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A Cutaneous Allergen Neutralisation Test That Correlates with the Duration of Venom Immunotherapy

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Key Words
Immunologic tests · Immunotherapy · Venom allergy · Skin test · Neutralising antibodies

Abstract
Background: Despite the well-documented efficacy of Hymenoptera specific immunotherapy (SIT), there is no safe method to reliably characterise the patient level of protection. Only poor correlations between protection and allergen-specific serum immunoglobulins have been found, and a sting challenge is the only means to evaluate the efficacy of immunotherapy. Therefore, we aimed to develop a cutaneous test that measures in vivo neutralisation of the Hymenoptera venom. ***Materials and Methods:*** Twenty-four patients with wasp venom allergy were included in the study. Wasp-specific serum IgE, IgG and IgG4 were measured by ImmunoCAP. Dilutions of the individual patient sera were intradermally injected into the forearm. Then, wasp venom extract was injected into these sites to quantitatively assess the formation of wheals and flares. ***Results:*** The results show that during the course of SIT, patient sera gained the capacity to neutralise skin reactions to wasp venom extracts in vivo. The test correlated with the duration of SIT as well as with the concentration of IgG and IgG4. ***Conclusion:*** The in vivo neutralisation test may become a promising tool in allergy diagnostics as well as in monitoring the success of SIT in patients undergoing allergen SIT.

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Introduction

Specific IgE antibodies against Hymenoptera venom can be determined in a large proportion of the general population. The sting allergy typically manifests with local reactions in 10–15% [1] and systemic anaphylactic sting reactions in 0.3–7.5% [2, 3] of the same population, whereby the prevalence is typically lower for children than for adults. Among beekeepers, anaphylaxis can occur in as much as 14–43% of the patients [4]. The annual mortality associated with Hymenoptera venom allergy is estimated between 0.03 and 0.48 per million [2]. The majority of fatalities occur in the elderly with cardiovascular diseases or those being treated with β-receptor blocking agents [5]. Patients suffering from systemic reactions after Hymenoptera stings should be evaluated allergologically, including skin tests and determination of venom-specific IgE in the serum.

Specific immunotherapy (SIT) represents a highly effective treatment of allergic diseases [6, 7]. SIT is the method of first choice for treating patients with a wasp venom allergy, as 95% of patients show ameliorated allergic symptoms after 5 years of treatment [8]. The mechanism by which SIT alleviates these allergic symptoms is currently unknown [6], but successful treatment is normally associated with a long-term reduction in the serum concentration of specific IgE [9] and a concomitant rise in the concentration of venom-specific IgG. Therefore, one possible mechanism of SIT is that allergen-specific

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IgG, also called blocking antibodies [10], would inhibit both the IgE-mediated release of inflammatory mediators from mast cells and basophils, as well as IgE-facilitated antigen uptake and presentation to T cells [10]. Another explanation may be that SIT produces tolerance to the allergen that is mediated by T cells [11], particularly regulatory CD4 T cells secreting immunosuppressive interleukin-10 [12]. Finally, SIT has been shown to reduce the numbers of mast cells, basophils and eosinophils. The mechanism underlying these changes is not known [7].

Provocation tests are considered the only reliable method to monitor the efficacy of SIT. However, in the case of Hymenoptera venom allergy, the provocation test is an actual sting challenge with a bee or wasp, with the risk of inducing severe life-threatening systemic allergic reactions including anaphylaxis. Intracutaneous tests or skin prick tests, as well as the determination of specific IgG or IgE in serum, have so far shown only limited value in predicting whether or not a Hymenoptera sting will still elicit an allergic reaction. Only the combination of a negative skin test and the absence of venom-specific IgE antibodies is associated with a diminished risk of an allergic reaction [3]. However, this situation rarely exists in the daily routine.

The goal of this study was to develop a functional skin test that would allow measuring the capacity of the allergic patients' serum to neutralise allergens. In a pilot trial, we investigated whether or not this test correlated with the duration of SIT against wasp venom.

Material and Methods

Patients

Twenty-four patients (11 males and 13 females) with a grade III or IV allergy to wasp venom [13] already undergoing SIT with wasp venom or considered medically eligible for initiation of SIT were recruited for this open, non-randomised pilot study that was conducted at the Allergy Unit of the Department of Dermatology, University Hospital of Zurich, Switzerland. The patients, with a mean age of 44 years (range 21–69), were divided into 4 groups of 6 patients according to their immunotherapy status. Group 1 had not yet begun SIT at the time of the baseline visit, group 2 had started SIT 1 month earlier, group 3 included patients 3 years through their SIT, and group 4 patients were 5 years through their SIT. The study protocol was reviewed and approved by the local ethics committee and notified by Swiss regulatory authorities (Swissmedic). Written informed consent was obtained from all patients prior to any study-related activities. The study conduct was performed according to the Guidelines of the International Conference on Harmonisation/Good Clinical Practice and the Declaration of Helsinki. The exclusion criteria were those advocated by the World Health Organisation [14].

