

Original Article

Microarray-Based Detection of Allergen-Reactive IgE in Patients with Mastocytosis

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What is already known about this topic? Patients with mastocytosis are at high risk to develop fatal anaphylaxis. Therefore, early detection of immunoglobulin E (IgE) sensitization is of particular importance in these patients.

What does this article add to our knowledge? A broad screen by allergen-chip profiling was found to identify multiple, potentially clinically relevant, IgE species in patients with mastocytosis in a single test.

How does this study impact current management guidelines? Allergen-chip profiling represents a useful tool to screen for allergen-specific IgE species in patients with mastocytosis and may thus help in early detection of life-threatening IgE- sensitivities in high-risk patients.

BACKGROUND: Because of a high risk to develop fatal anaphylaxis, early detection of immunoglobulin E (IgE)-dependent allergy is of particular importance in patients with mastocytosis.

OBJECTIVE: We examined whether microarray-based screening for allergen-reactive IgE (allergen-chip) is a sensitive and robust approach to detect specific IgE in patients with mastocytosis.

METHODS: Sera for 42 patients were analyzed, including 4 with cutaneous mastocytosis, 2 with mastocytosis in the skin, and 36 with systemic mastocytosis. In addition, sera from an age- and sex-matched control cohort (n = 42) were analyzed.

RESULTS: In 15 of 42 patients with mastocytosis (35.7%), specific IgE was detected by allergen-chip profiling. Ves v 5 and

Bet v 1 were the most frequently detected allergens (Ves v 5: 16.7% of patients; Bet v 1: 11.9% of patients). Allergen reactivity was confirmed by demonstrating upregulation of CD203c on blood basophils upon exposure to the respective allergen(s) in these patients. Specific IgE was identified by chip studies in 11 of 26 patients with mastocytosis with mediator-related symptoms (42.3%) and in 4 of 14 patients with mastocytosis without symptoms (28.6%). In the cohort with known allergy, 9 of 9 patients (100%) had a positive allergen-chip result. In patients with mastocytosis without a known allergy (n = 31), the chip identified 6 positive cases (19.5%). The prevalence of chip-positive patients was slightly lower in the mastocytosis group (35.7%) compared with age- and sex-matched controls (40.5%).

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Abbreviations used

ASM- Aggressive systemic mastocytosis
CM- Cutaneous mastocytosis
IgE- Immunoglobulin E
ISM- Indolent systemic mastocytosis
ISU- ImmunoCAP Solid-phase Allergen-Chip standardized units
mAb- Monoclonal antibody
MC- Mast cell
MFI- Mean fluorescence intensity
MIS- Mastocytosis in the skin
PE- Phycoerythrin
SM- Systemic mastocytosis
SM-AHN- Systemic mastocytosis with an associated hematologic neoplasm
SSM- Smoldering systemic mastocytosis

CONCLUSIONS: Although specific IgE may not be detectable in all sensitized patients with mastocytosis, allergy chip-profiling is a reliable screening approach for the identification of patients with mastocytosis suffering from IgE-dependent allergies. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

Key words: Allergy; IgE; Mastocytosis; Allergen-chip

INTRODUCTION

The term mastocytosis denotes a heterogeneous group of mast cell (MC) disorders defined by abnormal growth and pathological accumulation of clonal MCs in the skin and/or internal organs.¹⁻⁴ According to the classification of the World Health Organization, patients can be divided into cutaneous mastocytosis (CM) and systemic mastocytosis (SM). The group of SM includes indolent SM (ISM), smoldering SM (SSM), aggressive SM (ASM), MC leukemia, and SM with an associated hematologic neoplasm (SM-AHN).^{1,4-7} These disease variants differ from each other regarding symptoms, clinical course, and prognosis.⁸⁻¹⁰

Mediator-related symptoms are found in all variants of mastocytosis.¹⁻⁸ Although the MC burden is higher in advanced SM, the severity of mediator-related symptoms does not correlate with the SM variant.^{11,12} Examples for mediator-related symptoms are skin rash, flushing, diarrhea, and hypotension.¹¹⁻¹⁷ In patients with mastocytosis, allergic reactions can be severe or even life-threatening, especially when an immunoglobulin E (IgE)-dependent allergy is also present.¹⁴⁻¹⁸ These patients often suffer from an MC activation syndrome.¹⁹⁻²¹ A particularly high risk for anaphylaxis is found in patients allergic to bee or wasp venoms.¹⁶⁻¹⁸

Based on the high risk to develop fatal anaphylaxis, early detection of IgE sensitization is of particular importance in patients with mastocytosis. However, in these patients, results of conventional serologic tests measuring specific IgE are often negative, which may have several explanations. For example, parts or even most of the IgE may be fixed to MC surfaces via IgE receptors. Even the skin test may show false-negative results because of treatment with “antiallergic” drugs. Moreover, the skin test has a certain risk for anaphylaxis and new sensitizations. Nevertheless, it is important to detect patients with mastocytosis

at risk for IgE-dependent reactions and there is a need for specific diagnostic assays that cover most or all relevant allergens.

Chips containing microarrayed allergens are powerful tools for detecting IgE-dependent sensitization.^{22,23} Using this approach, IgE reactivity of patients' sera with more than 100 allergens can be assessed in a single test.²³ Allergen-chips have been validated successfully in several trials and found to be a valuable approach to screen for allergen-specific IgE in patients with allergy.²²⁻²⁶ However, so far, the allergen-chip technology has not been tested in patients with mastocytosis.

We analyzed sera of 42 patients suffering from mastocytosis as well as sera of 42 age- and sex-matched controls without a known mastocytosis or another malignant disease using the Immuno Solid-phase Allergen-Chip technology. Our results show that the allergen-chip is a robust approach to detect IgE sensitivities in patients with mastocytosis.

METHODS

Patients' characteristics

A total of 42 patients with mastocytosis (median age, 45.5 years; range, 21-73 years; female:male ratio, 1:0.7) and 42 age- and sex-matched controls without mastocytosis were analyzed for specific IgE in the allergen-chip. Patients with mastocytosis were classified according to World Health Organization criteria.^{1,2} Baseline characteristics of patients are summarized in Table I and in Table E1 in this article's Online Repository at www.jaci-inpractice.org. Patients were seen at the Medical University of Vienna between 1998 and 2018. Various disease-related symptoms, including mediator-related symptoms and bone pain (due to osteopenia/osteoporosis), were recorded during follow-up. ISM was the most common disease variant (23 patients, 54.8%). Five patients had SSM (11.9%), 5 SM-AHN (11.9%), 3 ASM (7.1%), and 4 CM (9.5%). In 2 patients, no bone marrow data were available; these patients were diagnosed as mastocytosis in the skin (MIS) (4.8%). The median serum tryptase level was 53.6 ng/mL (range, 6.1-1617 ng/mL). Hypersensitivity reactions were reported in 9 patients (21%). Eight of these patients had confirmed IgE-mediated allergies and suffered from mediator-induced symptoms. In 1 patient, the case history revealed a hypersensitivity reaction against tetracyclines. In 26 patients (61.9%), a history of mediator-related symptoms was documented. In all patients, the chip analysis was performed during routine blood examinations. In 2 patients (no. 5, ISM, and no. 26, SSM; see Table E1), the chip analysis was performed at 2 (no. 5) or 3 (no. 26) different time points.

Collection of serum samples

Serum samples were collected from patients with mastocytosis and from age- and sex-matched controls without mastocytosis (Austrian individuals). The study was approved by the Ethics Committee of the Medical University of Vienna (nos. 1549/2012 and 1787/2017) and conducted in accordance with the Declaration of Helsinki.

Monoclonal antibodies and other reagents

The anti-IgE monoclonal antibody (mAb) E124.2.8 and the phycoerythrin (PE)-conjugated mAb 97A6 (CD203c) were purchased from Immunotech (Marseille, France). The recombinant (r) allergens rBet v 1, rPhl p 1, and rPhl p 5 were obtained from Biomay (Vienna, Austria). Recombinant Der p 2 and rVes v 5 were expressed and purified as described.^{27,28}

TABLE I. Characteristics of the patients with mastocytosis analyzed on the chip (mechanisms for development of allergies)

Mastocytosis: Variants and subvariants	n (%)	F/M	Age (y), median (min-max)	Tryptase (ng/mL) median, (min-max)	WBC (cells/ μ L), median (min-max)	Eosinophils (cells/ μ L), Median (min-max)	Platelets (cells/ μ L), Median (min-max)	History of allergies, n (%)	Mediator symptoms, n (%)
Nonadvanced mastocytosis									
CM	4 (9.5)	3/1	37 (33-45)	13.05 (6-17)	7,210 (5,720-10,000)	164.4 (57.6-259.8)	266,500 (158,000-378,000)	2 (50.0)	2 (50.0)
MIS	2 (4.8)	2/0	33 (31-35)	13.5 (14-14)	7,800 (7,800-7,800)	0 (0-0)	305,000 (305,000-305,000)	0 (0.0)	1 (50.0)
ISM	23 (54.8)	14/9	45 (26-73)	46.5 (15-938)	6,620 (3,750-10,880)	102.2 (0-488)	265,000 (204,000-341,000)	6 (26.1)	14 (60.9)
SSM	5 (11.9)	4/1	48 (35-55)	352.5 (175-1,090)	4,860 (3,820-6,800)	0 (0-0)	212,000 (193,000-280,000)	1 (20.0)	4 (80.0)
Advanced mastocytosis									
ASM	3 (7.1)	0/3	59 (21-72)	285 (54-1,617)	5,030 (4,370-6,760)	349.6 (100.6-1,487.2)	115,000 (25,000-269,000)	0 (0.0)	2 (66.7)
SM-AHN	5 (11.9)	2/3	53 (47-72)	379 (78-615)	17,600 (2,950-35,490)	797.5 (59-2,515.5)	93,500 (54,000-287,000)	0 (0.0)	3 (60.0)
All patients	42	25/17	45.5 (21-73)	53.6 (6-1,617)	6,535 (2,950-35,490)	100 (0-2,515.5)	253,500 (274-378,000)	9 (21.0)	26 (65.0)

F, Female; M, male; WBC, white blood cell.

