

Surface membrane antigen alteration on blood basophils in patients with Hymenoptera venom allergy under immunotherapy

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Background: Venom immunotherapy (VIT) provides widespread protection against systemic anaphylactic reactions after a sting of the respective insect. This effect is attributed to a shift from T_H2 to T_H1 . However, because basophils also produce and release cytokines, such as IL-4 and IL-13, they may be part of the cytokine network. The cytokines may regulate basophilic granulocytes, as suggested by the presence of cytokine receptors IL-2R α , GM-CSFR α , IL-1RII, IL-3R, IL-4R, IL-5R, and IL-6R on basophils from nonallergic donors. **Objective:** The purpose of this study was to demonstrate that human basophils from subjects allergic to wasp venom undergoing VIT are regulated by cytokines, as shown by the alteration of the expression of cytokine receptors (and other markers). **Methods:** The expression of the surface interleukin receptors and activation antigens on basophils from 19 nonallergic subjects and 48 patients with wasp venom allergy was investigated before, immediately after, and 1 week after VIT (20 patients only). **Results:** Basophilic granulocytes in allergic subjects, compared with those in healthy persons, showed elevated expression of CD32 (Fc γ RII), CD122 (IL-2R β), CD124 (IL-4R α), CD130 (IL-6 and 11R β), CD154 (CD40L), and HLA-DR. Activation of basophils clearly increased during VIT indicated by increased expression of CD32, CD33, CD35 (CR1), CD63, CD116 (GM-CSFR α), CD122, CD124, CD130, and CD154. HLA-DR expression also tended to increase. The expression of IL-5R (CD125) decreased. A significant decrease of the basophilic surface antigens CD11c, CD32, CD35, CD63, CD116, CD122, CD124, CD130, and CD132 (interleukin receptor γ) was detected 1 week after the end of rush VIT. **Conclusion:** The rise in CD63 during VIT indicates a partial basophil degranulation with release of stored protein mediators, including IL-4. IL-4 may cause a transient upregulation of different surface antigens in an autocrine manner. Thereafter, cytokines released by T cells, which as a result of VIT have changed from a T_H2 type to a more T_H1 type, downregulate the activation of the basophilic granulocytes. (*J Allergy Clin Immunol* 2000;106:1190-5.)

Key words: Wasp venom allergy, basophils, immunotherapy, cytokine receptors, HLA-DR

Abbreviations used

PE: Phycoerythrin
VIT: Venom immunotherapy

Venom immunotherapy (VIT) is recommended as the method of choice for the treatment of wasp and bee venom allergy. It provides widespread protection against systemic anaphylactic reactions in over 80% of patients after a bee or wasp sting.¹

The mechanism of this protection is not well understood. Recent examinations suggest the T helper cell response shifts from a T_H2 type to T_H1 type.²⁻⁴ A T_H2 response supports antibody production, especially production of IgE.⁵

Bellinghausen et al² have shown that the surface expression of lymphocyte markers alters in subjects with venom allergy in response to immunotherapy. They detected decreased levels of IL-4R, CD23, CD40, and CD40L in lymphocytes 1 week after the initiation of VIT by using flow cytometry with purified PBMCs. No changes were found for the surface markers CD25 (IL-2R α), IL-12R, and HLA-DR. Moreover, after immunotherapy, the allergen-induced production of the T_H1 cytokines IL-10 and IFN- γ had increased, whereas the T_H2 cytokine IL-4 had decreased.

Basophilic granulocytes are considered to belong to the effector cells in the allergic reaction. Typically, they express the high-affinity IgE receptor. Cross-linkage of the IgE receptors with allergen-IgE complexes leads to release of preformed and newly synthesized mediators (eg, histamine) and leukotrienes. Already, after reaching the maintenance dose in VIT, the mediator release is altered but not the level of specific IgE.

CD32, the low-affinity Fc γ receptor (Fc γ RII), is another receptor expressed by basophils,^{6,7} although its significance for the allergic reaction has not been established.

The basophils are not only involved in IgE-mediated release of mediators, but they also produce the T_H2 -type cytokines IL-4 and IL-13.⁸ The extent to which they may increase the allergic response has not yet been quantified.

