Acta Derm Venereol 2009; 89: 466–469

INVESTIGATIVE REPORT

# Immunotherapy with Wasp Venom is Accompanied by Wide-ranging Immune Responses That Need Further Exploration

Jochen BRASCH and Tzanka MAIDUSCH
*Department of Dermatology, University Hospitals of Schleswig-Holstein, Campus Kiel, Kiel, Germany*

**Immunotherapy with wasp allergen leads to a variety of specific immunological changes. It is unknown, however, whether unspecific effects also occur, and which parameter shifts might indicate treatment success. Therefore, data of patients who had completed immunotherapy with wasp venom were analysed retrospectively for a change in the following parameters after therapy: threshold of skin tests with wasp venom, total and specific serum IgE, specific serum IgG and IgG4, and binding of IgE and IgG4 to major wasp venom allergens. Reactions to field stings were explored. A significant increase in the skin test threshold and a significant decrease in total serum IgE, specific serum IgE and major wasp allergens binding IgE were found. Concentrations of specific serum IgG and IgG4 increased. Patients with corresponding changes in at least three specific parameters did not report severe reactions to verified field stings after therapy. The marked decrease in total serum IgE indicates that wasp immunotherapy has wide-ranging immunological effects, and it appears reasonable to check combinations of several parameters for treatment control.** ***Key words: hypersensitivity; allergy; immunoglobulin E; immunological parameters; immunotherapy; venoms.***

(Accepted April 21, 2009.)

Acta Derm Venereol 2009; 89: 466–469.

Jochen Brasch, Klinik für Dermatologie, Venerologie und Allergologie, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Schittenhelmstr.7, DE-24105 Kiel, Germany. E-mail: jbrasch@dermatology.uni-kiel.de

Immunotherapy with wasp venom is an established therapy for the treatment of patients with severe IgE-mediated hypersensitivity to wasp allergens (1, 2). There is no doubt about its efficacy in general (2–4), but it is a matter of discussion how to evaluate the effect of treatment in individual cases (5). Although it is well known that immunotherapy with wasp allergens leads to a variety of specific immunological changes related to wasp venom, there is no distinct immunological parameter that is a sufficiently reliable indicator of protection after this immunotherapy (6). Instead, a broad spectrum of interacting effects may contribute to the therapeutic outcome, and such a broad response may include effects that are not restricted to wasp allergen. Considering recent findings, that immunotherapy with house dust mite allergens can mitigate atopic dermatitis (7, 8), one may speculate that immunotherapy with wasp allergen may also have some unspecific effects. The aim of this study was therefore to re-examine the changes in immunological parameters that had occurred in our patients treated previously with wasp allergen immunotherapy. We included the total serum IgE level as a marker for immunological activity not specific for wasp allergens. We also investigated whether a pattern of immunological changes could be helpful to determine the efficacy of immunotherapy with wasp allergens.

## MATERIALS AND METHODS

### *Patients*

Data files of our department were searched for patients who had been treated with wasp venom immunotherapy in the years 1992 to 2005. Out of 226 such patients, 89 were identified who had finished their therapy after a continuous treatment time of 3 years. None of them had taken relevant doses of steroids or other immunomodulating agents within this period. A decision for immunotherapy had been made if at least a generalized urticaria had occurred, which is in accordance with now common guidelines (9, 10). The mean age of the 89 patients was 40 years, and 53 of them were women. Fifty-four of the 89 patients had experienced urticaria and 35 had experienced bronchopulmonary or cardiovascular symptoms with or without urticaria, including 10 individuals with shock. All patients had been treated for 3 years and immunotherapy had always been performed with 100 μg Reless® wasp venom (ALK Scherax, Hamburg, Germany) applied subcutaneously once per month after tolerance had been achieved by use of a rush protocol. No severe side-effects had been observed. Time-points of start and termination of immunotherapy were evenly distributed over the seasons.