Mediator symptoms include flush, vascular instability, headache, bone pain, and diarrhea.

TABLE II. Allergens that were most frequently detectable by chip technology in the patient group with mastocytosis and in individuals of the control cohort

Allergen	Mastocytosis (n = 42)	Nonadvanced mastocytosis (n = 34)	Advanced mastocytosis (n = 8)	Controls (n = 42)
Ves v 5	16.7	17.6	12.5	16.7
Bet v 1	11.9	14.7	0	9.5
Aln g 1	9.5	11.8	0	7.1
Cor a 1.0401	7.1	8.8	0	7.1
Phl p 1	9.5	11.8	0	14.3
Phl p 5	7.1	8.8	0	14.3
Ole e 1	7.1	8.8	0	7.1

Values represent % of patients. Sera obtained from 42 patients with mastocytosis and 42 controls without mastocytosis were analyzed for specific IgE by the chip. Results for the chip-positive cases are shown as a percentage of all patients in each group. Allergen designation: *Aln g 1*, pathogenesis-related protein (PR-10) from alder; *Bet v 1*, PR-10, pollen from birch; *Cor a 1.0401*, PR-10 from hazelnut; *Ole e 1*, common olive group 1 (olive); *Phl p 1*, beta-expansin, pollen from timothy grass; *Phl p 5*, pollen from timothy grass; *Ves v 5*, antigen 5, venom from yellow jacket.

Analysis of serum samples using microarrayed allergens

Sera were analyzed for the presence of IgE directed against microarrayed allergens using ImmunoCAP Solid-phase Allergen-Chip technology capable of analyzing specific IgE for more than 100 allergens (Thermo Fisher/Phadia AB, Uppsala, Sweden) essentially as described.²³ A list of allergens spotted on the chip is presented in Table E2 in this article's Online Repository at www.jaci-inpractice.org. After microarrays had been washed, aliquots of undiluted, centrifuged sera (35 μ L per sample) were applied and incubated in a humid chamber for 2 hours.²³ Thereafter, arrays were again washed and dried. Then, 35 μ L of detection antibody was applied for 30 minutes in a humid chamber. After rinsing, washing, and drying, arrays were scanned in a confocal laser-scanner (LuxScan-10 K microarray scanner, CapitalBio, Beijing, People's Republic of China) and evaluated by Microarray Image Analyzer v3.1.2 software (Thermo Fisher/Phadia AB). Each of the allergen molecules was spotted in triplicates on the chip. Fluorescent signal intensities of the dots were coded, with red dots representing high levels of allergen-specific IgE and blue dots corresponding to low IgE levels, measured by a confocal laser-scanner (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). Levels of specific IgE were semi-quantified by Microarray Image Analyzer v3.1.2 software and expressed by ImmunoCAP Solid-phase Allergen-Chip standardized units (ISU). A positive result was defined by a minimal cutoff level of 0.3 ISU. Grading by ISU was as follows: <0.3 ISU, below cutoff; ≥ 0.3 to <1 ISU, low; ≥ 1 to ≤ 10 ISU, moderate to high; >10 ISU, very high.

Upregulation of CD203c on basophils

Peripheral blood was obtained in 7 patients with mastocytosis, namely 4 with specific IgE (3 with documented allergy, 1 sensitized patient) and 3 in whom no specific IgE was detected by chip analysis. In the basophil CD203c activation test, 45 μ L of heparinized peripheral blood was incubated with 5 μ L of recombinant allergens (rBet v 1, rDer p 2, rPhl p 1, rPhl p 5, rVes v 5; each 1 μ g/mL), anti-IgE antibody E124.2.8 (1 μ g/mL) as positive control or control buffer, and 2.5 μ L of PE-conjugated mAb 97A6 (CD203c) at 37°C for 15 minutes.^{29,30} Then, samples were subjected to erythrocytelysis using 1 mL FACS Lysing Solution (BD Biosciences, San Jose, Calif). Thereafter, cells were washed and analyzed by multi-color flow cytometry on a FACS Calibur (BD Biosciences). Basophils were detected by their forward side-scatter characteristics and expression of CD203c (see Figure E2 in this article's Online

Repository at www.jaci-inpractice.org). Antibody reactivity was measured using FlowJo-Software (BD Biosciences).^{29,30} Allergen-induced upregulation of CD203c was calculated from mean fluorescence intensities (MFIs) obtained with stimulated (MFI_{stim}) and unstimulated (MFI_{control}) cells and expressed as stimulation index (MFI_{stim}/MFI_{control}) as described.^{29,30} Antibody reactivity was controlled by isotype-matched PE-labeled mouse IgG1K mAb MOPC-21 (BD Biosciences).

RESULTS

Chip-based detection of specific IgE in patients with mastocytosis

Of the 42 patients with mastocytosis tested, 15 (35.7%) showed IgE reactivity in the allergen-chip. Most of these patients (8 of 15 [53.3%]) were found to be polysensitized against multiple allergens. The most frequently detected allergen sensitivities in our mastocytosis patients were Ves v 5 (16.7%) and Bet v 1 (11.9%), followed by Aln g 1 (9.5%), Phl p 1 (9.5%), Cora 1.0401 (7.1%), Phl p 5 (7.1%), and Ole e 1 (7.1%) (Table II; see Figure E3 in this article's Online Repository at www.jaci-inpractice.org). Detailed results for all patients are presented in Table E3 in this article's Online Repository at www.jaci-inpractice.org.

Comparison with an age- and sex-matched control population

In our control cohort, specific IgE was detected in 17 of 42 individuals (40.5%) by chip-profiling. In most of these cases (64.7% [11 of 17]), reactivity against several different allergens was detected. The prevalence of chip-positive cases was slightly higher in healthy controls compared with patients with mastocytosis, where 35.7% of the cases displayed specific IgE by chip-profiling ($P > .05$). Indeed, for most allergens tested, the numbers of IgE reactivities in the chip (number of allergens detected) was higher in controls compared with our patients with mastocytosis (Table II). In both groups, most chip-positive cases were found to be polysensitized against various allergens (mastocytosis, 53.3%; controls, 64.7%). As shown in Figure E3 (and Table E4) in this article's Online Repository at www.jaci-inpractice.org, some of the allergens, such as Phl p 6, Der p 2, or Bet v 2, were recognized only in controls but not in patients with mastocytosis.

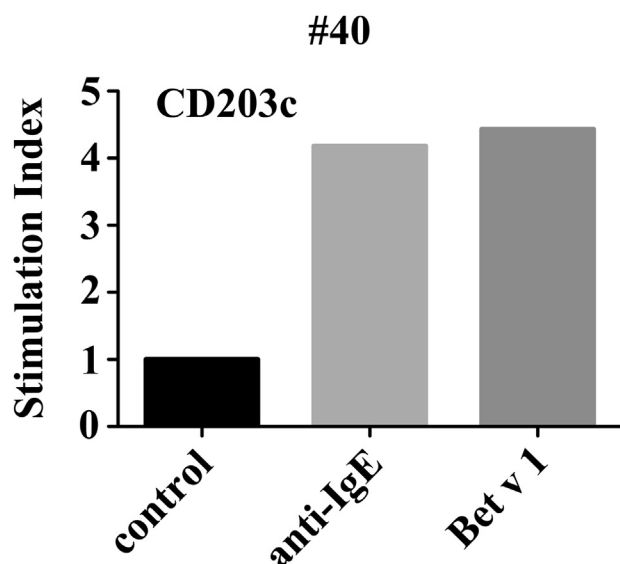


FIGURE 1. Confirmation of specific IgE reactivity detected in the chip by basophil CD203c activation test. Heparinized blood from patient number 40 was incubated with recombinant Bet v 1 (1 μ g/mL), anti-IgE (1 μ g/mL) or control buffer and PE-conjugated antibody 97A6 (CD203c) for 15 minutes (37°C). Basophils were detected by side-scatter characteristics and expression of CD203c. Allergen-induced upregulation of CD203c was calculated from MFIs obtained with stimulated (MFI_{stim}) and unstimulated ($MFI_{control}$) cells and expressed as stimulation index ($MFI_{stim}/MFI_{control}$).

Correlation between chip results, type of mastocytosis, and disease burden

The prevalence of “chip-positive” cases was 50.0% in CM (2 of 4), 50.0% in MIS (1 of 2), 47.8% in ISM (10 of 23), 20% in SSM (1 of 5), 0% in ASM (0 of 3), and 20% in SM-AHN (1 of 5). Two patients, 1 with SSM and 1 with ISM, were also analyzed during follow-up and became chip-positive after several years of observation. Overall, the percentage of chip-positive cases was substantially higher in nonadvanced mastocytosis (CM/MIS/ISM/SSM: 41.2%) compared with advanced SM (ASM/SM-AHN: 12.5%). We also examined correlations between chip-positivity and serum tryptase levels, a marker of the total burden of MC. Median serum tryptase levels were higher in the cohort without specific IgE ($n = 27$; median, 69.7 ng/mL; range, 6.1–1617 ng/mL) compared with those with specific IgE against at least 1 allergen ($n = 15$; median, 40 ng/mL, range, 13.7–1090 ng/mL). Overall, a moderate inverse correlation between total IgE and tryptase levels was found (Pearson correlation coefficient: $r = 0.41$; $P < .05$; see Figure E4 in this article’s Online Repository at www.jaci-inpractice.org). However, no correlation between specific IgE detected by microarray and total IgE levels was found in our patients ($r = 0.24$).

Allergen-induced upregulation of CD203c on basophils

To confirm the functional relevance of IgE reactivities detected by chip-profiling, we performed basophil activation

experiments. In these experiments, recombinant allergens (rVes v 5, rBet v 1, rPhl p 1, rPhl p 5, rDer p 2) were applied to examine their ability to upregulate CD203c on basophils derived from patients with mastocytosis. In all chip-positive patients tested, the recombinant allergen(s) increased the expression of CD203c on basophils (see Figure 1 and Figure E5, A, in this article’s Online Repository at www.jaci-inpractice.org). For example, in patient number 8, specific IgE against Bet v 1, Phl p 1, Phl p 5, and Ves v 5 was detected in the chip, and all these allergens were all found to induce upregulation of CD203c on basophils (see Figure E5, A). Patient number 38 showed low IgE reactivity against Phl p 5 (0.56 ISU) in the chip, but rPhl p 5 was still able to promote CD203c expression. Interestingly, in this patient, rPhl p 1 and rBet v 1 also augmented CD203c expression on basophils although the chip did not detect Phl p 1— or Bet v 1—specific IgE (see Figure E5, A). We also tested 3 other patients with mastocytosis in whom no IgE reactivity was detected by chip-profiling. In these patients, no allergen-induced upregulation of CD203c on basophils was found (see Figure E5, B).