Cytokines also regulate release of basophil mediators. For instance, IL-3, IL-5, and GM-CSF increase the IgE-dependent activation of basophils. Moreover, IL-3, together with C5a, can activate basophils in an IgE-

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TABLE I. Surface antigen expression on basophilic granulocytes (percentage of positive cells): Comparison of allergic and nonallergic (healthy) subjects

Surface antigen	Allergic subjects (n = 46)	Healthy subjects (n = 19)	Significance (t test)
CD11c	85.46 ± 14.5	78.92 ± 11.9	.0854
CD25	46.08 ± 22.6	38.32 ± 19.1	.1911
CD32	78.56 ± 20.0	51.75 ± 24.9	.0008
CD33	51.72 ± 21.9	47.01 ± 22.1	.4681
CD35	79.11 ± 20.6	88.32 ± 10.5	.0236
CD63	93.83 ± 5.9	81.28 ± 13.0	.0006
CD116	24.68 ± 18.4	16.67 ± 12.6	.0679
CD122	13.03 ± 13.1	1.79 ± 1.1	<.0001
CD123	98.63 ± 2.3	98.18 ± 1.1	.1991
CD124	13.40 ± 12.9	2.16 ± 1.7	<.0001
CD125	67.10 ± 25.2	53.05 ± 17.9	.0116
CD130	33.74 ± 21.6	7.57 ± 5.0	<.0001
CD132	71.25 ± 27.3	51.89 ± 19.7	.0037
CD154	18.28 ± 15.4	4.06 ± 2.9	<.0001
HLA-DR	20.34 ± 14.4	7.11 ± 5.7	.0002

Values are given as mean ± SD where shown.

independent manner. IL-3 is the most important growth factor for the basophilic granulocytes.⁹

Other cytokines may also regulate basophilic granulocytes, as suggested by the presence of cytokine receptors IL-2R α , GM-CSFR α , IL-1RII, IL-3R, IL-4R, IL-5R, and IL-6R on enriched basophils from nonallergic donors and from patients with chronic myeloid leukemia (by means of radioreceptor assays and flow cytometry).^{6,7,10} However, IL-6R β ,⁷ IL-2R β ,^{7,10} and HLA-DR^{7,10} were not detected on basophils from nonallergic donors.

Furthermore, the complement receptors CD11b/CD18 (CR3 and C3bIR), CD11c/CD18 (CR4 and C3bIR), and CD35 (CR1, C3bR, and C4bR) were found on basophils from healthy donors,^{6,7,11} suggesting interactions with, for example, immune complexes.

CD33, a marker for myeloid cells with no known function, also occurs on human blood basophils.¹²

Recently, it was shown that CD63 is an activation marker on basophils. CD63 (the function of which in regard to basophils is unknown) is located inside the granule membrane in resting basophils. It is expressed on their surface after degranulation. Knol et al¹³ showed a high correlation between CD63 and histamine release.

Therefore we investigated the expression of the surface membrane antigens CD11c, CD25, CD32, CD33, CD35, CD63, CD116, CD122, CD123, CD124, CD125, CD130, CD132, CD154, and HLA-DR on basophils from patients with wasp venom allergy before, immediately after, and 1 week after rush VIT and compared the results to the expression of the surface markers on basophils from healthy donors.

METHODS

Subjects and immunotherapy protocol

Forty-eight patients (21 men and 27 women; age, 35.2 ± 13.1 years), all of whom had been treated with rush VIT (rush therapy, reaching a maintenance dose of 100 mg of venom injected subcuta-

neously within 1 week), were enrolled in the study. Blood was drawn into vacutainer heparinized tubes before initiation of VIT, at the end of rush VIT, and, in 20 patients, 1 week after the end of VIT.

Clinical severity of reactions was graded according to the scale of Mueller¹⁴ as follows: grade 0 involves local reaction only; grade I involves urticaria, anxiety, and/or malaise (12 patients); grade II involves angioedema, chest tightness, nausea, vomiting, diarrhea, abdominal pain, and/or dizziness (22 patients); grade III involves shortness of breath, wheeze, stridor, dysphonia, and/or confusion (11 patients); and grade IV involves hypotension, loss of consciousness, and/or incontinence (3 patients). Nineteen patients were also sensitized to bee venom, and 1 patient was sensitized to bee and hornet venom. Total IgE levels were significantly higher in patients with allergy to wasp and bee venom than in patients with allergy to wasp venom only (270 vs 90 kU/L, $P = .0185$). Of the included patients, 3 were atopic, with grass pollen rhinoconjunctivitis (2) and house dust mite asthma (1).

