incubated for 15 min

at 37°C with either PBS (negative control) or serial dilutions of anti-IgE antibody, bee and wasp venom. After staining with E-NPP3-specific antibody 97A6, the cells were analyzed by flow cytometry. Figure 1a shows a maximum of E-NPP3 expression (median fluorescence intensity of 97A6+ population) after stimulation with 1 µg/ml of either anti-IgE antibody (SI = 8.1 ± 3.1), bee allergen or wasp allergen, whereas concentrations of 10 µg/ml were less effective. To determine the time dependence of basophil activation, blood cells from healthy subjects were stimulated with 1 µg/ml anti-IgE antibody for defined time intervals (0.5–120 min) prior to staining with antibody 97A6. Figure 1b shows that the highest E-NPP3 expression is reached after 15 min of stimulation. Longer exposure times result in decreased levels of E-NPP3 expression.

Response in Patients Allergic to Wasp Venom. In the next step, we analyzed allergen-induced E-NPP3 upregulation in 16 patients allergic to wasp venom. In 15 patients, E-NPP3 expression on basophils significantly increased when challenged with wasp venom (table 1). The SI varied from 4.2 to 13.5 (SI = 8.2 ± 2.8), and the percentage of activated basophils ranged from 59 to 92% (median = $79 \pm 12\%$). Figure 2 shows E-NPP3 upregulation in a representative sample of a patient allergic to wasp venom (No. 14). Compared to the control (fig. 2a), stimulation with bee venom (fig. 2c) did not result in an increased E-NPP3 expression. In contrast, when the cells were activated by anti-IgE antibody (fig. 2b) or wasp

venom (fig. 2d), a 9.1-fold increase of E-NPP3 expression on 91% of the basophils was observed. In 1 patient (No. 19), no change of E-NPP3 expression was induced by anti-IgE antibody or wasp venom. Interestingly, 3 patients (No. 1, 13, 20) were negative or had a nonspecific baseline reaction for specific IgE and skin test, but were classified as allergic to wasp venom according to Mueller. In these cases, the basophil activation test was of particular confirmatory value. The basophils of 5 patients allergic to wasp venom (No. 3, 4, 5, 6, 9) were also activated by bee venom, indicating cross-sensitization in these patients (not shown).

Response of Patients Allergic to Bee Venom. Only 6 of the 22 patients tested were allergic to bee venom (table 1). In 5 patients, upregulation of E-NPP3 expression on basophils was similar ($SI = 8.3 \pm 1.7$) to that observed in patients allergic to wasp venom. In 1 patient (No. 18), no increase of E-NPP3 expression could be induced by either anti-IgE antibody or bee venom. Notably, the basophils of 3 patients allergic to bee venom (No. 7, 12, 15) were also activated by wasp venom, a feature that indicates cross-sensitization.

Response in Healthy Individuals. Thirteen healthy control individuals without a known history of allergic reactions were analyzed. As expected, 10/13 individuals responded exclusively to the stimulation with anti-IgE antibody but not with allergen (table 2). One individual (No. 6) neither responded to anti-IgE antibody nor to allergen activation. Notably, 2 individuals (No. 4 and 5) who reported recent wasp stings responded also to wasp allergen. Further analyses showed that individual No. 4 was also positive for specific IgE, whereas individual No. 5 was negative or nonspecific for both, IgE and skin test.

Correlation between E-NPP3 and CD63 Upregulation in Responders and Nonresponders

Previous reports have shown that activation of basophils in patients allergic to cypress pollen allergen is associated with CD63 upregulation [6]. This prompted us to compare E-NPP3 and CD63 upregulation in selected individuals who either responded to anti-IgE stimulation (responders) or did not show an elevated E-NPP3 expression after stimulation (nonresponders). For this purpose, the basophils of 2 responders (control No. 1 and patient No. 22) and 2 nonresponders (control No. 6 and patient No. 19) were stimulated with anti-IgE antibody, double stained with anti-IgE-FITC and CD63-PE or anti-IgE-FITC and 97A6-PE, and analyzed by flow cytometry. Figure 3a shows control individual No. 1 (responder), whose activated basophils not only show an increased E-NPP3