Correlation of clinical data with results from the allergen-chip

In patients with mastocytosis presenting with MC mediator-induced symptoms, 11 of 26 cases (42.3%) showed IgE reactivity in the chip. In those without mediator-related symptoms, the percentage of patients with specific IgE was lower (4 of 14 [28.6%]) although the difference was not significant ($P > .05$). In 1 of the 4 patients with specific IgE without mediator-related symptoms, a hypersensitivity reaction to tetracycline was reported in the case history, and in 2 patients no information on mediator-related symptoms was available (Figure E6, A, in this article’s Online Repository at www.jaci-inpractice.org).

All 9 patients with mastocytosis with a history of allergies had a positive chip result (see Figure E6, B). An example (patient no. 2, ISM) with specific IgE against Aln g 1, Api g 1, Bet v 1, Cor a 1.040, Mal d 1, and Pru p 1 is shown in Figure 2. In another patient with a reported reaction to tetracyclins (presumably IgE-independent), specific IgE against Api m 1 was detected. In the mastocytosis cohort without allergies in their case history ($n = 31$), the percentage of positive cases in the chip was 19.3% (6 of 31). In this cohort, 3 patients (62.5%) reported suffering from MC mediator-induced symptoms. We also compared the predictive value of the allergen-chip (frequency of positive cases with allergies) in mastocytosis with published data published in individuals with allergy or healthy controls. As presented in Table E5 in this article’s Online Repository at www.jaci-inpractice.org, the frequency of positive cases was similar in the control cohorts compared to patients with mastocytosis.

Use of the allergen-chip in the follow-up studies of patients with mastocytosis

Next, we asked whether the microarray results are reproducible in individual patients and are able to detect specific IgE after sensitization. To address this point, sera from 2 patients (no. 5 and no. 26) were examined at different time points. Patient number 5 was stung by a wasp 3 years after diagnosis, and samples were taken at diagnosis and 3.5 years later. In this patient, Ves v 5 was detected by chip-profiling in 2005 but not in

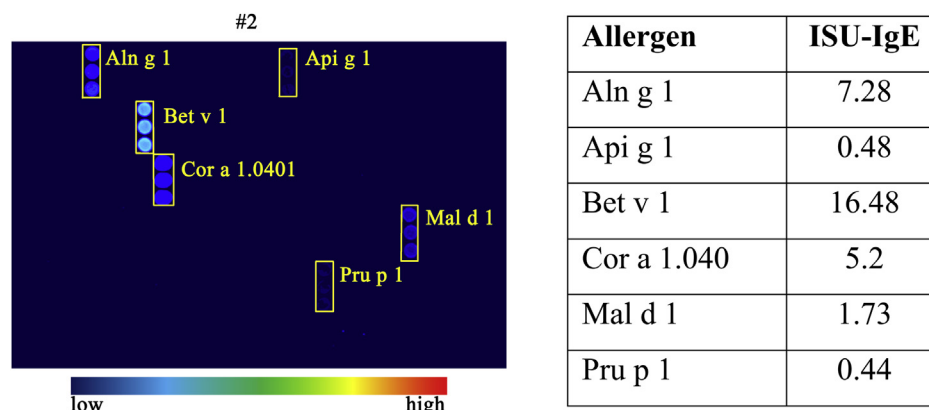


FIGURE 2. Results from the chip approach in a patient with confirmed history of allergies. Sera from patient number 2 (ISM) with known allergy against birch pollen, hazelnut, and wasp was analyzed by chip-profiling (triplicates). IgE reactivity was quantified by Microarray Image Analyzer v3.1.2 software and expressed by ISU. ISU values are listed in the right panel. ISU score: <0.3 ISU, below cutoff; ≥ 0.3 to <1 ISU, low; ≥ 1 to ≤ 10 ISU, moderate to high; >10 ISU, very high. For more details, see Table E3.

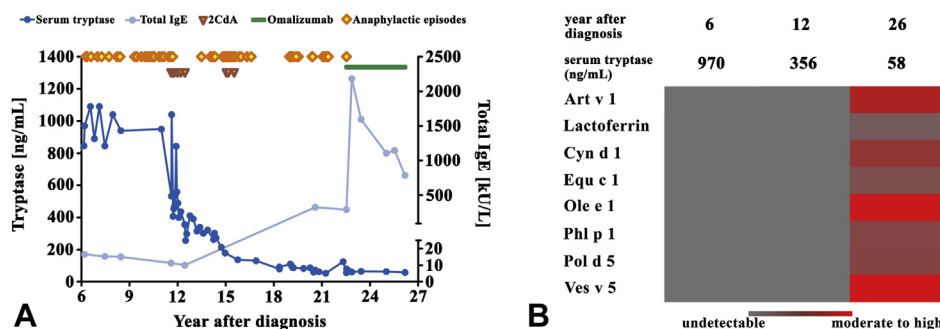


FIGURE 3. Follow-up in a case of SSM with a history of recurrent episodes of life-threatening anaphylactic reactions. (A) Serum tryptase levels (—●—) and IgE (—●—) in patient number 26 (SSM) suffering from recurrent life-threatening anaphylaxis (—♦—). Following 2 series of 2CdA-cycles (—▼—), a temporary control of symptoms was achieved. After recurrence of severe anaphylactic episodes, omalizumab-therapy (—■—) was initiated, followed by marked improvement. (B) Allergen-chip was performed 6 years after diagnosis, before 2CdA, 12 years after diagnosis, and 26 years after diagnosis. 2CdA, 2-Chlorodeoxyadenosine (=cladribine).

2001. No other IgE reactivities were detected in this patient by chip-profiling. Thirteen years after diagnosis the patient was stung again by a wasp and developed anaphylaxis. Patient number 26 was suffering from SSM with a high MC burden and recurrent anaphylaxis, requiring resuscitation and hospitalization (at least 3 times a year) (Figure 3, A).³¹ The first chip-reactivity patterns were obtained 6 and 12 years after diagnosis and showed a negative result (Figure 3, B). Because of the severity of the symptoms, the high MC burden, and suspected progression to ASM, cytoreductive therapy with 2-chlorodeoxyadenosine (=cladribine) was initiated (Figure 3, A). Treatment resulted in a substantial decrease in the MC burden and in the basal serum tryptase level (Figure 3, A). During and shortly after therapy, no severe symptoms were observed. However, 12 months after the last cycle of cladribine, severe reactions (MC activation syndrome) were again recorded. Later, a second series of cladribine cycles was given, followed again by a symptom-free period. In addition, serum tryptase levels further decreased. Three years later, when symptoms were again severe and life-threatening, treatment with omalizumab was initiated and resulted in a

substantial improvement (see Figure 3, A, and Table E6 in this article's Online Repository at www.jaci-inpractice.org). Twenty-six years after diagnosis, chip analysis was repeated and revealed specific IgE against Ole e 1, Ves v 5, and Art v 1 (Figure 3, B). At that time, total IgE levels were also markedly higher compared with those found in samples taken 6 and 12 years after diagnosis. A detailed description of the case report is provided in this article's Online Repository at www.jaci-inpractice.org.^{32,33}

DISCUSSION

Patients with mastocytosis suffering from a concomitant hymenoptera venom allergy, other allergies, or drug hypersensitivity reactions have an increased risk for severe anaphylaxis.¹⁴⁻¹⁸ Early detection of such concomitant allergies is therefore of particular importance. In the current study, the presence of specific IgE was analyzed in the sera of patients with mastocytosis by chip testing. The prevalence of chip-positive cases was higher in patients with mastocytosis with mediator-related symptoms compared with those without symptoms. Moreover, all patients with

mastocytosis with a known IgE-dependent allergy were positive in the allergen-chip.

The prevalence of IgE-dependent allergies in the Western world is increasing, which may be due to several factors, including changes in our lifestyle, industrialization, pollution, and climate changes. Today, the prevalence of IgE-dependent allergies in Europe is considered to range between 20% and 30%. A similar prevalence has been reported for the Austrian population (25%-27%).³⁴ It has also been described that IgE reactivity alone may not necessarily lead to an overt allergic disease.³⁵ In our study, 40.5% of the age- and sex-matched Austrian controls had a positive chip result, and in most of these cases, the chip detected 2 or more IgE-reactive allergens. The criterion to select cases for the control cohort was the absence of mastocytosis and the absence of other neoplastic disorders. Otherwise, these controls were randomly collected without asking for allergies in the case history. These results suggest that the chip technology is a sensitive test detecting low quantities of IgE, including clinically "silent" IgE sensitivities, which is supported by results from birth cohorts where clinically silent sensitization often preceded "aeroallergen" symptomatic allergy.^{25,36,37}

So far, little is known about the overall IgE-reactivity status of patients with mastocytosis. In our study, 42 patients with confirmed mastocytosis were examined by the chip technology. Compared with other methods to detect IgE, a large panel of allergens (>100) can be tested with a small amount of patient-derived serum in this allergen-chip. Probably the most important result obtained in our study was that the prevalence of chip-positive cases was lower in our patients with mastocytosis compared with our age- and sex-matched controls (35.7% compared with 40.5%). The lower percentage of affected patients with mastocytosis (compared with controls) was found in most subgroups defined by specific allergens. One explanation for this phenomenon would be that in patients with mastocytosis, most of the IgE is fixed to the high-affinity IgE receptor expressed on neoplastic MCs. IgE measurements in our patient receiving cytoreductive therapy (cladribine) confirmed this hypothesis. An alternative explanation would be that in symptomatic patients with a high MC burden, IgE is often (or even permanently) internalized together with IgE receptors and is then degraded. Finally, it cannot be excluded that in patients with mastocytosis, treatment with glucocorticosteroids or cytoreductive drugs suppresses growth and function of plasma cells producing IgE. However, even in patients receiving cytoreductive drugs such as cladribine, the allergen-chip still disclosed positive results.