The allergy diagnosis was based on the clinical history of an allergic reaction to a wasp sting (Mueller grade >1), a positive skin test response (Prick-test, performed with wasp venom Reless; Scherax Arzneimittel GmbH, Hamburg, Germany), and a positive specific IgE measurement (CAP System; CAP class 3.02 ± 1.16) to wasp venom. Patients with a positive clinical history, a positive skin test response, and a CAP class of at least 2 (mean, 3.06) were included in the study.

The treatment was performed between November 1998 and May 1999 in an inpatient department. Reless wasp venom was used with a standardized protocol over 7 days. Initially, each patient received a dose of 1 pg of venom, which was increased to a maintenance dose of 100 μ g before discharge.

Nonallergic subjects

Nineteen healthy nonallergic persons without sensitization to wasp venom (specific IgE <0.35 kU/L) and no evocative clinical history to wasp sting served as control subjects.

Specific IgE

Quantification of serum wasp venom-specific IgE was performed by a CAP assay (Pharmacia & Upjohn). Results were expressed as kilounits per liter. A serum concentration of less than 0.35 kU/L was considered negative.

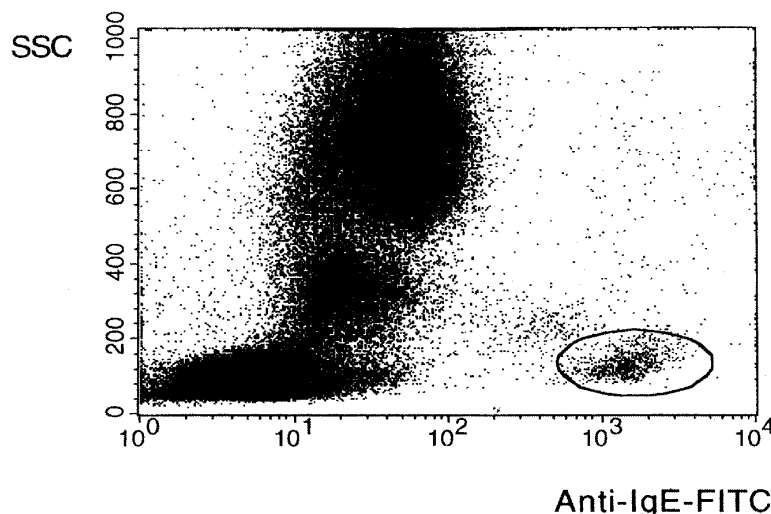


FIG 1. Identification of basophilic granulocytes by means of flow cytometry. The position of IgE-positive cells in the side scatter is shown. SSC, Side scatter.

Flow cytometry

For the 2-color flow cytometry, 100 μ L of heparinized blood was incubated for 20 minutes at room temperature together with 5 μ L of FITC-conjugated anti-IgE (Dako) and phycoerythrin (PE)-conjugated mAb. The erythrocytes were lysed with 2 mL of FACSlysing agent (Becton Dickinson) for 10 minutes.

The samples were analyzed on a flow cytometer FACScan with Cellquest software. The basophilic granulocytes were characterized through the high IgE expression and the side light scatter behavior of lymphocytes. Five hundred to 1000 basophils were measured, and the percentage of PE-positive cells in this population was assessed.

The following PE-conjugated antibodies were used: HLA-DR and CD11c from Becton Dickinson; CD123, CD125, CD130, CD132, and CD154 from Pharmingen; CD116, CD122, CD124, CD125, CD32, CD33, and CD63 from Coulter/Beckmann; and CD35 from Serotec.

Statistical analysis

The Wilcoxon matched pairs signed-rank test for comparing changes from the same individuals and the *t* test for unpaired variances (different populations) were applied.

RESULTS

Characterization of basophils

Basophils were characterized by their position in the side scatter and their high IgE-binding density. Fig 1 shows the gating of basophilic cells. In this example, 688 of 100,000 cells were defined as basophils. On the graph, the basophils are located in the side scatter on the level of lymphocytes. The intensity of CD45 expression of the gated cells was within the range of the granulocyte population (data not shown). Cytospin preparations showed the staining for basophils (not shown).

In patients with wasp venom allergy, on average, there were 15,000 basophils per 10^6 lymphocytes ($\pm 70\%$), and in healthy subjects there were 2000 basophils per 10^6 lymphocytes ($\pm 90\%$). Only subjects with more than 500 basophils per 10^6 lymphocytes were enrolled in the

study. Under VIT, there were no significant changes in the number of basophils.