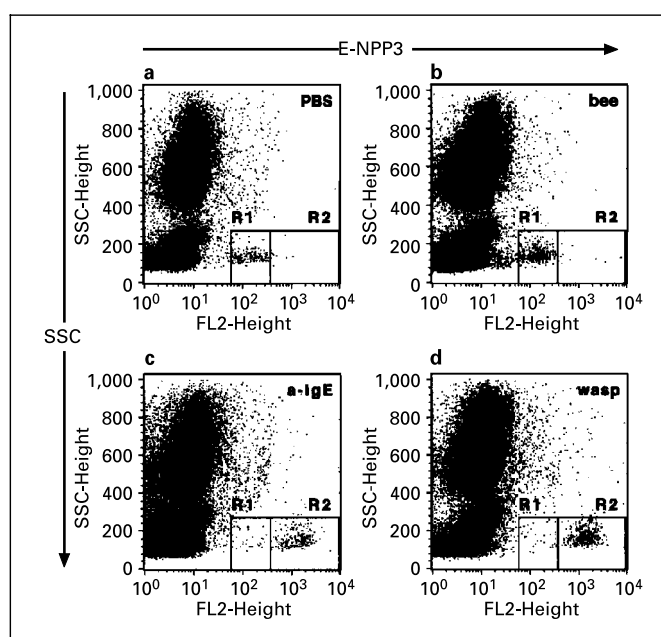


Fig. 2. E-NPP3 expression on basophils upregulated by allergen and anti-IgE antibody. One hundred microliters of whole blood cells from a patient allergic to wasp venom (No. 14) were incubated for 15 min with either PBS (**a**; negative control), 1 μ g/ml of anti-IgE antibody (**b**; positive control), bee allergen (**c**), or wasp allergen (**d**). After staining with 97A6-PE and red blood cell lysis, the cells were analyzed on a FACSCalibur flow cytometer. The SSC versus FL2 (97A6-PE) plots result from 50,000 analyzed cells. Nonstimulated basophils reside in R1 and stimulated basophils in R2.

(R2) but also an elevated CD63 (R3) expression. Similar results were obtained with the second analyzed responder (patient No. 22; figure not shown). In both individuals, the E-NPP3+ population consisted of more cells (68 and 78%) than the CD63+ population (31 and 45%).

E-NPP3 is not upregulated on the basophils of 1 healthy individual (control No. 6; table 2) and 2 patients with hymenoptera allergy (No. 18 and 19; table 1). The unresponsiveness to allergen and anti-IgE antibody in these individuals was not due to the lack of cell-bound IgE because the cells stained well with anti-IgE antibody. As shown in figure 3b, neither E-NPP3 nor CD63 expression was upregulated in control individual No. 6. Likewise, a failed upregulation of CD63 was also observed in the other 2 nonresponders (patients No. 18 and 19; not shown).

Comparison of Histamine Release in Responders and Nonresponders

To analyze whether the failed E-NPP3 or CD63 upregulation in nonresponders is associated with inhibited