When correlating chip results with clinical parameters, we found that only 21.4% of our patients reported having an allergy in their case history had a positive chip result, whereas 35.7% of all patients with mastocytosis tested positive in the chip. These data might argue for a high sensitivity of the chip regarding detection of allergen-specific IgE. Because early detection of patients at risk for allergic reactions is of importance, especially in patients with mastocytosis, the high sensitivity might represent a major advantage of the chip. Indeed, recently published data have shown that in patients suffering from hymenoptera venom allergy, severe reactions may be observed already when Ves v 5-specific IgE levels are below 1 ISU.²⁸ In our study, the most prevalent allergen in the mastocytosis group was Ves v 5 (16.7%; median, 1.7 ISU). In this regard, it is noteworthy that 1 of our

patients was tested positive for Ves v 5 9 years before a life-threatening anaphylactic reaction to a wasp sting.

There are several limitations of currently used chip-based assays to detect IgE in patients with SM. One is that the assays may not cover all relevant hymenoptera venom species. For example, the chip assay applied did not detect Ves v 2, Ves v 3, Api m 3, Api m 5, or Api m 10. In addition, the chip did not detect drugs known to cause hypersensitivity reactions, such as penicillin.³⁸ However, severe drug reactions are rare in these patients and may have different etiologies, including non-IgE-dependent mechanisms.^{39,40} Another disadvantage is that in patients with SM who have a very high MC burden, a negative result by chip testing does not exclude the presence of an IgE-mediated allergy, because most of the IgE is fixed to the MC surface and is therefore not detectable by a serologic test, especially when the levels of produced IgE are low and the burden of MCs is high. However, as exemplified in 1 patient with SSM in this study, specific IgE may become detectable when the burden of neoplastic MCs decreased substantially after cytoreductive therapy. Indeed, in patient number 26, an increase in total IgE was already observed after cladribine before the administration of omalizumab, which typically increases serum IgE levels. To confirm the clinical value of the chip, we performed functional experiments with basophils in 7 patients. In these analyses, the recombinant allergens were found to promote CD203c expression on basophils in all patients carrying specific IgE detected by chip-profiling. On the other hand, however, the chip was not able to detect all allergen reactivities that were documented in the CD203c assay. For example, in patient number 38 (ISM), moderate IgE reactivity for Phl p 5 was found in the chip and was confirmed by allergen-induced CD203c upregulation on basophils. However, in the same patient, recombinant Phl p 1 and Bet v 1 also induced CD203c upregulation on basophils although the chip did not detect IgE against Phl p 1 or Bet v 1. Another interesting observation was that the chip was positive in 6 of 31 patients with mastocytosis (3 symptomatic and 3 non-symptomatic) in whom no previous allergy had been documented. Unfortunately, we were not able to apply skin tests or the CD203c assay in these cases.

Together, the allergen-chip approach represents a novel, simple, and easily applicable tool for detection of specific IgE in patients with mastocytosis. This assay is able to early detect patients with mastocytosis with IgE-dependent sensitization to a large panel of different allergens, including venom allergens, which are the most relevant allergens in mastocytosis, in a single test. Whether this approach is able to predict allergies in all patients with mastocytosis remains to be determined in future studies.

REFERENCES

1. Valent P, Horny HP, Escribano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001;25:603-25.
2. Valent P, Sperr WR, Schwartz LB, Horny HP. Diagnosis and classification of mast cell proliferative disorders: delineation from immunologic diseases and non-mast cell hematopoietic neoplasms. *J Allergy Clin Immunol* 2004;114:3-11.
3. Metcalfe DD. Mast cells and mastocytosis. *Blood* 2008;112:946-56.
4. Arock M, Valent P. Pathogenesis, classification and treatment of mastocytosis: state of the art in 2010 and future perspectives. *Expert Rev Hematol* 2010;3:497-516.
5. Horny HP, Akin C, Arber DA, Peterson LA, Tefferi A, Metcalfe DD, et al. Mastocytosis. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA,

- Stein H, et al., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Volume 3. Lyon, France: IARC Press; 2017. p. 62-9.
6. Valent P, Akin C, Hartmann K, Nilsson G, Reiter A, Hermine O, et al. Advances in the classification and treatment of mastocytosis: current status and outlook toward the future. *Cancer Res* 2017;77:1261-70.
 7. Valent P, Akin C, Metcalfe DD. Mastocytosis. 2016 updated WHO classification and novel emerging treatment concepts. *Blood* 2017;129:1420-7.
 8. Arock M, Sotlar K, Akin C, Broesby-Olsen S, Hoermann G, Escribano L, et al. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 2015;29:1223-32.
 9. Longley BJ, Tyrrell L, Lu SZ, Ma YS, Langley K, Ding TG, et al. Somatic *c-kit* activating mutation in urticaria pigmentosa and aggressive mastocytosis: establishment of clonality in a human mast cell neoplasm. *Nat Genet* 1996;12:312-4.
 10. Fritsche-Polanz R, Jordan JH, Feix A, Sperr WR, Sunder-Plassmann G, Valent P, et al. Mutation analysis of *C-KIT* in patients with myelodysplastic syndromes without mastocytosis and cases of systemic mastocytosis. *Br J Haematol* 2001;113:357-64.
 11. Valent P, Akin C, Arock M. Diagnosis and treatment of anaphylaxis in patients with mastocytosis. *Curr Treat Options Allergy* 2014;1:247-61.
 12. Akin C. Anaphylaxis and mast cell disease: what is the risk? *Curr Allergy Asthma Rep* 2010;10:34-8.
 13. Theoharides TC, Valent P, Akin C. Mast cells, mastocytosis, and related disorders. *N Engl J Med* 2015;373:163-72.
 14. Brockow K, Jofer C, Behrendt H, Ring J. Anaphylaxis in patients with mastocytosis: a study on history, clinical features and risk factors in 120 patients. *Allergy* 2008;63:226-32.
 15. Valent P. Risk factors and management of severe life-threatening anaphylaxis in patients with clonal mast cell disorders. *Clin Exp Allergy* 2014;44:914-20.
 16. Bonadonna P, Bonifacio M, Lombardo C, Zanotti R. Hymenoptera anaphylaxis and C-kit mutations: an unexpected association. *Curr Allergy Asthma Rep* 2015;15:49.
 17. Bonadonna P, Bonifacio M, Lombardo C, Zanotti R. Hymenoptera allergy and mast cell activation syndromes. *Curr Allergy Asthma Rep* 2016;16:5.
 18. Niedoszytko M, de Monchy J, van Doormaal JJ, Jassem E, Oude Elberink JN. Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom immunotherapy. *Allergy* 2009;64:1237-45.
 19. Akin C. Mast cell activation syndromes. *J Allergy Clin Immunol* 2017;140:349-55.
 20. Valent P, Akin C, Arock M, Brockow K, Butterfield JH, Carter MC, et al. Definitions, criteria and global classification of mast cell disorders with special reference to mast cell activation syndromes: a consensus proposal. *Int Arch Allergy Immunol* 2012;157:215-25.
 21. Valent P. Mast cell activation syndromes: definition and classification. *Allergy* 2013;68:417-24.
 22. Anto JM, Bousquet J, Akdis M, Auffray C, Keil T, Momas I, et al. Mechanisms of the Development of Allergy (MeDALL): introducing novel concepts in allergy phenotypes. *J Allergy Clin Immunol* 2017;139:388-99.
 23. Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. *Methods* 2014;66:106-19.
 24. Asarnoj A, Hamsten C, Lupinek C, Melén E, Andersson N, Anto JM, et al. Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: the BAMSE population-based birth cohort. *J Allergy Clin Immunol* 2017;140:587-90.
 25. Asarnoj A, Hamsten C, Wadén K, Lupinek C, Andersson N, Kull I, et al. Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: a BAMSE/MeDALL study. *J Allergy Clin Immunol* 2016;137:813-21.
 26. Sørensen M, Klingenberg C, Wickman M, Sollid JUE, Furberg AS, Bachert C, et al. *Staphylococcus aureus* enterotoxin sensitization is associated with allergic poly-sensitization and allergic multimorbidity in adolescents. *Allergy* 2017;72:1548-55.
 27. Chen KW, Focke-Tejkl M, Blatt K, Kneidinger M, Gieras A, Dall'Antonia F, et al. Carrier-bound non allergenic Der p 2 peptides induce IgG antibodies blocking allergen-induced basophil activation in allergic patients. *Allergy* 2012;67:609-21.
 28. Gattinger P, Lupinek C, Kalogiros L, Silar M, Zidarn M, Korosec P, et al. The culprit insect but not severity of allergic reactions to bee and wasp venom can be determined by molecular diagnosis. *PLoS One* 2018;13:e0199250.
 29. Hauswirth AW, Natter S, Ghannadan M, Majlesi Y, Schernthaner GH, Sperr WR, et al. Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol* 2002;110:102-9.
 30. Kneidinger M, Schmidt U, Rix U, Gleixner KV, Vales A, Baumgartner C, et al. The effects of dasatinib on IgE receptor-dependent activation and histamine release in human basophils. *Blood* 2008;111:3097-107.
 31. Boehm T, Reiter B, Ristl R, Petroczi K, Sperr W, Stimpfl T, et al. Massive release of the histamine-degrading enzyme diamine oxidase during severe anaphylaxis in mastocytosis patients. *Allergy* 2019;74:583-93.
 32. Eckl-Dorna J. Omalizumab's impact on total and allergen-specific IgE levels: a polyclonal story. *Int Arch Allergy Immunol* 2016;169:69-70.
 33. Mizuma H, Tanaka A, Uchida Y, Fujiwara A, Manabe R, Furukawa H, et al. Influence of omalizumab on allergen-specific IgE in patients with adult asthma. *Int Arch Allergy Immunol* 2015;168:165-72.
 34. Österreichische Gesundheitsbefragung 2014. *Hauptergebnisse des Austrian Health Interview Survey (ATHIS) und methodische Dokumentation*. Wien: Statistik Austria; 2015:245. Available from: <https://broshuerenservice.sozialministerium.at/Home/Download?publicationId=542>. Accessed July 27, 2017.
 35. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Cramer R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens: a GA2LEN project. *Allergy* 2006;61:671-80.
 36. Westman M, Lupinek C, Bousquet J, Andersson N, Pahr S, Baar A, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. Mechanisms for the Development of Allergies Consortium. *J Allergy Clin Immunol* 2015;135:1199-206.
 37. Wickman M, Lupinek C, Andersson N, Belgrave D, Asarnoj A, Benet M, et al. Detection of IgE reactivity to a handful of allergen molecules in early childhood predicts respiratory allergy in adolescence. *EBioMedicine* 2017;26:91-9.
 38. Castells M, Khan DA, Phillips EJ. Penicillin allergy. *N Engl J Med* 2019;381:2338-51.
 39. Hamilton RG, Oppenheimer J. Serological IgE analyses in the diagnostic algorithm for allergic disease. *J Allergy Clin Immunol Pract* 2015;3:833-40.
 40. Warrington R, Silviu-Dan F, Wong T. Drug allergy. *Allergy Asthma Clin Immunol* 2018;14:60.