Antibody Determination

Serum from each patient was prepared during the course of SIT and analysed for total IgE, as well as venom-specific IgE, IgG and IgG4 with the CAP (i3) system as described by the provider (Pharmacia) [15].

Wasp Venom Threshold Determination

The dose at which the wasp venom produced a positive skin reaction (threshold dose) was defined in an intracutaneous test. Serial dilutions of the wasp venom extract also containing 0.5% phenol (ALK 802; ALK-Abelló, Hoersholm, Denmark) were intradermally employed 2 cm apart to the left forearm of each patient. A positive skin reaction was defined by a swelling that produced wheals larger than 5 mm or at least 25% of the wheal induced by simultaneous histamine dihydrochloride (1 mg/ml) pricking and erythemas larger than 10 mm [16]. The threshold doses as well as the group medians are illustrated in figure 1. Note that 5% of the non-sensitised population reacts to wasp venom with a threshold dose at 0.1 μg.

Venom Neutralisation Test

The patients were given six intradermal injections into the right forearm with 100 μl of their own serum, serially diluted in 0.9% NaCl (1:2, 1:4, 1:8, 1:16, 1:32 and 1:64). After 10 min, the previously determined wasp venom threshold dose was injected (6×50 μl) into the sites of the serum injections. Areas of wheal and erythema were assessed after 15 min. To obtain a permanent record, the sizes of the wheal and erythema were outlined on the skin with a pen, then blotted onto a cellophane strip and stored on paper. Both the areas of the wheal and the erythema were assessed by planimetry [17]. The reactions were scanned and the surface areas are measured using commercial software.

Assessment of Wheal and Flare Reactions

The response of the intracutaneous test was measured by the size of the wheal and the surrounding flare or erythema. This test allowed classifying patients as inhibitors (negative) or responders (positive) with respect to wasp venom. The test was considered negative when wheal formation was inhibited (≤5 mm in diameter).

Statistical Analyses

Normally distributed (parametric) data were presented as mean ± 95% confidence limits and compared using a Student t test or ANOVA, unless otherwise indicated. Non-parametric correlations were analysed by Spearman's ρ test for the correlation coefficient, while parametric data were analysed using Pearson's correlation. The significance level was set at 5%.

Results

When giving patients intracutaneous injections of their own serum in serial dilutions and challenging these sites with the threshold dose of wasp venom, cutaneous wheal and erythema formations were strongly dependent on the SIT status. As summarised in table 1, serum samples taken from patients before initiation of the SIT

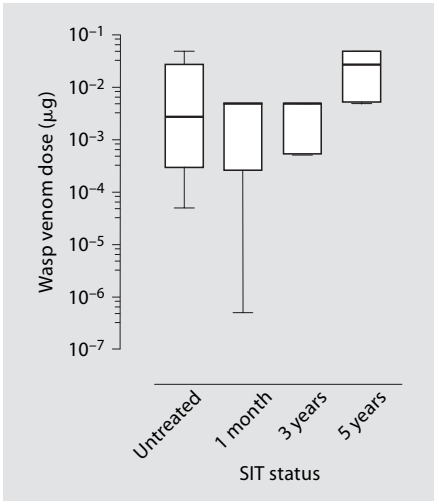


Fig. 1. Wasp venom threshold determination. The dose at which the wasp venom produced a positive skin reaction was defined by intracutaneous administration of serial dilutions of the venom to the left forearm of each patient (n = 6). A positive skin reaction was defined by a swelling that produced wheals larger than 5 mm and erythemas larger than 10 mm [16]. The box blots illustrate the 25th and the 75th percentile with the median line, while the whiskers show the minimum and maximum outliers.

Table 1. Wasp venom threshold determination

Serum dilution	Number of patients with neutralising serum			
	group 1	group 2	group 3	group 4
1:64	0	0	0	0
1:32	0	0	0	2
1:16	0	0	0	2
1:8	0	0	0	2
1:4	0	0	0	2
1:2	0	2	3	6

Skin reaction in patients (6 patients per group) as a function of SIT status and serum dilution. A positive skin reaction was defined by cutaneous swelling of more than 5 mm for the wheals and more than 10 mm for the erythemas.