### *Immunodiagnostics*

Before the start of treatment, an IgE-mediated allergy to wasp venom had been diagnosed by a combination of a positive skin test with wasp venom (Reless®, ALK Scherax), demonstration of specific serum IgE to wasp venom and, in many cases, by detecting IgE binding to distinct major allergens of wasp venom. Intracutaneous skin tests had been performed with 0.05 ml Reless® (ALK Scherax) wasp venom in concentrations from 0.0001μg/ml to 1μg/ml and a positive reaction to a concentration < 0.1μg/ml was considered to indicate sensitization. Levels of total serum IgE, specific serum IgE, specific serum IgG, and specific serum IgG4 had been measured by use of the ImmunoCAP® system (Phadia Diagnostics, Uppsala, Sweden) or by the preceding diagnostic systems offered by the same manufacturer. Antibody binding to three major wasp allergens

(hyaluronidase, phospholipase A1, antigen 5) had been determined with the AlaBLOT® system (DPC Biermann GmbH, Bad Nauheim, Germany). All patients with completed immunotherapy were questioned about reactions to field stings, and in the case of a field sting followed by more than a local skin reaction, further diagnostic measures were taken as appropriate in the individual case.

### *Statistics*

The data used for comparisons were obtained before start of immunotherapy and after 3 years of therapy. The data evaluated did not follow a Gaussian distribution (Kolmogorov-Smirnov test) and, for some parameters, no analogue data were measured. Accordingly, the Wilcoxon rank-sum test (Mann-Whitney *U* test) was used for statistical evaluations and the Wilcoxon matched pairs test was applied for intra-individual comparisons. $p < 0.05$ was considered statistically significant.

## RESULTS

### *Skin test threshold*

Results of skin test thresholds with wasp venom were available for 76 identical patients before and after therapy. Related to these 76 individuals, before therapy the skin threshold was 0.0001 μg/ml in 3 cases, 0.001 μg/ml in 27 cases, 0.01 μg/ml in 42 cases and 0.1 μg/ml in 2 cases. After therapy, no patient out of these 76 ones reacted to 0.0001 μg/ml or to 0.001 μg/ml, 17 reacted to 0.01 μg/ml, 40 to 0.1 μg/ml and 19 to 1.0 μg/ml. This means that 89.5% of the 76 patients had an increase in their skin test threshold and no change in the skin test threshold was seen in the others. A lowering of the skin test threshold did not occur.

### *Serological findings*

Serum levels of total serum IgE had been measured before and after therapy in 73 identical patients. The median of their total serum IgE was 143 kU/l before therapy, and had decreased to 71 kU/l after therapy ($p < 0.001$) (Fig. 1). Serum levels of specific IgE had been measured before and after therapy in 84 identical patients. The median of their specific serum IgE was 16.4 kU/l before therapy and it had decreased to 4.8 kU/l after therapy ($p < 0.001$) (Fig. 2).

In 40 patients IgE antibodies to distinct major wasp venom allergens had been determined before therapy. Of these, 22 had IgE binding to three major allergens, 5 to two major allergens, 9 to one major allergen, and 4 to none of the three major allergens assessed by the test. After therapy, data were available for 74 patients; 15 had positive IgE binding to three major allergens, 20 to two major allergens, 13 to one major allergen, and 26 to none of the three major allergens. This means that the percentage of patients with IgE binding to three major allergens had decreased from 55% before therapy to 20% after therapy ($p < 0.03$). In 74 patients, binding