ONLINE REPOSITORY

CASE REPORT

We report on a 48-year-old female patient suffering from smoldering systemic mastocytosis with a history of multiple ($n = 64$) episodes of life-threatening anaphylactic reactions. In 1998, therapy with H1- and H2-receptor blockers (desloratadine and ranitidine) was initiated. Since 2001, she also received levocetirizine (see [Table E6](#)). Despite this therapy, recurrent life-threatening anaphylactic reactions were recorded. The severity and frequency of these anaphylactic reactions as well as the high MC burden present in this patient (as assessed by bone marrow histology and serum tryptase levels) prompted us to initiate cytoreductive therapy with 2-chlorodeoxyadenosine (cladribine) (2CdA). Between December 2003 and October 2004, 6 subsequent cycles of 2CdA (0.13 mg/kg/d, days 1-5) were given. Treatment resulted in a marked decrease in the serum tryptase level ([Figure 3, A](#)) and a decrease in the MC burden confirmed by a bone marrow biopsy. In the next

11 months, no further severe symptoms were observed. However, 12 months after the last cycle of 2CdA she again started suffering from recurrent severe anaphylactic reactions. In May 2007, 30 months after the end of the first series of 2CdA cycles, a second series of 3 cycles of therapy with 2CdA (0.13 mg/kg/d; days 1-5) was initiated followed by a period without severe symptoms. In addition, a further decrease in serum tryptase level was observed ([Figure 3, A](#)). Similar to the first series, recurrence of severe events was observed 20 months after the completion of this series of 2CdA cycles. In 2014, treatment with omalizumab (300 mg subcutaneous every 3-4 weeks) was initiated and resulted in a marked improvement (see [Table E6](#)). No life-threatening events were recorded during the last 4 years when the patient received omalizumab. Serial measurements of total serum immunoglobulin (IgE) showed a clear increase following cytoreduction induced by 2CdA ([Figure 3, A](#)). Serum tryptase remained at relatively low levels for more than 6 years after cytoreductive therapy with 2CdA (despite recurrence of anaphylaxis).

TABLE E1. Characteristics of patients with mastocytosis

Patient no.	Age (y)	Sex	Diagnosis (WHO)	Tryptase (ng/mL)	WBC (cells/ μ L)	Hemoglobin (g/dL)	Platelets (cells/ μ L)	Total IgE (kU/L)	History of allergic reactions	Type of allergen(s)	Severity of symptoms
1	35	F	SSM	202	6,800	13.8	216,000	12.10	No	—	—
2	46	F	ISM	938	6,450	13.6	279,000	NA	Yes*	Birch, nuts, food, wasps	Anaphylaxis
3	47	F	ISM	47.9	7,300	14.5	237,000	10.20	No	—	—
4	55	F	SSM	352.5	4,860	13.1	280,000	<2.00	NK	—	—
5	34	F	ISM	45.6	5,910	12.3	288,000	22.40	Yes*	Wasps	Anaphylaxis
6	35	F	MIS	NA	NA	NA	NA	8.44	No	—	—
7	36	M	ISM	34.4	8,400	15.6	341,000	47.80	Yes*	Wasps, bees	Not reported
8	36	F	ISM	23.3	10,880	13.9	329,000	473	Yes	Birch, timothy grass, nuts	Anaphylaxis
9	45	M	ISM	90.6	7,250	12.5	242,000	6.80	No	—	—
10	26	F	ISM	68	3,750	13.3	204,000	10.40	No	—	—
11	37	F	ISM	69.7	7,400	12.7	272,000	33.90	No	—	—
12	37	M	ISM	33.5	7,280	14.8	307,000	336	No	—	—
13	53	M	SM-AHN	204.0	35,490	16.4	287,000	5.54	No	—	—
14	33	F	ISM	15.5	9,220	15	251,000	5.55	No	—	—
15	38	F	CM	13.7	8,660	13.6	378,000	177	No	—	—
16	59	M	ASM	285	6,760	9.6	115,000	15.6	No	—	—
17	33	M	CM	12.4	10,000	15.8	158,000	90.6	No	—	—
18	37	F	ISM	46.5	5,190	13.9	221,000	12.4	No	—	—
19	31	F	ISM	17.9	6,620	13.5	256,000	9.95	No	—	—
20	53	F	ISM	53.7	5,110	12.4	205,000	70.2	No	—	—
21	36	F	CM	17.1	5,760	13.6	264,000	39.1	Yes	Birch, mugwort	Rhinitis, conjunctivitis
22	31	F	MIS	13.5	7,800	14.1	305,000	87.5	NK	—	—
23	58	M	ISM	108	6,100	14.2	307,000	11.3	No	—	—
24	42	M	ISM	14.5	5,450	14.3	265,000	5.52	No	—	—
25	56	M	ISM	34.8	7,960	13.5	320,000	17.5	No	—	—
26	48	F	SSM	1090	5,250	11.2	212,000	17.0	Yes	Timothy grass, mugwort, food	Anaphylaxis
27	64	M	ISM	164	10,480	16.1	206,000	NA	No	—	—
28	72	M	SM-AHN	77.7	2,950	12.2	101,000	16.3	No	—	—
29	21	M	ASM	53.6	5,030	12.9	269,000	29.2	No	—	—
30	62	F	ISM	25.6	5,410	14.8	257,000	46.7	No	—	—
31	47	F	SM-AHN	NA	NA	NA	NA	<2.00	No	—	—
32	50	F	SSM	175	3,820	12.8	193,000	18.3	No	—	—
33	72	M	ASM	1,617	4,370	9.1	25,000	12.6	No	—	—
34	40	M	SSM	937	3,980	12.8	201,000	5.91	No	—	—
35	52	F	SM-AHN	379	7,250	10.2	86,000	16.9	No	—	—
36	56	M	ISM	650	10,480	14.4	329,000	NA	No	—	—

37	53	M	SM-AHN	615	27,950	8.8	54,000	41.9	No	—	—
38	73	M	ISM	49.9	5,130	14.2	265,000	60.1	Yes	Timothy grass	Not reported
39	53	M	ISM	17.1	4,360	15.1	215,000	8.63	No	—	—
40	48	F	ISM	78.5	4,690	13.6	237,000	6.65	Yes	Birch	Not reported
41	45	F	CM	6.1	5,720	13.2	269,000	124	No	—	—
42	33	F	ISM	45.5	8,960	12.1	274,000	4.90	No	—	—

ASM, Aggressive systemic mastocytosis; *CM*, cutaneous mastocytosis; *f*, female; *ISM*, indolent systemic mastocytosis; *m*, male; *MIS*, mastocytosis in the skin; *NA*, not available; *NK*, not known; *SM-AHN*, systemic mastocytosis with an associated hematologic neoplasms; *SSM*, smoldering systemic mastocytosis; *WBC*, white blood cell count; *WHO*, World Health Organization.

*In these patients, hypersensitivity reactions against bee or wasp were recorded. In patient number 5, a previous sting did not result in anaphylaxis but the second exposure resulted in a severe hypersensitivity reaction. In the other 2 patients, no history about previous stings was available.

TABLE E2. List of allergens spotted on the chip used in this study

Allergen source	Allergen
Kiwi fruit	Act d 1
Kiwi fruit	Act d 2
Kiwi fruit	Act d 5
Kiwi fruit	Act d 8
Alder	Aln g 1
Alternaria plant rot fungus	Alt a 1
Alternaria plant rot fungus	Alt a 6
Ragweed	Amb a 1
Cashew	Ana o 1
Cashew	Ana o 2
Cashew	Ana o 3
Herring worm	Ani s 1
Herring worm	Ani s 3
Celery	Api g 1
Honeybee	Api m 1
Honeybee	Api m 2
Honeybee	Api m 4
Peanut	Ara h 1
Peanut	Ara h 2
Peanut	Ara h 3
Peanut	Ara h 6
Peanut	Ara h 8
Peanut	Ara h 9
Mugwort	Art v 1
Mugwort	Art v 3
Common mold	Asp f 1
Common mold	Asp f 3
Common mold	Asp f 6
Brazil nut	Ber e 1
European white birch	Bet v 1
European white birch	Bet v 2
European white birch	Bet v 4
German cockroach	Bla g 1
German cockroach	Bla g 2
German cockroach	Bla g 5
German cockroach	Bla g 7
Storage mite	Blo t 5
Domestic cattle	Bos d 4
Domestic cattle	Bos d 5
Domestic cattle	Bos d 6
Domestic cattle	Bos d 8
Domestic cattle	BSA
Milk	aS1-casein
Milk	aS2-casein
Milk	b-casein
Milk	K-casein
Milk	Lactoferrin
Dog	Can f 1
Dog	Can f 2
Dog	Can f 3
Dog	Can f 4
Dog	Can f 5
Dog	Can f 6
Goosefoot	Che a 1

(continued)

TABLE E2. (Continued)