(group 1) showed no neutralisation of wasp venom. Positive skin reactions (wheal >5 mm, erythema >10 mm) were observed at all serum concentrations. One month after starting the SIT (group 2), serum exerted neutralisation capacity at its lowest dilution of 1:2. The neutralising capacity further increased in patients having undergone 3 years of SIT (group 3). Five years of SIT (group 4) produced strongly neutralising sera that blocked skin reactions to wasp venom even at 1:32 dilutions, with all patients showing neutralisation capacity.

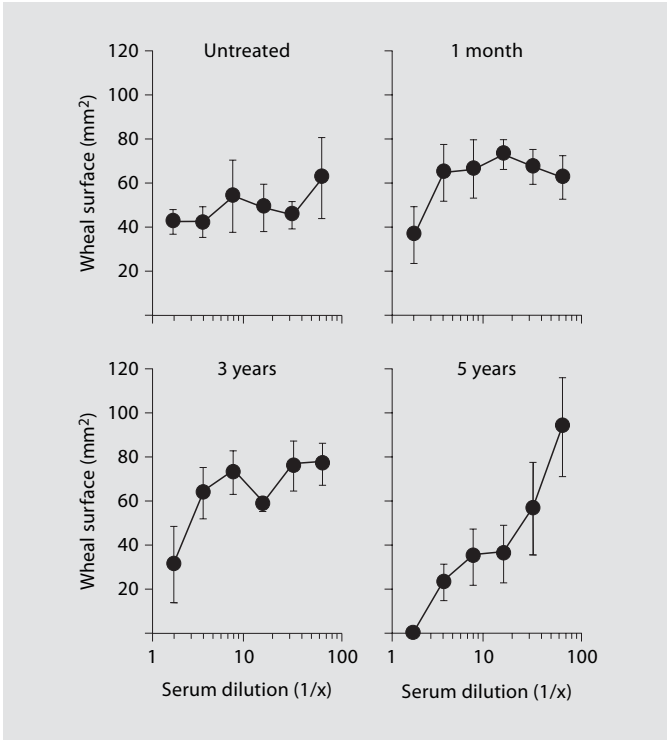


Fig. 2. The average size ($mm^2 \pm$ SE) of the skin wheals as a function of the stage of SIT (prior to, as well as 1 month and 3 and 5 years after started SIT). Patients were challenged with wasp venom intracutaneously 10 min after an injection of the patient's own serum at different dilutions (1:2 to 1:64) to the same injection site. Wheals were recorded 15 min after the challenge (n = 6).

Serum neutralisation of wasp venom is also illustrated in figure 2 (wheal) and figure 3 (erythema), which show the absolute swelling of the skin at the different dilutions for the different investigational groups. Again, no dose-dependent neutralisation was observed in the untreated group. For patients treated 1 month, a minor neutralisation was observed at the lowest dilution, whereas after 3 and especially 5 years of SIT, a strong and dose-dependent inhibition of wheal and erythema was measured.

To test if the development of the neutralising capacity of the sera was accompanied by a particular pattern of serum immunoglobulins, wasp venom-specific IgE, IgG, IgG4 as well as total IgE were measured in all patient sera using ImmunoCAP (fig. 4). SIT had no statistically significant effect on total IgE and venom-specific IgE. However, an increase in wasp venom-specific IgG was observed after 3 or 5 years of SIT. Total venom-specific IgG increased from an average of 5.54 mg/l at time zero to