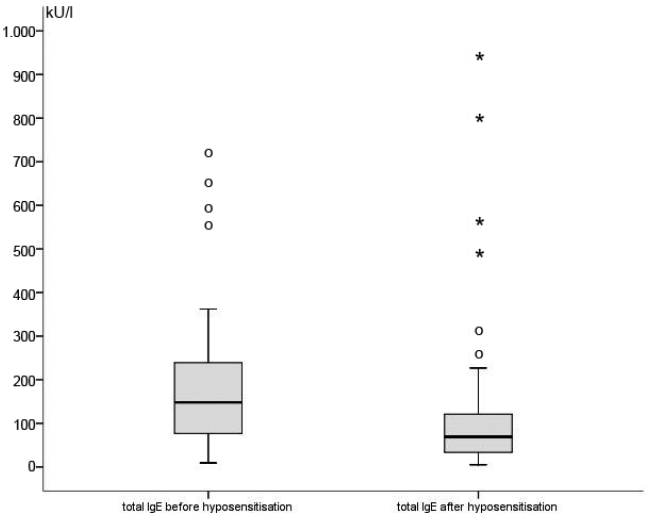


*Fig. 1.* Total serum IgE before and after 3 years of immunotherapy with wasp venom. Box-and-whisker plots with medians; $n = 73$. Fifty percent of the data are within the box, the box length indicates the interquartile range and the thick horizontal line inside the box marks the median of the data. The lengths of the vertical lines (whiskers) correspond to the 1.5-fold interquartile ranges. Circles mark outliers, and asterisks mark extreme values.

of IgG4 to major wasp allergens had been determined after therapy (no measurements had been made before therapy). Of these, 55 had IgG4 binding to three major allergens, 13 to two major allergens, 6 to one major allergen, and 0 to none of the three major allergens.

Serum levels of specific IgG had been measured in 57 identical patients before and after therapy. The median of their specific IgG was 6.3 mg antibody/l before therapy and it had increased to a median of 13.5 mg antibody/l

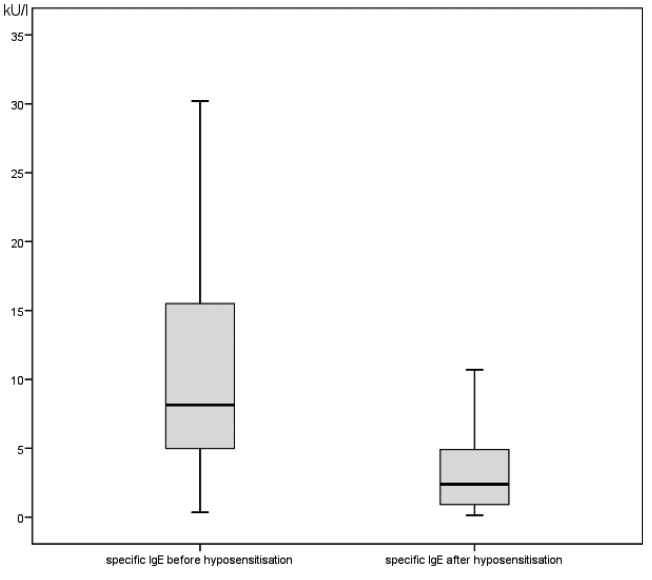


*Fig. 2.* Specific serum IgE before and after 3 years of immunotherapy with wasp venom. Box-and-whisker plots with medians; $n = 84$. Fifty percent of the data are within the box, the box length indicates the interquartile range and the thick horizontal line inside the box marks the median of the data. The lengths of the vertical lines (whiskers) correspond to the 1.5-fold interquartile ranges.

after therapy ($p < 0.01$). Because the measurements of IgG4 had only recently been established in our laboratory, serum levels of specific IgG4 antibodies to wasp venom had not been measured before treatment in patients who had completed their therapy at the time of our analysis. However, in 44 patients with completed therapy, the median of the specific serum IgG4 was 3.4 mg antibody/l. For comparison, data of 42 patients were obtained from other patients with wasp allergy before their therapy was started. These patients were matched to the group with completed treatment with regard to age, sex and atopy. The median of their serum level of specific IgG4 was only 0.5 mg antibody/l ($p < 0.001$).