Allergen source	Allergen
Fungus of plants	Cla h 8
Hazelnut	Cor a 1.0401
Hazelnut	Cor a 8
Hazelnut	Cor a 9
Sugi	Cry j 1
Bermuda grass	Cyn d 1
Cypress	Cup a 1
American house-dust mite	Der f 1
American house-dust mite	Der f 2
European house-dust mite	Der p 1
European house-dust mite	Der p 2
European house-dust mite	Der p 4
European house-dust mite	Der p 5
European house-dust mite	Der p 7
European house-dust mite	Der p 10
European house-dust mite	Der p 11
European house-dust mite	Der p 14
European house-dust mite	Der p 15
European house-dust mite	Der p 18
European house-dust mite	Der p 21
European house-dust mite	Der p 23
Domestic horse	Equ c 1
Domestic horse	Equ c 3
Common buckwheat	Fag e 2
Cat	Fel d 1
Cat	Fel d 2
Cat	Fel d 4
Baltic cod	Gad c 1
Chicken	Gal d 1
Chicken	Gal d 2
Chicken	Gal d 3
Chicken	Gal d 5
Soybean	Gly m 4
Soybean	Gly m 5
Soybean	Gly m 6
Latex	Hev b 1
Latex	Hev b 3
Latex	Hev b 5
Latex	Hev b 6.01
Latex	Hev b 8
English walnut	Jug r 1
English walnut	Jug r 2
English walnut	Jug r 3
Storage mite	Lep d 2
Apple	Mal d 1
Annual mercury	Mer a 1
Mouse	Mus m 1
Bromelain	MUXF3
Olive	Ole e 1
Olive	Ole e 5
Olive	Ole e 6
Olive	Ole e 7
Olive	Ole e 8
Olive	Ole e 9

(continued)

TABLE E2. (Continued)

Allergen source	Allergen
Olive	Ole e 10
Timothy grass pollen	Phl p 1
Timothy grass pollen	Phl p 2
Timothy grass pollen	Phl p 4
Timothy grass pollen	Phl p 5
Timothy grass pollen	Phl p 6
Timothy grass pollen	Phl p 7
Timothy grass pollen	Phl p 11
Timothy grass pollen	Phl p 12
Pellitory-of-the-Wall	Par j 2
Black tiger shrimp	Pen m 1
Black tiger shrimp	Pen m 2
Black tiger shrimp	Pen m 4
Pistachio	Pis v 3
London plane tree	Pla a 1
London plane tree	Pla a 2
London plane tree	Pla a 3
London plane tree	Pla l 1
Mediterranean paper wasp	Pol d 5
Peach	Pru p 1
Peach	Pru p 3
Almond	Pru du 3
Almond	Pru du 4
Almond	Pru du 6
Almond	Pru du 6.01
Almond	Pru du 6.02
Sesame	Ses i 1
Wheat	Tri a 14
Wheat	Tri a 19.0101
Wheat	Tri a 36
Yellow jacket	Ves v 1
Yellow jacket	Ves v 5

IUIS, International Union of Immunological Societies.

The sources and names of the allergens according to IUIS are shown.

TABLE E3. Heat map of IgE reactivities detected in patients with mastocytosis as assessed by allergy chip-profiling (only those allergens for which at least 1 patient showed a positive result are shown). A detailed description of allergens is presented in [Table E4](#).

Patient no.	Act d 1	Aln g 1	Amb a 1	Api g 1	Api m 1	Api m 4	Ara h 1	Ara h 8	Art v 1	Bet v 1
1	0	0	0	0	0	0	0	0	0	0
2	0	7.28†	0	0.48*	0	0	0	0.1	0	16.48‡
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0.25	0	0	0	0	0	0.17	0	0.91*
7	0	0	0	0	0	0	0	0	0	0
8	0.18	0.15	0	0	0	0	0	0	0	0.71*
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0.12	0.35*	0.19	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0.18	0	0	0	0.75*	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
21	0	0.32*	6.17†	0	0	0	0	0	0	0.56*
22	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	1.22†§	0
27	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0
40	0	2.14†	0	0	0	0	0	0.19	0	8.15†
41	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0
Patient no.	Can f 3	Cor a 1.0401	Cyn d 1	Cup a 1	Der f 2	Der p 2	Fel d 2	Gal d 1	Lep d 2	Mal d 1
1	0	0	0	0	0	0	0	0	0	0
2	0	5.2†	0	0	0	0	0	0	0	1.73†
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	0	0.09	0	0	0	0	0	0	0	0
7	0	0	0	0	0.08	0.11	0	0	0	0
8	0.24	1.18†	0.12	0	0	0	0.26	0	0	0.22
9	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0

(continued)

TABLE E3. (Continued)

Patient no.	Can f 3	Cor a 1.0401	Cyn d 1	Cup a 1	Der f 2	Der p 2	Fel d 2	Gal d 1	Lep d 2	Mal d 1
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0.12	0	0
16	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0
19	0	0	0.34*	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
21	0	0.2	0	0.11	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0
26	0	0	0.84*§	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0.08	0	0
30	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0
40	0	0.75*	0	0	0.21	0.29	0	0	0.34*	0
41	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0

Patient no.	Ole e 1	Phl p 1	Phl p 4	Phl p 5	Pol d 5	Pru p 1	Pru du 3	Ves v 5
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0.44*	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0.87*
6	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	2.2†
8	1.01†	0.76*	0	1†	0.27	0.15	0	0.58*
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0
12	0	0	0.2	0	0	0	0	0.32*
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0.21	0
16	0	0	0.2	0	0	0	0	0.12
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0.09	0	0	1.74†
19	0	0.68*	0	0.67*	0	0	0	0
20	0	0	0.17	0	0	0	0	0.1
21	0	0	0	0	0	0.11	0	0

(continued)

TABLE E3. (Continued)

Patient no.	Ole e 1	Phl p 1	Phl p 4	Phl p 5	Pol d 5	Pru p 1	Pru du 3	Ves v 5
22	0	0	0	0	0	0	0	0.1
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	1.63 ^{†§}	0.58 ^{*§}	0.71 ^{*§}	0	0.6 ^{*§}	0	0	1.66 ^{†§}
27	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0.11	0	1.37 [†]	0.08	0	1.88 [†]
38	0	0	0	0.56 [*]	0	0	0	0
39	0	0.31 [*]	0	0	0	0	0	0
40	0.32 [*]	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0

Sera obtained from 42 patients with mastocytosis were analyzed using microarrayed allergens. Levels of specific IgE were semi-quantified by Microarray Image Analyzer v3.1.2 software and expressed by ISU. For positivity, a cutoff of 0.3 ISU was used. Chip scoring system: <0.3 ISU below cutoff. A detailed description of allergens is presented in the Table E4.

*Class 1: ≥ 0.3 to <1, ISU low.

[†]Class 2: ≥ 1 to ≤ 10 ISU, moderate to high.

[‡]Class 3: >10 ISU, very high.

[§]In this patient, allergen-chip detected sensitization against various allergens after treatment-induced reduction of the MC burden.

TABLE E4. Description of allergens shown in [Figure E3](#)

No.	Allergen source	Name/function of the protein	Allergen
1	Kiwi fruit	Thaumatococcus-like protein	Act d 2
2	Alder	PR-10, Bet v 1 family member	Aln g 1
3	Alternaria plant rot fungus	Acidic glycoprotein	Alt a 1
4	Ragweed	Pectate lyase	Amb a 1
5	Celery	PR-10, Bet v 1 family member	Api g 1
6	Honeybee	Phospholipase A2	Api m 1
7	Honeybee	Hyaluronidase	Api m 4
8	Peanut	PR-10, Bet v 1 family member	Ara h 8
9	Mugwort	Defensin-like protein	Art v 1
10	European white birch	PR-10, Bet v 1 family member	Bet v 1
11	European white birch	Profilin	Bet v 2
12	European white birch	Polcalcin	Bet v 4
13	Dog	Lipocalin	Can f 1
14	Goosefoot	Ole e 1—related protein (trypsin inhibitor)	Che a 1
15	Hazelnut	PR-10, Bet v 1 family member	Cor a 1.0401
16	Japanese cedar	Pectate lyase	Cry j 1
17	Cypress	Cypress	Cup a 1
18	Bermuda grass	Beta-expansin	Cyn d 1
19	American house-dust mite	NPC2 family	Der f 2
20	European house-dust mite	NPC2 family	Der p 2
21	Cat	Uteroglobin	Fel d 1
22	Latex	Profilin	Hev b 8
23	English walnut	Vicilin seed storage protein	Jug r 2
24	Storage mite	NPC2 family	Lep d 2
25	Apple	PR-10, Bet v 1 family member	Mal d 1
26	Annual mercury	Profilin	Mer a 1
27	Olive	Ole e 1—related protein (trypsin inhibitor)	Ole e 1
28	Timothy grass pollen	Beta-expansin	Phl p 1
29	Timothy grass pollen	Grass group 2/3	Phl p 2
30	Timothy grass pollen	Grass group 4 (Berberine bridge enzyme)	Phl p 4
31	Timothy grass pollen	Grass group 5	Phl p 5
32	Timothy grass pollen	Grass group 5/6	Phl p 6
33	Timothy grass pollen	Polcalcin	Phl p 7
34	Timothy grass pollen	Ole e 1—related protein (trypsin inhibitor)	Phl p 11
35	Timothy grass pollen	Profilin	Phl p 12
36	London plane tree	Polygalacturonase	Pla a 2
37	English plantain	Ole e 1—related protein (Pectate lyase)	Pla l 1
38	Mediterranean paper wasp	Antigen 5	Pol d 5
39	Peach	PR-10, Bet v 1 family member	Pru p 1
40	Almond	Profilin	Pru du 4
41	Yellow jacket	Antigen 5	Ves v 5

IUIS, International Union of Immunological Societies; PR-10, pathogenesis-related protein 10.

The sources and names of the allergens according to IUIS are shown.