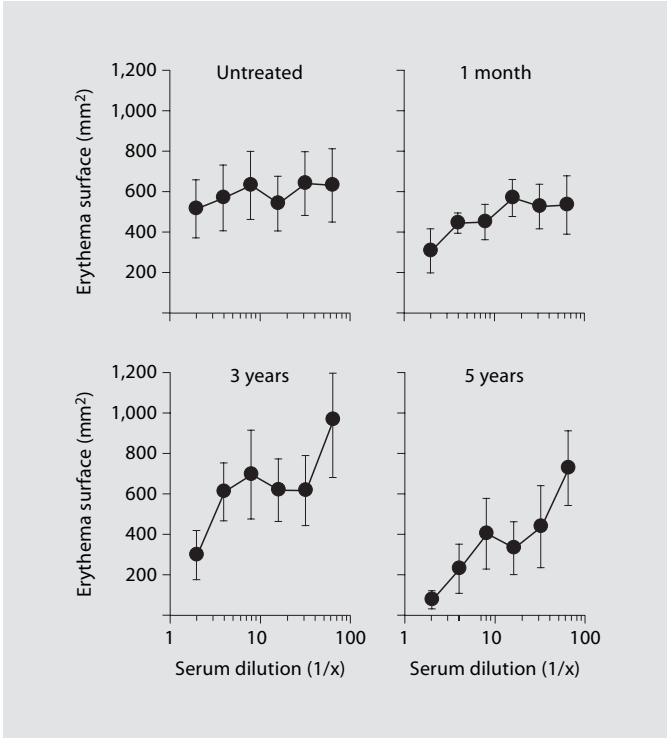


Fig. 3. The average size (mm^2 ± SE) of the skin erythema as a function of the stage of SIT (prior to, as well as 1 month and 3 and 5 years after started SIT). Patients were challenged with wasp venom intracutaneously 10 min after an injection of the patient's own serum at different dilutions (1:2 to 1:64) to the same injection site. Erythema was recorded 15 min after the challenge (n = 6).

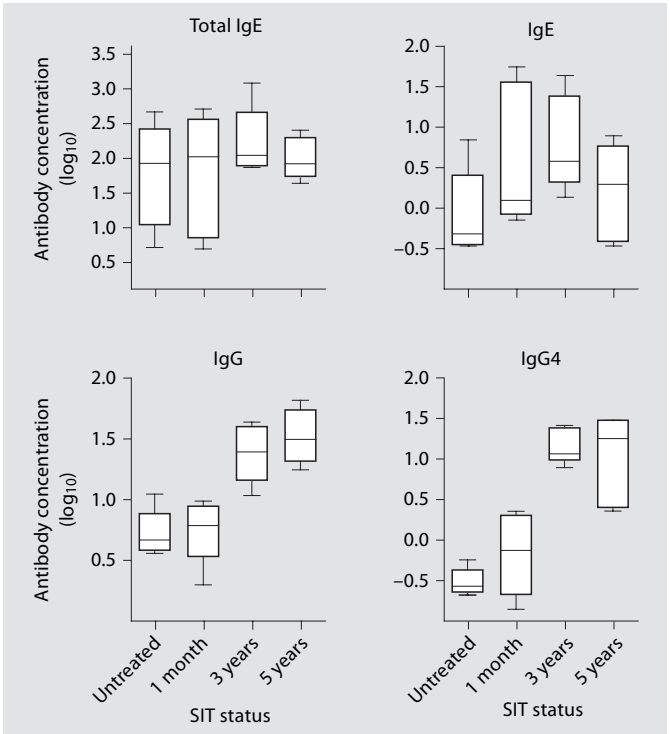


Fig. 4. Serum concentration of total IgE and wasp venom-specific IgE, as well as venom-specific IgG and IgG4 in groups of patients (n = 6) before starting SIT and after 1 month, 3 years and 5 years (IgE, kU/ml; IgG and IgG4, mg/l). The antibodies were analysed using an ImmunoCAP, and the box blots illustrate the 25th and the 75th percentile with the median line, while the whiskers show the minimum and maximum outliers.

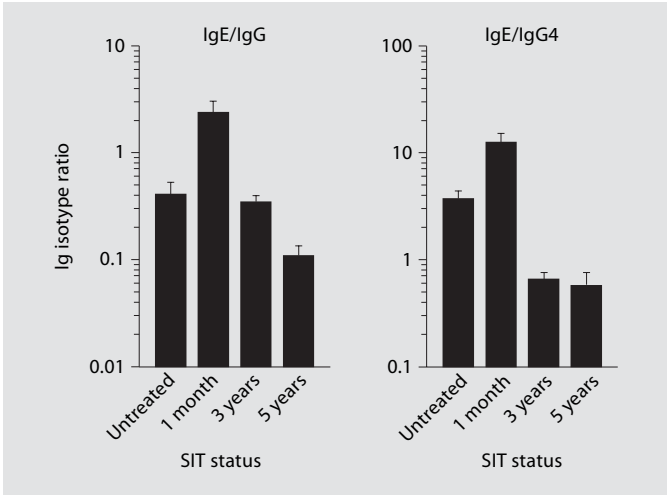


Fig. 5. Ratio of wasp venom-specific IgE to IgG or IgG4 in groups of patients (n = 6) before starting SIT and after 1 month, 3 years and 5 years. The antibodies were analysed using the ImmunoCAP, and ratios (± SE) are extracted from the data in figure 4.