### *Field sting reports*

Field stings were reported by 24 out of all patients with completed immunotherapy. Nineteen of these 24 reported no abnormal reaction. Three patients reported symptoms suggesting urticaria. Two patients had experienced some cardiovascular reactions after field stings, which were thought to be wasp stings. These reactions were clearly milder than their reactions prior to treatment, but they were considered as possible allergic responses to wasp venom. In these patients immunotherapy had been continued, with subcutaneous injections of 100 μg wasp venom every 4 weeks. No adverse effects were observed during this additional treatment and no further stings were reported. An assessment of treatment response after the first 3 years of therapy based on combined immunological parameters was not possible in these 2 patients because of incomplete data. Taken together, no patient with at least 3 of the following shifts in immunological parameters had reacted with a systemic reaction to a verified field sting by a wasp after 3 years of immunotherapy: increase in the skin test threshold, decrease in specific serum IgE, decrease in the number of IgE-binding major allergens, and an increase in specific serum IgG or IgG4.

## DISCUSSION

This is a retrospective study with a restricted number of patients and an inclusion period of 14 years that necessitated some minor methodological changes in serological assays due to the course of time. Due to this study design and the fact that only patients with immunotherapy completed at our department were evaluable for our analysis, the significance of the results is limited and needs to be checked by further investigations. Nevertheless, we would like to address some interesting aspects that either corroborate previous findings or may be promising subjects for prospective studies.

First, our results confirm that immunotherapy with wasp venom is a very effective and safe treatment (11, 12). We also found the expected changes in specific immunological parameters, including raised skin test thresholds and shifts of specific serum immunoglobulins. It is in fact well known that immunotherapy with wasp allergen induces corresponding variations in these immune parameters (13).

However, an interesting and new result of our analysis is the marked and significant decrease in the total serum IgE level by 72 kU/l within 3 years of immunotherapy. This decrease is quite conspicuous, and clearly exceeds a decrease that according to some observations might be expected due to ageing of the patients (14–16). Although it is associated with changes in specific IgE, it can also not completely be explained by the decrease in the specific serum IgE, because the reduction of specific IgE by 11.6 kU/l is too small for this. Venom-specific immunotherapy, as well as specific immunotherapy for atopic dermatitis is, at present, interpreted as antigen-specific immune therapy. As a hypothesis, however, it may be discussed whether an immunotherapy with wasp venom might not only modulate the specific immune response to wasp allergens but in addition may have attenuating effects on other IgE-mediated immune reactions. This idea relates to the concept that allergen-specific immunotherapy may improve atopic dermatitis (7). Considering, however, what was said at the beginning of the discussion, our finding certainly needs to be checked by future studies.

Positive specific provocation tests with wasp stings can demonstrate an actual failure of immunotherapy with wasp venom, but genuine wasp stings were not applied in our patients. For various reasons real wasp stings are not used as a matter of routine in Germany nowadays and because the amount of venom injected by an individual wasp during a provoked sting is variable this test should, in fact, not be taken as unfailing as well. Ideally, a distinct serum parameter should mirror the efficacy of immunotherapy. Our findings show that even when a reduced reaction to a field sting indicates that immunotherapy was clearly effective it is not obligatory that each of the distinct parameters evaluated in our study has changed in the expected way. This corroborates previous reports that evaluation of treatment efficacy can not rely on one parameter alone, but that a combination of parameters should be considered (6). Based on our results, we suggest that the success of an immunotherapy carried into effect for 3 years may be assumed if at least three of the following shifts of parameters occur: increase in the skin threshold, decrease in specific serum IgE, increase in specific serum IgG or IgG4, and decrease in the number of IgE-binding major allergens. Instead of the latter an increase in IgG4 binding to major allergens may in the future turn out to be a better parameter. This proposal to consider at least three parameters is made as kind of a preliminary start point. Our data suggest this option, but they are not sufficient for a definite statement. In particular, our data

pool did not comprise a meaningful group of patients with unambiguous systemic responses to proven wasp stings after therapy that would have allowed sound statistic comparisons with successfully treated patients. Nevertheless, we think that a multifactor evaluation makes sense and hope that corresponding prospective analyses with larger number of cases will be performed. The surprising decline in total (unspecific) serum IgE during wasp venom immunotherapy shows, in fact, that the spectrum of immunological parameters changing under therapy is broad and probably comprises some relevant factors that are still unexplored. It therefore appears reasonable to check combinations of several parameters for treatment control.