TABLE E5. Evaluation of allergen-microarray in various cohorts

Study population	Allergic individuals among all patients, n (%)	Positive IgE reactivity by allergen-microarray testing in patients, n (%)	Mediator-induced symptoms of patients with allergies (previous studies) or mastocytosis (this study)	Reference
BAMSE	546/786 (69.5%)*	409/786 (52%)*	Asthma, rhinitis, asthma and/or rhinitis	Wickman et al ^{E1}
BAMSE	122/686 (17.8%)*	173/686 (25.2%)*	Rhinitis	Westman et al ^{E2}
EGEA	197/340 (57.9%)†	166/340 (48.8%)	Asthma, rhinitis	Siroux et al ^{E3}
ECA	83/132 (62.9%)*	132/265 (49.8%)*	Asthma	Skrindo et al ^{E4}
	109/132 (83.1%)*	132/265 (49.8%)*	Rhinitis	
Mastocytosis	9/42 (21%)‡	15/42 (35.7%)	Mediator-related symptoms§	Smiljkovic et al (this study)

BAMSE, Barn/Children, Allergy, Milieu, Stockholm, Epidemiological study; ECA, environment and childhood asthma; EGEA, asthma, bronchial hyperresponsiveness and atopy.

*Samples tested at age 16 y and allergic status confirmed with asthma, rhinitis, asthma, and/or rhinitis at age 16 y.

†Allergic sensitization determined on the basis of ≥ 1 positive skin prick test result among the 11 allergen sources.

‡Based on reported allergy in patient history.

§Mediator-related symptoms: flush, vascular instability, anaphylaxis, rhinitis, headache, diarrhea.

TABLE E6. Therapy of the patient during follow-up

Month of follow-up	Therapy
73	
114	
139	2CdA, 0.13 mg/kg/d
140	2CdA, 0.13 mg/kg/d
141	2CdA, 0.13 mg/kg/d
144	2CdA, 0.13 mg/kg/d
146	2CdA, 0.13 mg/kg/d
149	2CdA, 0.13 mg/kg/d
177	
179	Prednisolone 12.5mg/d po
180	2CdA, 0.13 mg/kg/d
182	2CdA, 0.13 mg/kg/d
186	2CdA, 0.13 mg/kg/d
234	
270	
314 (last visit)	Omalizumab 300 mg in 3-4-wk intervals SC

Levocetirizine

Desloratadine

Ranitidine

Montelukast

po, Per os (by mouth).

2CdA was given intravenously 0.13 mg/kg/d for 5 d per cycle. Orally administered medication was ranitidine 300 mg twice daily, desloratadine 5 mg once daily, montelukast 10 mg once daily, and levocetirizine 5 mg once daily. Omalizumab 300 mg was given subcutaneously (SC) in 3-4-wk intervals.

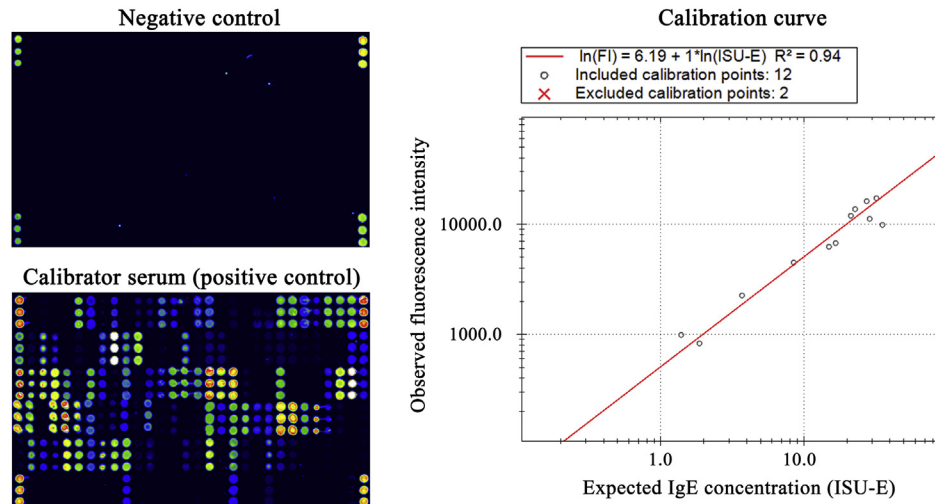


FIGURE E1. Calibration curve plus positive and negative controls for microarray analyses. Fluorescence signal intensities of the dots are encoded, with red dots representing high levels of allergen-specific IgE and blue color corresponding to low IgE levels as measured by a confocal laser-scanner. Levels of specific IgE were semi-quantified by Microarray Image Analyzer v3.1.2 software and expressed by ImmunoCAP Solid-phase Allergen-Chip standardized units for IgE. *FI*, fluorescence.

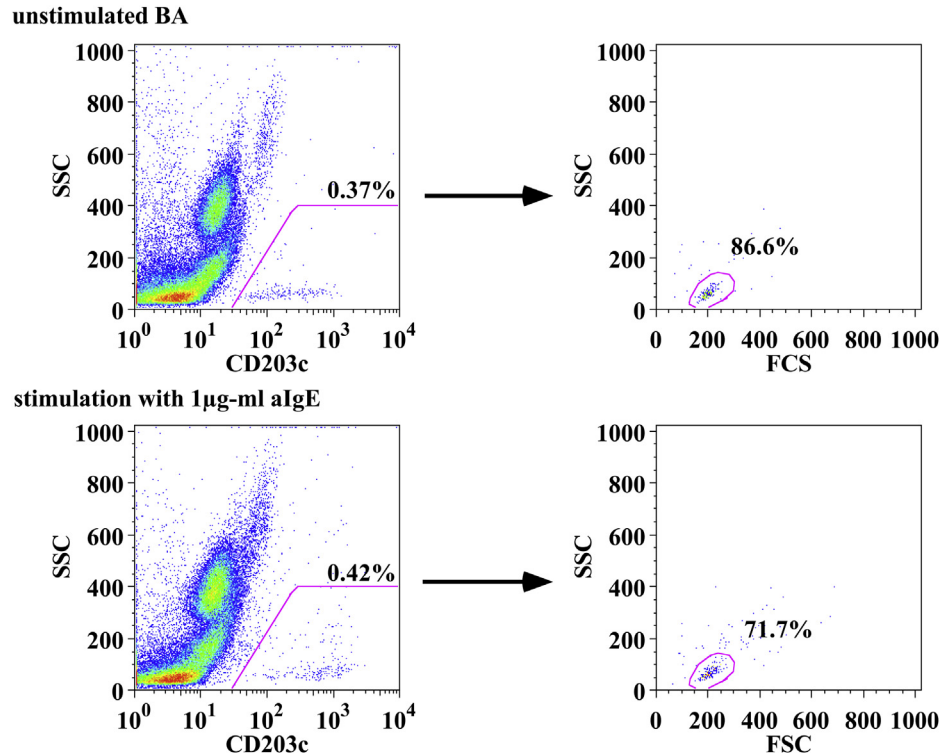


FIGURE E2. Gating strategy for basophils in the basophil CD203c activation test. Allergen-induced upregulation of CD203c was determined by multicolor flow cytometry on a FACS Calibur. For these experiments, cells were stained with the phycoerythrin -labeled CD203c antibody 97A6. Basophils were detected on the basis of their SSC and FSC characteristics and expression of CD203c (purple line). *BA*, Basophils; *FSC*, forward scatter; *SSC*, side scatter.

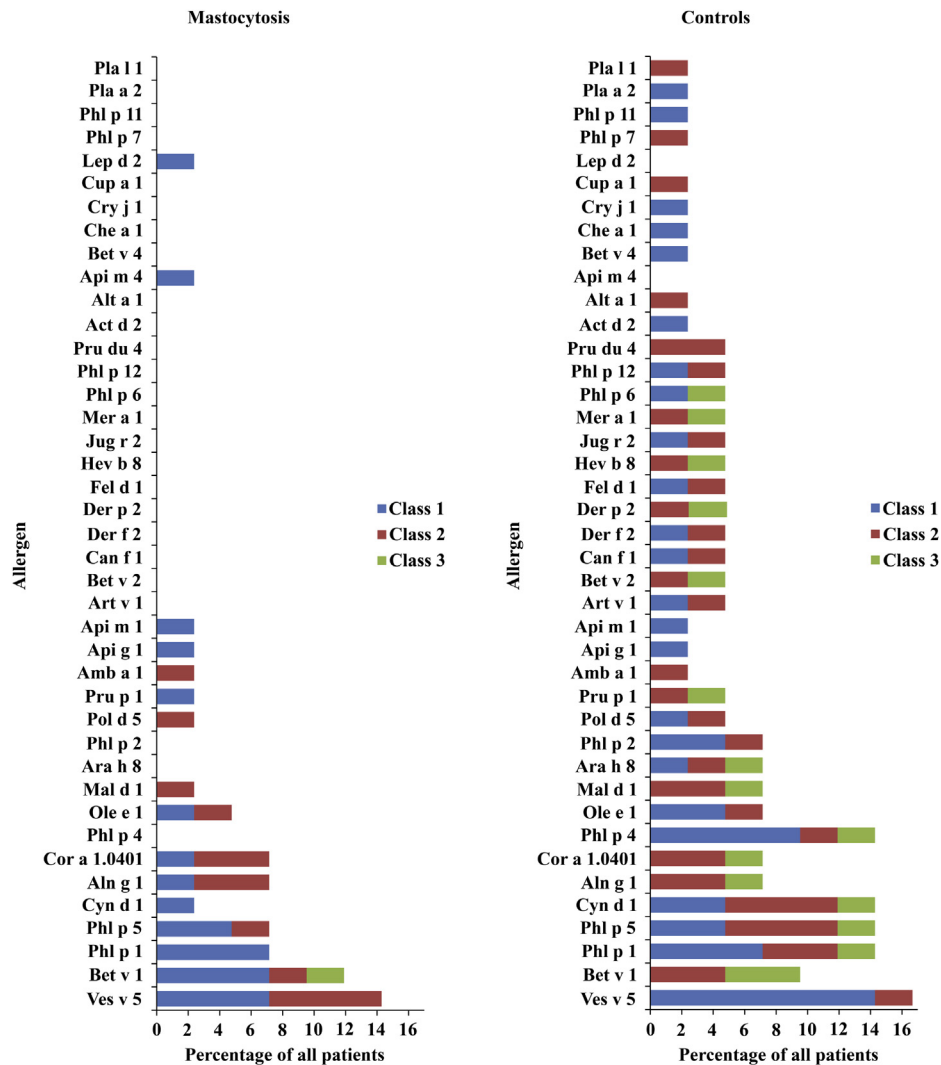


FIGURE E3. Profiling of specific IgE reactivities for allergens detected in the sera of patients with mastocytosis compared with age-matched controls. Sera obtained from 42 patients with mastocytosis and 42 age- and sex-matched controls without mastocytosis were analyzed by chip-profiling. Results are expressed as a percentage of patients with mastocytosis or percentage of controls testing positive in the chip assay. Levels of specific IgE are shown as: class 1: $\geq 0.3 < 1$ ISU, class 2: $\geq 1 \leq 10$ ISU, and class 3: > 10 ISU. A detailed description of allergens is presented in [Table E4](#).