26.53 mg/l after 3 years ($p < 0.01$) and to 36.08 mg/l after 5 years ($p < 0.01$). A similar increase was observed for venom-specific IgG4 with serum concentrations rising from an average of 0.31 mg/l at time zero to 15.16 mg/l after 3 years ($p < 0.01$) and to 17.65 mg/l after 5 years ($p < 0.01$). The increase in IgG and IgG4 also correlated positively at a 99% significance level ($p < 0.01$). As a consequence, figure 5 illustrates that the ratio of venom-specific IgE to IgG or IgG4 overall decreased as a function of SIT time ($p < 0.1$), suggesting polarisation of the T-cell response from Th2 towards Th1. While the IgE-to-IgG ratio reached its minimum after 3 years of SIT, the ratio of IgE-to-IgG4 further decreased between 3 and 5 years.

Finally, the increase in venom-specific IgG and IgG4 inter-correlated, and their increase also correlated negatively with wheal and erythema produced in the skin test at the 1:2 dilutions as illustrated in figure 6 ($p < 0.01$ using non-parametric Spearman's ρ tests for correlation coefficients); the neutralisation of the skin reaction was cal-

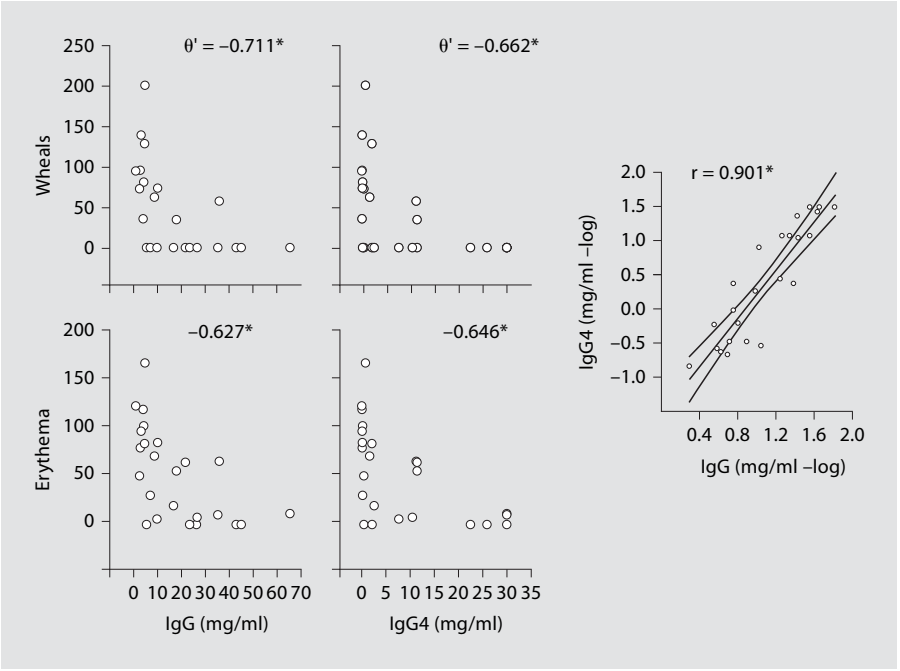


Fig. 6. Scatter plot showing the correlation between IgG or IgG4 and wheal or erythema formation as well as that between IgG and IgG4 (including 95% confidential intervals). The neutralisation of the skin reaction *(sr)* was calculated as the quotient *(q)* of the wheal or erythema using the highest (1:64) and the lowest (1:2) serum dilutions for all patients in the study (n = 24). The quotient represents the change in skin reaction (Δ*sr*) at the two serum dilutions. * Correlations are significant at the 0.01 level (two-tailed) as analysed by Spearman's ρ.

$$q = \Delta sr \times 100 = \frac{sr_{low}}{sr_{high}} \times 100$$

culated as the quotient of the wheal or erythema using the highest (1:64) and the lowest (1:2) serum dilutions for all patients as one population (n = 24).

Discussion

SIT is now practised for nearly a century [18, 19] and has proved very successful in treating patients with allergy to certain inhalant allergens such as grass pollen [20] and house dust mite [21]. For allergy to Hymenoptera venom, the success rate after 5 years of treatment is as high as 95% [22–26]. Nonetheless, the mechanism whereby SIT alleviates these allergic symptoms is not well understood, although SIT induces a series of immunological changes, whereby some may be responsible for symptom relief, but others may be only epiphenomena [10, 27].