Comparison of surface membrane antigens on basophils from healthy donors and patients with wasp venom allergy

We measured several surface membrane molecules on basophils from healthy donors, as characterized by extensive anamnesis and low levels of wasp venom-specific IgE, and compared these with the same molecules on basophils from subjects with wasp venom allergy selected for VIT. As Table I shows, the cell numbers expressing surface antigens differed significantly. The percentages of basophils expressing Fc γ RII, IL-2R β , IL-4R, IL-5R, the β chain of IL-6R, IL-11R, the common γ chain of at least 5 interleukin receptors, CD40L, CD63, and HLA-DR were significantly higher in the blood of the allergic persons compared with that of healthy control subjects. We found insignificant higher expression of CR4 and GM-CSFR α in allergic subjects. The complement receptor 1 is expressed significantly more frequently on the basophils of healthy persons compared with the basophils of subjects with wasp venom allergy. In patients with wasp venom allergy, we could not find any correlation of clinical parameters with surface marker expression.

Change of surface antigen expression after 1 week of immunotherapy

Forty-eight patients with wasp venom allergy were tested for surface antigens on basophils before and after rush VIT. The standardized protocol consisted of 3 to 5 injections of wasp venom on 7 subsequent days, with increasing doses to a maintenance dose of 100 μ g. The treatment was performed during the winter.

Blood was taken from patients before VIT (day of admission) and after VIT (on day of discharge).

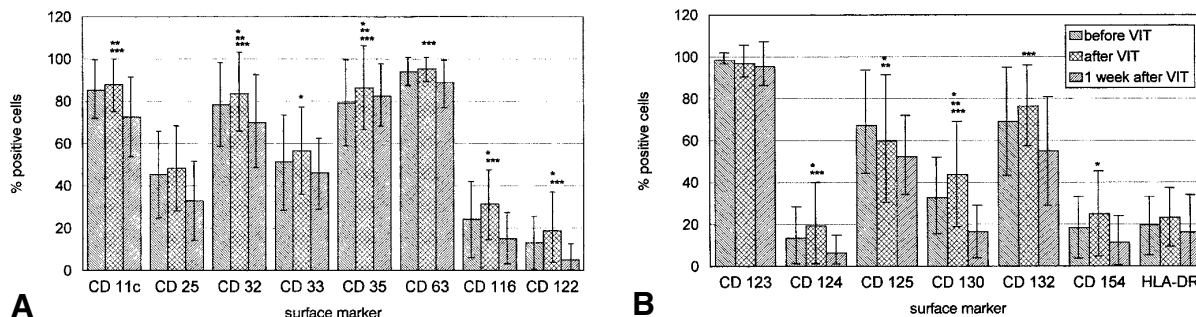


FIG 2. Expression of surface markers on basophils. **A**, CD11c, CD25, CD32, CD33, CD35, CD63, and CD116 (bars as defined in **B**) expression. **B**, CD122, CD123, CD124, CD125, CD130, CD132, CD154, and HLA-DR expression. Asterisks indicate statistically significant differences between the following: before and immediately after VIT (*), before and 1 week after VIT (**), and immediately and 1 week after VIT (***).

As Fig 2 shows, expression of the receptors CD32, CD33, CD35, CD116, CD122, CD124, CD130, CD132, and CD154 was significantly increased after VIT compared with levels at the first measurement. HLA-DR was slightly increased, whereas CD125 expression was significantly decreased. CD11c, CD25, CD123, and CD132 expression did not change.

Control of surface marker expression on basophils 1 week after completion of treatment

We assessed the expression of surface markers in 20 patients 1 week after the end of treatment. Fig 3 shows some typical dot plots of a selected patient. As shown in Fig 2, expression in all markers was reduced compared with 1 week before. CD33, CD116, CD122, CD124, and CD154 expression approached the values before VIT, and CD11c, CD32, CD35, CD125, and CD130 expression was significantly decreased. The expression of CD25, CD63, CD123, and HLA-DR did not change significantly during the entire course of therapy.

DISCUSSION

Most of the reported investigations on basophils have been performed on separated cells. This study used heparinized whole blood. The basophils were characterized, by means of flow cytometry, by their high IgE surface density and their localization in the side scatter in the region of the lymphocytes.

Our method differs from the current CD45/anti-IgE labeling.^{15,16} To allow results to be compared, we demonstrated that the cells located in the gate of lymphocytes in the side scatter with high expression of IgE receptors also express CD45 but with lower density than lymphocytes (data not shown). Like Laudy-Sainte et al,¹⁷ we acquired free fluorescent channels for multiple staining by using our method of single anti-IgE labeling for basophil definition. Interpretation of only the gate site scatter/anti-IgE^{bright} is simpler. This method enables a representative number of basophilic cells to be examined phenotypically.