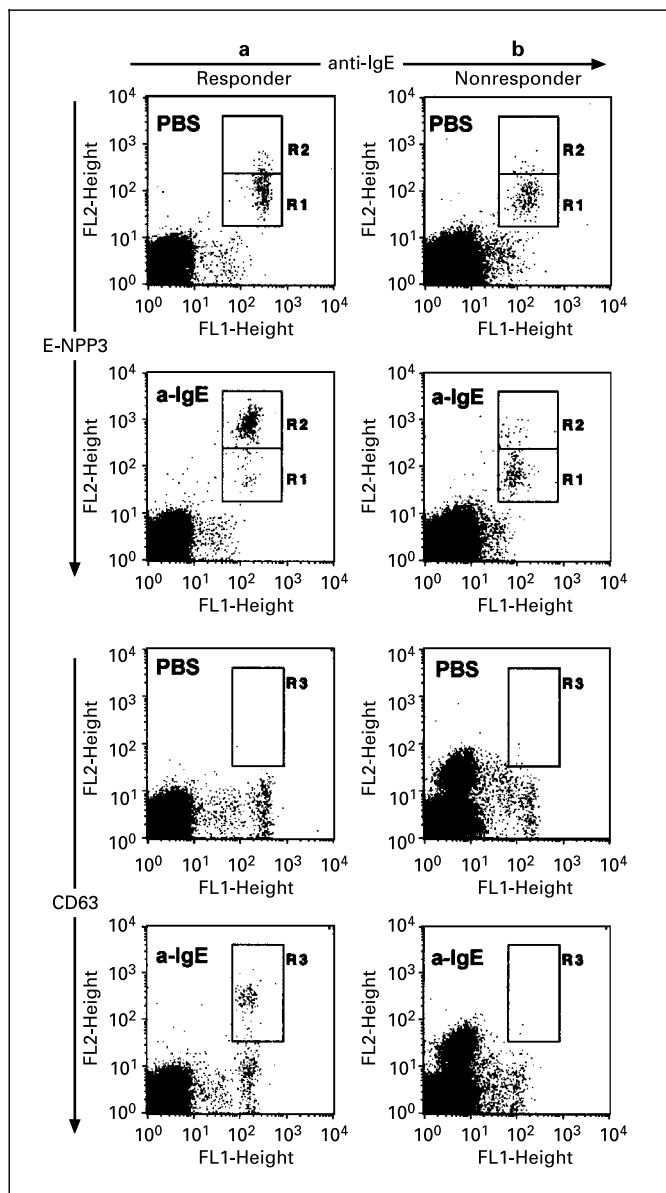


Fig. 3. Comparison of E-NPP3 and CD63 upregulation on stimulated basophils. Whole blood cells were incubated for 15 min with either PBS or anti-IgE and double-stained with antibodies 97A6-PE and anti-IgE-FITC (top) or CD63-PE and anti-IgE-FITC (bottom). After red blood cell lysis and washing, cells were analyzed on a FAC-SCalibur flow cytometer. **a** Cells of a responder (control No. 1). **b** A nonresponder (control No. 6). Note that both E-NPP3 and CD63 are exclusively upregulated on the basophils of responders.

degranulation, histamine release experiments were performed in 2 normal responders (patient No. 22, control No. 2), 1 low responder (control No. 5), and 2 nonresponders (patient No. 19, control No. 6). Our analyses revealed that nonresponders were not only unable to upregulate

either E-NPP3 or CD63 expression, but also failed to release histamine. In contrast, the basophils of the responders secreted significant amounts of histamine (>10% of total histamine) that correlated well with the CD203c SI. The good responders (SI = 9.9 and 10.6) released 12.5 and 15.6% of total histamine, the low responder (SI = 1.9) 5.0%, and the nonresponders (SI = 0.9 and 1.2) 1.9 and 1.2%. It is not known whether the failure to release histamine, to upregulate E-NPP3 or to induce CD63 cell surface expression is the result of inhibited degranulation.

Discussion

In this study, we described the development of a novel flow cytometric assay for basophil activation using E-NPP3-specific monoclonal antibody 97A6. We demonstrated that in vitro stimulation of basophils with allergen or anti-IgE antibody resulted in an increased E-NPP3 expression. Our study also showed that E-NPP3 upregulation was induced in a dose-dependent manner. Based on these data, we suggest that in most cases, the E-NPP3 (CD203c) activation assay is suitable for the detection of immediate-type hypersensitivity and to monitor patients under immunotherapy or other treatments.

In our flow cytometric assay, the basophils were activated with the same commercially available test allergens that are also used for diagnostic skin tests. However, these allergens are not single compounds but rather a mixture of molecules. The rather crude wasp venom preparations, which contain phospholipase A₁, hyaluronidase, and Ves v5 (antigen 5) as their major allergens [18–20], as well as the bee venom preparations, which contain phospholipase A₂, hyaluronidase, acid phosphatase, and mellitin as major allergens, are poorly defined [21, 22]. These impure mixtures may at least in part explain the variability in E-NPP3 upregulation due to the undefined concentrations of the single venom compounds. To unambiguously define the specificity of allergen-induced E-NPP3 upregulation, recombinant allergens are the preferred reagents for stimulation. Studies using molecularly defined bee and wasp venom components are in progress [Binder et al., in preparation].