One obvious hypothesis regarding the immunological mechanism of SIT is that the allergen-specific IgE decreases. However, the current consensus is that SIT initially increases IgE concentration in the patient's serum. Subsequently, IgE decreases with time, but long-term treatment is required to return it to pre-treatment level or lower. Also, in the present study, IgE was not found to decrease over the time period of SIT. IgE specific to wasp venom increased during the first month of therapy and then slowly returned to pre-treatment levels after 5 years.

Alternatively, venom SIT might work through allergen neutralisation and especially that produced by IgG antibodies, which may block the allergen before it encounters IgE and triggers an allergic reaction. Indeed, while allergic patients have high levels of venom-specific IgE antibodies, sera of beekeepers that are 'naturally desensitised' by frequent stings usually contain high levels of venom-specific IgG [28–30], mainly of the IgG4 subclass [31, 32]; beekeepers that are repeatedly stung are virtually at no risk for developing systemic reactions to honey bees [4]. These observations, in combination with the protective results of passive immunotherapy trials with beekeeper γ-globulin [33, 34], indicate a protective role of IgG in venom allergy. Similarly, we observed that IgG specific to wasp venom increased over 5 years by nearly 10-fold. Furthermore, this preferential interaction of the allergen with IgG and not IgE is supported stoichiometrically by the fact that normal serum contains several orders of magnitude more IgG (12 mg/ml) than IgE (50 ng/ml). Hence, intruding allergens most probably bind to an antibody of an IgG isotype before encountering IgE and thereby inducing a systemic allergic reaction. Especially IgG4 has received recent attention as an important IgG subclass with respect to blocking, and thereby preventing, an allergic reaction [10, 35].

If IgG and its subclasses were crucial in the protection against wasp and other insect venom allergies, it should be possible for the serum to neutralise the allergen and

thereby inhibit an allergic reaction. To test this, we developed the in vivo neutralisation method presented here. To better reflect a real challenge, dilutions of the sera were administered prior to the venom instead of administering the mixture of serum and venom. Strong skin reactions in terms of wheals and erythemas were observed in untreated patients and in patients having received 1 month of SIT. In contrast, patients with 5 years of SIT produced sera that fully inhibited the skin reactions, indicating that the in vivo neutralisation skin test may well correlate with the protection against wasp venom allergy. Obviously, a sting challenge would have been the best method to test whether the in vivo neutralisation test correlated with sting protection. However, both ethical and personal (study subjects and clinicians) restrains in performing such a sting challenge – especially on the untreated subjects in this study – disabled the possibility of direct correlation. Nonetheless, wasp venom was chosen as a model allergen due to its well-documented SIT protection efficiency [22, 24, 26]. This allowed us to assume protection in at least 5 out of 6 patients with 5 years of SIT. Therefore, the sting provocation should not be necessary, and the data strongly suggest that the neutralisation test is descriptive of both the SIT and protective status.

We observed a particularly strong increase in IgG4 during SIT. Although other potential allergen-neutralising subclasses of IgG were not analysed, it is possible that the increase in IgG4 may produce the observed neutralisation, which is in line with previous studies [10, 35]. However, this is contradicted by studies failing to find a correlation between the concentration of IgG4 and the neutralising activity associated with SIT [35]. Here, it is argued that the antibody response to immunotherapy is initially characterised mainly by IgG1 formation, while IgG4 becomes more prominent only after 1–2 years and only as long as the therapy is maintained [32]. However, this contraction regarding the role of IgG4 may partly be explained by the fact that the radio-allergosorbent test (RAST) technique previously utilised when measuring allergen-specific antibodies in the serum may have been inadequate [36, 37]. The RAST method has now largely been replaced by the more sensitive ImmunoCAP technique [38, 39], which was used in the present study.

More importantly, the presented allergen neutralisation skin test is different from serum IgG and IgG4 determination in so far that it also accounts for the affinity of antibodies, while ELISA, CAP or RAST detect low- and high-affinity antibodies with the same sensitivity. Increased avidity and affinity of antibodies have been postulated to provide increased in vivo effectiveness and function of antibodies in antibody-mediated protection against viral infections [40–42]. This phenomenon is likely to be valid also for neutralising antibodies in allergy prevention. Hence, although absolute titers may drop after termination of SIT, the matured affinity may compensate for the drop and still provide protection. Another reason why the correlation of IgG or IgG4 levels and clinical outcome could not be clearly established so far [43] is that such analysis determines IgG also against Hymenoptera venom components, against which the patients may not have any IgE, i.e. against components the patient is not allergic to. In contrast, the presented skin test measures neutralisation of an allergic reaction, i.e. against the relevant components of the allergen extract.