The presented data clearly indicate that the basophils in subjects with wasp venom allergy show a higher expression of many surface membrane molecules than do those in healthy persons. This is especially remarkable because the examinations were usually performed up to 6 months, but at least 8 weeks, after the previous sting during a time when no insects were present. The significantly increased expression of CD32, CD122, CD124, CD130, CD154, and HLA-DR suggests persistent activation of these cells.

Somewhat unexpected was the finding of HLA-DR expression on basophils from allergic donors. However, our data concerning HLA-DR on basophils from healthy persons (7.1%) are in accordance with those of Toba et al,¹⁰ who found that 1.4% of major basic protein-positive cells carry HLA-DR. In contrast, 20.3% of basophils of donors with wasp venom allergy were HLA-DR positive. This suggests that stimulated basophils are capable of presenting antigens. This capability has already been shown for mast cells.¹⁸ The capacity to present antigens was supposed for the first time after the detection of MHC class II molecules on mast cells in rats.¹⁹

The increased expression of CD40L on activated basophils indicates a helper cell-like function. The (stimulated) human basophilic cell line KU812 (but not the mast cell line HMC-1) induces the IgE synthesis in B cells, as was interpreted by the expression of CD40L by these cells.²⁰ This molecule is not expressed on basophils from healthy persons,^{7,10} or it is expressed in such a low quantity that no allergic reaction is induced (7.1% as found in this study). We can only speculate about the reason for this obvious activation of basophils in allergic persons. The increased expression of cytokine receptors may indicate an interaction with cytokines. In T and B cells IL-4 increases the expression of its own receptor,^{21,22} as well as that of IL-3R on monocytes.²³ On T cells again, the IL-1R is increased by IL-4.²⁴ The same might be true for basophils.

Because basophils are able to produce and store IL-4,²⁵ an autocrine stimulation is possible. IL-4 is also responsible for the MHC II expression on mast cells.²⁶ The same effect (the IL-4-induced MHC II expression) can be assumed for basophils. The expression of HLA-