Our in vitro assay confirmed sensitization in 20/22 patients with hymenoptera allergy. The remaining 2 patients with positive skin tests and/or specific IgE, were nonresponders in terms of E-NPP3 and CD63 upregulation, as well as histamine release. Of the tested nonallergic individuals, 1 healthy donor failed to respond to anti-IgE

stimulation. In this context, it is of interest that about 10% of the healthy or allergic individuals fail to release histamine from basophils in response to IgE receptor cross-linkage [23]. Thus, our test is suitable to analyze basophil activation and to confirm sensitization in responders. The absent E-NPP3 upregulation in nonresponders is probably caused by defective signaling of adaptor molecules downstream the FcεRI pathway. A possible lack of cell-bound IgE could be excluded because the basophils of all nonresponders stained well with anti-IgE antibody. Maybe mast cells compensate this defect of nonresponder basophils in allergic disease. Remarkably, in 2 individuals with no reported allergy, E-NPP3 upregulation was also induced by wasp allergen. One individual had specific IgE, whereas the other individual was negative for specific IgE and nonspecific on the skin test. It is of interest that these donors had a recent history of wasp stings. It remains unknown whether the E-NPP3 upregulation is indicative of allergy or represents a prephase of allergy or is a transient phenomenon.

Recently, Pâris-Köhler et al. [6] described a flow cytometric test for cypress pollen diagnosis that relies on the upregulation of CD63 on activated basophils. Since CD63 is not a selective marker for basophils, but is also expressed on activated platelets and monocytes [24–26], fluorochrome-conjugated anti-IgE antibodies are additionally required to define the basophil subset. However, anti-IgE antibodies not only detect basophils with considerable selectivity, but are also known to activate basophils by cross-linking high-affinity IgE receptors via cell-bound IgE [27]. This activity of anti-IgE antibodies may therefore interfere with the test. In addition, IgE receptors are also expressed on monocytes and eosinophils in allergic patients [28, 29]. When comparing E-NPP3 and CD63 upregulation, the percentage of activated basophils was higher when E-NPP3 was analyzed. One explanation is that CD63 may be expressed at a later stage of basophil activation than E-NPP3, possibly because E-NPP3 but not CD63 molecules may be expressed on granule membranes that display E-NPP3 molecules on the cell surface as soon as the basophils start to degranulate. Studies are in progress to address the question whether CD63 and E-NPP3 upregulation follow different kinetic behaviors.

The use of antibody 97A6 to analyze basophil activation offers several advantages over other tests: first, the test is rapid and requires only minute amounts of peripheral blood. Second, the test can be applied to detect immediate-type hypersensitivity and possibly to monitor the success of hyposensitization. Third, antibody 97A6 simultaneously defines basophils and distinguishes activated

and resting basophils on the basis of quantitative differences of E-NPP3 expression. Therefore, in contrast to the Basotest™, the addition of anti-IgE antibodies to define the basophil subset is not required.

E-NPP3 is a multifunctional ecto-enzyme that combines phosphodiesterase I and nucleotide pyrophosphatase activities and catalyzes the cleavage of phosphodiester and phosphosulfate bonds of a variety of molecules including deoxynucleotides, NAD, and nucleotide sugars [10–13]. In addition to the catalytic site, E-NPP3 contains a somatomedin B-like domain, a cell adhesive (Arg-Gly-Asp) and an EF-hand motif [10]. At present, we can only speculate about the role of this enzyme in basophil function(s). The closely located somatomedin-B-like and cell-adhesive domains of E-NPP3 may be responsible for binding to cells expressing insulin-like receptors. The catalytic domain and the EF-hand, Ca²⁺Mg²⁺-binding motif, may be responsible for the clearance and degradation of nucleotides produced during an allergic reaction. Since E-NPP3 also exists as a secreted molecule [10, 11], activated basophils may not only cleave foreign molecules in close vicinity to their plasma membranes but also in their extracellular surroundings.

Apart from the usefulness of analyzing E-NPP3 function and to detect immediate-type hypersensitivity, antibody 97A6 is also a suitable reagent to define and purify viable and pure basophils, including their precursor cells [10], using paramagnetic separation procedures [30]. In summary, antibody 97A6 is a useful reagent to classify sensitization of individuals allergic to hymenoptera venoms, to monitor the success of hyposensitization, to analyze basophil activation, and to isolate basophils. To our knowledge, this is the first flow cytometry-based assay to analyze basophil activation which depends on the use of only one antibody.

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