In conclusion, we developed a method for testing the capacity of the patient sera to neutralise the allergen using a skin test. We found that during the course of SIT, patient sera gained the ability to neutralise skin reactions to wasp venom extracts in vivo. Further studies will evaluate whether or not this skin test will be able to replace the sting challenge. If so, the presented in vivo neutralisation test may become a promising tool to monitor the success of SIT with Hymenoptera venom and may even shorten the required duration of SIT for patients reacting fast to SIT.

References

1 Incorvaia C, Senna G, Mauro M, Bonadonna P, Marconi I, Asero R, Nitti F: Prevalence of allergic reactions to Hymenoptera stings in northern Italy. Allerg Immunol (Paris) 2004; 36:372–374.

2 Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JNG: Diagnosis of Hymenoptera venom allergy. Allergy 2005;60: 1339–1349.

3 Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U: Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005;60:1459–1470.

4 Day JH, Buckeridge DL, Welsh AC: Risk assessment in determining systemic reactivity to honeybee stings in sting-threatened individuals. J Allergy Clin Immunol 1994;93: 691–705.

5 Hepner MJ, Ownby DR, Anderson JA, Rowe MS, Sears-Ewald D, Brown EB: Risk of systemic reactions in patients taking beta-blocker drugs receiving allergen immunotherapy injections. J Allergy Clin Immunol 1990;86:407–411.