## ACKNOWLEDGEMENT

We thank Dipl. Inform. J. Hedderich, Institute of Medical Informatics and Statistics, University Hospitals of Schleswig-Holstein, Campus Kiel, Kiel, for his advice and statistical calculations.

*The authors declare no conflict of interest.*

## REFERENCES

1. Roesch A, Boerzsoenyi J, Babilas P, Landthaler M, Szeimies RM. Outcome survey of insect venom allergic patients with venom immunotherapy in a rural population. J Dtsch Dermatol Ges 2008; 6: 292–297.
2. Rueff F, Przybilla B. Venom immunotherapy: side effects and efficacy of treatment. Hautarzt 2008; 59: 200–205.
3. Pasaoglu G, Sin BA, Misirligil Z. Rush hymenoptera immunotherapy is efficious and safe. J Investig Allergol Clin Immunol 2006; 16: 232–238.
4. Scribner TA, Bernstein DI. Rapid venom immunotherapy update. Curr Opin Allergy Clin Immunol 2003; 3: 295–298.
5. Bilò BM, Bonifazi F. Advances in hymenoptera venom immunotherapy. Curr Opin Allergy Clin Immunol 2007; 7: 567–573.
6. Ott H, Wosnitza M, Merk HF. Immunologic control parameters during specific immunotherapy. Hautarzt 2008; 59: 551–556.
7. Bussmann C, Böckenhoff A, Henke H, Werfel T, Novak N. Does allergen-specific immunotherapy represent a therapeutic option for patients with atopic dermatitis? J Allergy Clin Immunol 2006; 118: 1292–1298.
8. Niebuhr M, Kapp A, Werfel T. Specific immunotherapy (SIT) in atopic dermatitis and food allergy. Hautarzt 2008; 59: 544–550.
9. Przybilla B, Rueff F, Fuchs T, Pfeiffer C, Rakoski J, Stolz W, Vieluf D. Leitlinien der Deutschen Gesellschaft für Allergologie und Klinische Immunologie (DGAI). Allergo J 2004; 13: 186–190.
10. Bonifazi F, Jutel M, Bilo BM, Bimbaum J, Muller U. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. Allergy 2005; 60: 1459–1470.
11. Lee H, Roediger C, Bauer A, Zuberbier T. Worm M. Prospective safety analysis of an ultrarush specific immunotherapy in adults with wasp venom allergy. Allergy 2006; 61: 1237–1238.
12. Hafner T, DuBuske L, Kosnik M. Long-term efficacy of venom immunotherapy. Ann Allergy Asthma Immunol 2008; 100: 162–165.
13. Pereira Santos MC, Pedro E, Spínola Santos A, Branco Ferriera M, Palma Carlos ML, Palma Carlos AG. Immunoblot studies in allergic patients to hymenoptera venom before and during immunotherapy. Eur Ann Allergy Clin Immunol 2005; 37: 273–278.
14. Criqui MH, Seibles J, Hamburger RN, Coughlin SS, Gabriel S. Epidemiology of immunoglobulin E levels in a defined population. Ann Allergy 1990; 64: 308–313.
15. Mediaty A, Neuber K. Total and specific serum IgE decreases with age in patients with allergic rhinitis, asthma and insect allergy but not in patients with atopic dermatitis. Immun Ageing 2005; 2: 9.
16. Jarvis D, Luczynska C, Chinn S, Potts J, Sunyer J, Janson C, et al. Change in prevalence of IgE sensitization and mean total IgE with age and cohort. J Allergy Clin Immunol 2005; 116: 675–682.