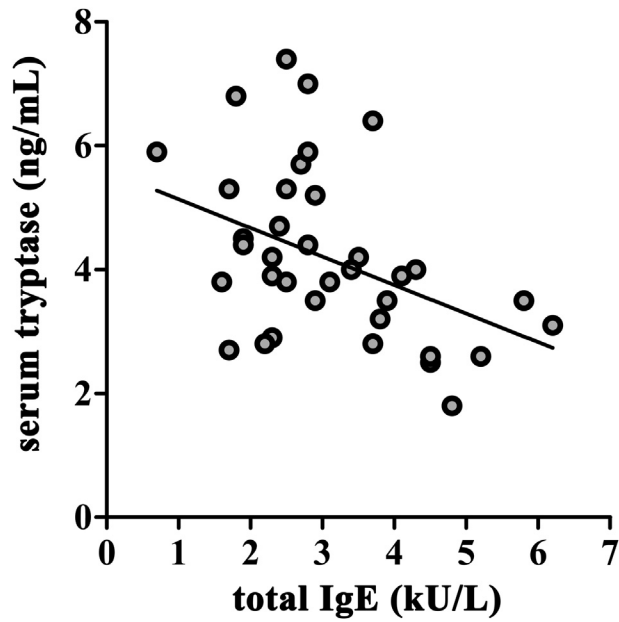


FIGURE E4. Correlation between serum tryptase levels and total IgE in patients with mastocytosis. Serum tryptase levels and total serum IgE levels were measured in 37 patients with mastocytosis in whom both markers were determined. Results are expressed in natural log units. A significant negative correlation between serum tryptase levels and total IgE levels was found (Pearson correlation coefficient: $r = 0.41$; $P < .05$).

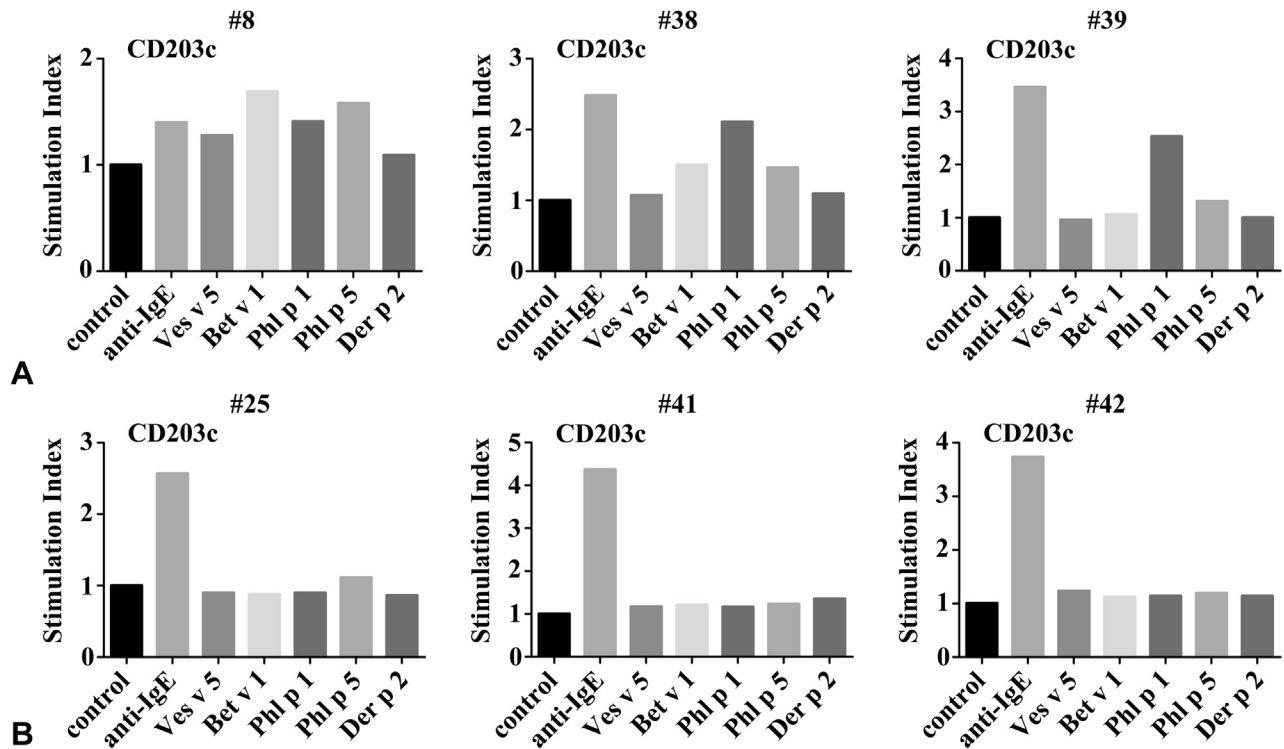


FIGURE E5. Basophil CD203c activation test with some of the most frequent allergens performed in our patients with mastocytosis. Patients with mastocytosis with (A) or without (B) specific IgE by allergen-chip-profiling were analyzed: patient number 8 had IgE against Bet v 1, Phl p 1, Phl p 5, and Ves v 5, number 38 IgE against Phl p 5, and number 39 IgE against Phl p 1. Blood cells were incubated with allergens (1 μ g/mL), anti-IgE (1 μ g/mL), or control buffer for 15 minutes (37°C). Allergen-induced upregulation of CD203c was determined as described in the text.

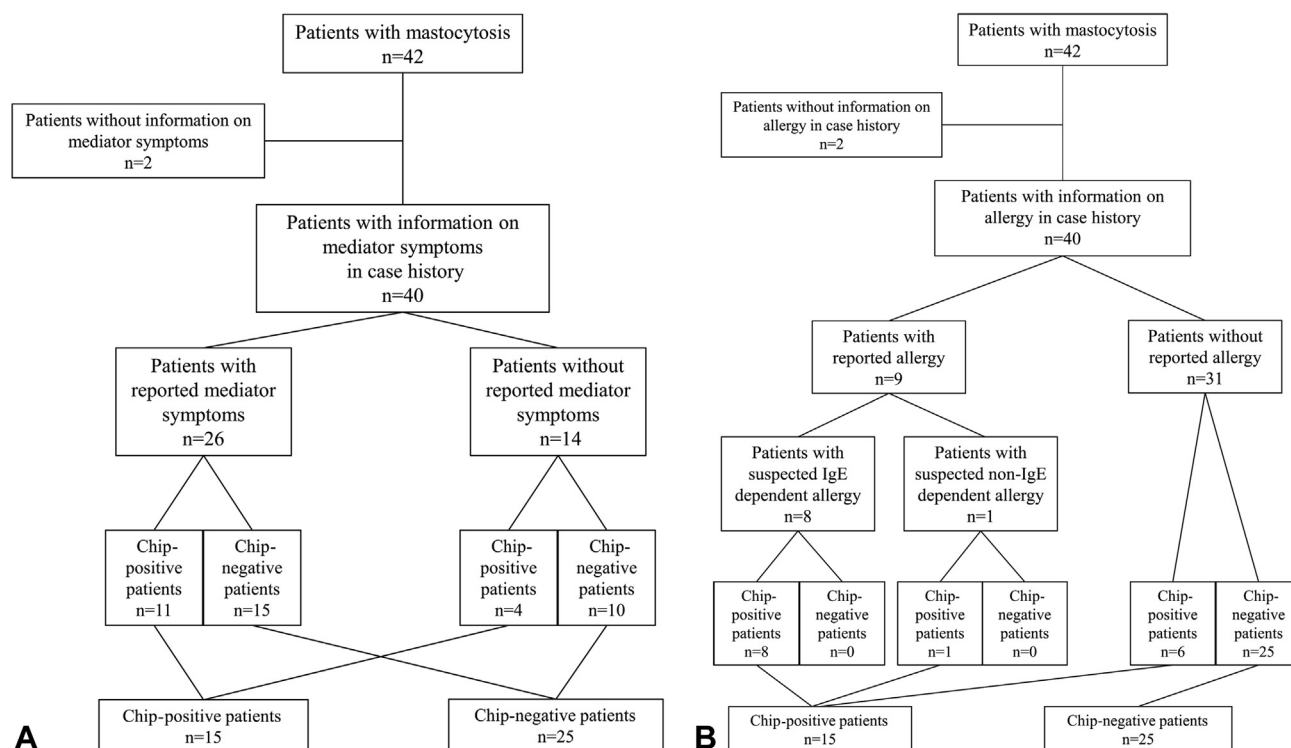


FIGURE E6. Flowchart diagram of patients included in the study. Forty-two patients with mastocytosis were included. In 2 patients no information about mediator-related symptoms (**A**) and no information on allergies in the case history (**B**) were available. Positive results in the allergen-chip were found in 15 patients, whereas in 25 patients the allergen-chip showed negative results.

REFERENCES

- E1. Wickman M, Lupinek C, Andersson N, Belgrave D, Asarnoj A, Benet M, et al. Detection of IgE reactivity to a handful of allergen molecules in early childhood predicts respiratory allergy in adolescence. *EBioMedicine* 2017;26:91-9.
- E2. Westman M, Lupinek C, Bousquet J, Andersson N, Pahr S, Baar A, et al. Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence. *J Allergy Clin Immunol* 2015;135:1199-206.e1-11.
- E3. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: the EGEA study. *J Allergy Clin Immunol* 2017;139:643-654.e6.
- E4. Skrandio I, Lupinek C, Valenta R, Hovland V, Pahr S, Baar A, et al. The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol* 2015;26:239-46.