6 Bousquet J, Lockey R, Malling HJ, Alvarez-Cuesta E, Canonica GW, Chapman MD, Creticos PJ, Dayer JM, Durham SR, Demoly P, Goldstein RJ, Ishikawa T, Ito K, Kraft D, Lambert PH, Lowenstein H, Muller U, Norman PS, Reisman RE, Valenta R, Valovirta E, Yssel H: Allergen immunotherapy: therapeutic vaccines for allergic diseases. World Health Organization. American Academy of Allergy, Asthma and Immunology. Ann Allergy Asthma Immunol 1998;81:401–405.
7 Norman PS: Immunotherapy: 1999–2004. J Allergy Clin Immunol 2004;113:1013–1023.
8 Golden DB: Discontinuing venom immunotherapy. Curr Opin Allergy Clin Immunol 2001;1:353–356.
9 Cantani A, Micera M: Significant decrease of IgE antibodies after a three-year controlled study of specific immunotherapy to pollen allergens in children with allergic asthma. Eur Rev Med Pharmacol Sci 2005;9:103–111.
10 Wachholz PA, Durham SR: Mechanisms of immunotherapy: IgG revisited. Curr Opin Allergy Clin Immunol 2004;4:313–318.
11 Akdis CA, Blesken T, Akdis M, Alkan SS, Wuthrich B, Heusser CH, Blaser K: Induction and differential regulation of bee venom phospholipase A2-specific human IgE and IgG4 antibodies in vitro requires allergen-specific and nonspecific activation of T and B cells. J Allergy Clin Immunol 1997;99:345–353.
12 Robinson DS, Larche M, Durham SR: Tregs and allergic disease. J Clin Invest 2004;114:1389–1397.
13 Wuthrich B: Classification of sting insect sensitivity. J Investig Allergol Clin Immunol 2001;11:132.
14 Bousquet J, Lockey RF, Malling HJ: WHO position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998;53:1–42.
15 Meier P, Muller U: Evaluation of IgG RAST FEIA for the assay of venom-specific IgG antibodies during venom immunotherapy. Int Arch Allergy Immunol 1998;117:46–51.
16 Brockow K, Romano A, Blanca M, Ring J, Pichler W, Demoly P: General considerations for skin test procedures in the diagnosis of drug hypersensitivity. Allergy 2002;57:45–51.
17 Sussman GL, Harvey RP, Schocket AL: Evaluation of skin test response using two techniques of measurement. Ann Allergy 1982;48:75–77.
18 Freeman J: Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. Lancet 1911;178:814–817.
19 Noon L: Prophylactic inoculation against hay fever. Lancet 1911;177:1572–1573.
20 Eng PA, Borer-Reinhold M, Heijnen IA, Gnehm HP: Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. Allergy 2006;61:198–201.
21 Pichler CE, Helbling A, Pichler WJ: Three years of specific immunotherapy with house-dust-mite extracts in patients with rhinitis and asthma: significant improvement of allergen-specific parameters and of nonspecific bronchial hyperreactivity. Allergy 2001;56:301–306.
22 Golden DB, Kagey-Sobotka A, Valentine MD, Lichtenstein LM: Dose dependence of Hymenoptera venom immunotherapy. J Allergy Clin Immunol 1981;67:370–374.
23 Golden DB, Kagey-Sobotka A, Norman PS, Hamilton RG, Lichtenstein LM: Outcomes of allergy to insect stings in children, with and without venom immunotherapy. N Engl J Med 2004;351:668–674.
24 Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM: A controlled trial of immunotherapy in insect hypersensitivity. N Engl J Med 1978;299:157–161.
25 Muller U, Helbling A, Berchtold E: Immunotherapy with honeybee venom and yellow jacket venom is different regarding efficacy and safety. J Allergy Clin Immunol 1992;89:529–535.
26 Wyss M, Scheitlin T, Stadler BM, Wuthrich B: Immunotherapy with aluminum hydroxide adsorbed insect venom extracts (Alutard SQ): immunologic and clinical results of a prospective study over 3 years. Allergy 1993;48:81–86.
27 Pereira C, Botelho F, Tavares B, Lourenco C, Baeta C, Palma-Carlos AG, Lima J, Chieira C: Kinetics and dynamic evaluation of specific immunotherapy. Allerg Immunol (Paris) 2004;36:375–386.
28 Yunginger JW, Jones RT, Leiferman KM, Paull BR, Welsh PW, Gleich GJ: Immunological and biochemical studies in beekeepers and their family members. J Allergy Clin Immunol 1978;61:93–101.
29 Golden DB, Lawrence ID, Hamilton RH, Kagey-Sobotka A, Valentine MD, Lichtenstein LM: Clinical correlation of the venom-specific IgG antibody level during maintenance venom immunotherapy. J Allergy Clin Immunol 1992;90:386–393.
30 Bousquet J, Coulomb Y, Robinet-Levy M, Michel FB: Clinical and immunological surveys in bee keepers. Clin Allergy 1982;12:331–342.
31 Wetterwald A, Skvaril F, Muller U, Blaser K: Isotypic and idiotypic characterization of anti-bee venom phospholipase A2 antibodies. Int Arch Allergy Appl Immunol 1985;77:195–197.
32 Aalberse RC, van der Gaag R, van Leeuwen J: Serologic aspects of IgG4 antibodies. 1. Prolonged immunization results in an IgG4-restricted response. J Immunol 1983;130:722–726.
33 Muller UR, Morris T, Bischof M, Friedli H, Skarvil F: Combined active and passive immunotherapy in honeybee-sting allergy. J Allergy Clin Immunol 1986;78:115–122.
34 Bousquet J, Fontez A, Aznar R, Robinet-Levy M, Michel FB: Combination of passive and active immunization in honeybee venom immunotherapy. J Allergy Clin Immunol 1987;79:947–954.
35 Wilson AB, Deighton J, Lachmann PJ, Ewan PW: A comparative study of IgG subclass antibodies in patients allergic to wasp or bee venom. Allergy 1994;49:272–280.
36 Bousquet J, Chanez P, Chanal I, Michel FB: Comparison between RAST and Pharmacia CAP system: a new automated specific IgE assay. J Allergy Clin Immunol 1990;85:1039–1043.
37 Ewan PW, Coote D: Evaluation of a capsulated hydrophilic carrier polymer (the ImmunoCAP) for measurement of specific IgE antibodies. Allergy 1990;45:22–29.
38 Jeep S, Kirchhof E, O'Connor A, Kunkel G: Comparison of the Phadebas RAST with the Pharmacia CAP system for insect venom. Allergy 1992;47:212–217.
39 Leimgruber A, Lantin JP, Frei PC: Comparison of two in vitro assays, RAST and CAP, when applied to the diagnosis of anaphylactic reactions to honeybee or yellow jacket venoms. Correlation with history and skin tests. Allergy 1993;48:415–420.
40 Bachmann MF, Kalinke U, Althage A, Freer G, Burkhart C, Roost H, Aguet M, Hengartner H, Zinkernagel RM: The role of antibody concentration and avidity in antiviral protection. Science 1997;276:2024–2027.
41 Foote J, Milstein C: Kinetic maturation of an immune response. Nature 1991;352:530–532.
42 Milstein C: Affinity maturation of antibodies. Immunol Today 1991;12:93–94.
43 Ewan PW, Deighton J, Wilson AB, Lachmann PJ: Venom-specific IgG antibodies in bee and wasp allergy: lack of correlation with protection from stings. Clin Exp Allergy 1993;23:647–660.