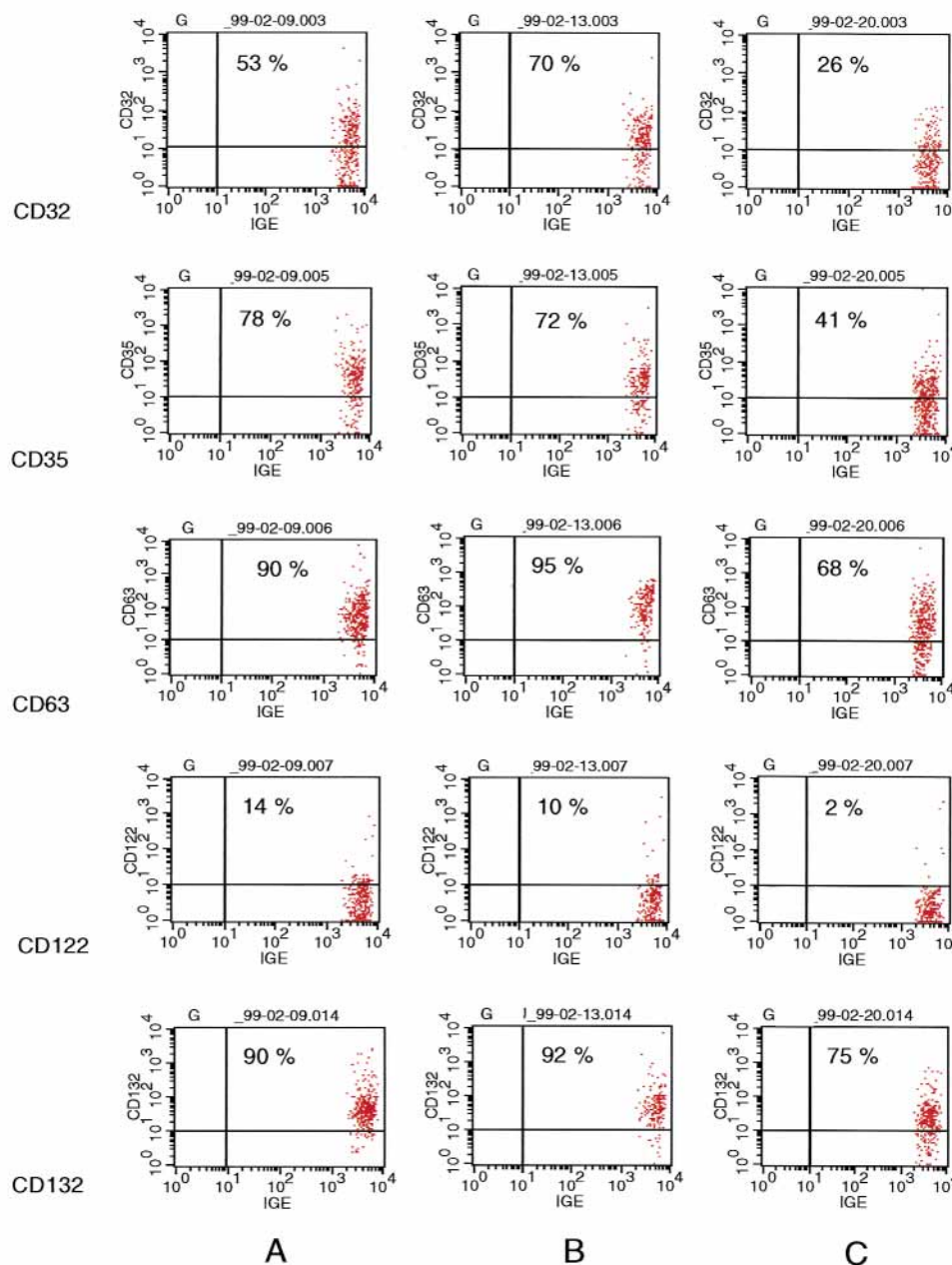


FIG 3. Expression of the membrane receptors CD32, CD35, CD63, CD122, and CD132 on basophils in patient 18 before VIT (A), immediately after VIT (B), and 1 week after VIT (C).

DR on the one hand and CD154 (CD40L), together with the secretion of IL-4 on the other, tempt us to consider activated basophils as antigen-presenting (allergen-presenting) T_H2 -like cells.

In any case the long-lasting activation of basophils in subjects with wasp venom allergy clearly did not need any additional allergen boosting. Initiation of the immunotherapy increased the activation of basophils, as demonstrated by the increase in the number of nearly all examined surface markers. Unexpectedly, the expression of IL-5R decreased. Inclusion of a control group of

patients with wasp venom allergy that were left untreated was impossible for ethical reasons. These patients are vitally threatened by their sensitization, and VIT represents the only approved successful therapy for them. However, in a few patients the described parameters did not change between diagnosis (after sting) and the start of VIT (data not shown).

Most of the markers investigated had clearly decreased 1 week after the rush immunotherapy. With the exception of CD40L, HLA-DR, and CD25, the percentages of the respective marker-positive cells tended to

approach the values of healthy persons. Whether this normalization continues over time and whether the other receptors will follow this tendency has to be examined.

The behavior of the basophils and PBMCs (mostly T cells) under immunotherapy is different.² On PBMCs, IL-4R, as well as CD40L, levels decreased immediately. The same molecules on basophils increased after rush VIT and then decreased in the week after therapy, when wasp venom was not applied. This effect may have been a result of the high cumulative doses of wasp venom administered during immunotherapy. The increased expression of CD63 suggests a partial degranulation as a consequence of exposure to the allergen. Stored IL-4 was also released during degranulation, which may have further activated the basophils. If so, we may be able to explain another observation. As known, immunotherapy causes specific IgE titers to decrease and IgG4 titers to increase when TH2 shifts to TH1 followed by the induction of IL-10 production.² More precisely, specific IgE levels initially increase and later decrease, as shown for wasp venom²⁷ and birch pollen immunotherapy.²⁸ This initial rise in IgE may be caused by IL-4, which is released by the basophils during VIT. Of course, basophils are only one player in the cytokine orchestra, which is directed by T cells. The tendency of basophils to aggravate the allergic reaction is superimposed by T cell-mediated mechanisms. This also may explain why clinical (skin reactivity) and laboratory (levels of specific antibodies) parameters change much more slowly than markers on basophils.

Overall, we found that basophils in persons with wasp venom allergy are permanently activated, as illustrated by the expression of different activation markers, such as cytokine receptors and HLA-DR molecules. Venom immunotherapy immediately increases this activation, which subsequently decreases to the normal levels seen in nonallergic persons.

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