Sensitization to Hymenoptera venoms is common, but systemic sting reactions are rare

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Background: Sensitization to Hymenoptera venom without systemic sting reactions (SSRs) is commonly observed in the general population. Clinical relevance for a future sting has not yet been investigated.
Objective: We aimed to evaluate the effect of these debatable sensitizations with deliberate sting challenges and to monitor serologic changes for up to 2 years.
Methods: One hundred thirty-one challenges with bees and wasps were performed in 94 subjects with a hitherto irrelevant sensitization. The clinical outcome was recorded, and results of specific IgE (sIgE) determinations, skin tests, and basophil activation tests were correlated to the sting reaction. sIgE levels were monitored in reactors and nonreactors after 3 hours, 1 week, 4 weeks, and 1 year.
Results: Only 5 (5.3%) patients had SSRs, but 41 (43.6%) had large local reactions (LLRs) after the sting. Compared with the general population, there was a 9.5-fold higher risk for LLRs but not for SSRs. Three hours after the sting, sIgE levels slightly decreased, but none of the 94 subjects' results turned negative. After 1 week, sIgE levels already increased, increasing up to 3.5-fold (range, 0.2- to 34.0-fold) baseline levels after 4 weeks. To assess the clinical relevance of this increase, we randomly selected 18 patients for a re-sting. Again, 50% had an LLR, but none had an SSR.
Conclusion: Although sensitization to Hymenoptera venoms was common, the risk of SSRs in sensitized subjects was low in our study. The sIgE level increase after the sting was not an indicator for conversion into symptomatic sensitization. Currently available tests were not able to distinguish between asymptomatic sensitization, LLRs, and SSRs. (J Allergy Clin Immunol 2014;133:1635-43.)

Key words: *Asymptomatic sensitization, basophil activation test, component-resolved diagnosis, IgE determination, intradermal test, large local reaction, sting challenge, systemic sting reaction, total IgE*

Abbreviations used
AS: Asymptomatic sensitization
BAT: Basophil activation test
CCD: Cross-reactive carbohydrate determinant
CRD: Component-resolved diagnosis
IDT: Intradermal test
LLR: Large local reaction
sIgE: Specific IgE
SSR: Systemic sting reaction
tIgE: Total IgE

Depending on the climate, the prevalence of Hymenoptera stings ranges from 56.6% to 94.5% in the general adult population.[1] In the general population 0.3% to 7.5% are reported to have experienced systemic sting reactions (SSRs), and 2.4% to 26.4% have had large local reactions (LLRs) to Hymenoptera stings.[2] Recently, we carried out the first epidemiologic telephone survey on Hymenoptera venom allergy in Austria and found that 3.3% have experienced SSRs and 4.6% have experienced LLRs, respectively.[3]

Asymptomatic sensitization (AS) to bee and wasp venom occurs frequently in *in vitro* tests, and 27.1% to 40.7% of the general population are reported to have detectable specific IgE (sIgE) to Hymenoptera venoms.[4,5] Furthermore, AS is related to total IgE (tIgE) levels; in healthy subjects with high tIgE levels, sIgE to Hymenoptera venoms was demonstrable in up to 66.7% of investigated subjects.[5] Therefore current criteria to diagnose Hymenoptera venom allergy cannot accurately predict the occurrence or severity of anaphylactic symptoms after a sting. The main cause of AS in subjects double sensitized to bee and wasp venom is the presence of sIgE to cross-reactive carbohydrate determinants (CCDs) in the serum.[6,7] Many of the bee venom allergens and some of the wasp venom allergens bear CCDs; therefore sensitization to CCDs can mimic double sensitization to Hymenoptera venoms.[7] However, sIgE to CCDs as a cause for AS in monosensitized subjects is typically not observed.[5]

Nevertheless, a large portion of subjects are sensitized to nonglycosylated venom allergens and tolerate Hymenoptera stings well. Given that the prevalence of SSRs in Austria is 3.3% and that 40.7% of the general population are sensitized to at least 1 venom, it can be assumed that the majority of sensitized subjects will not experience SSRs. However, sensitization could have been converted into clinically relevant hypersensitivity after the recent sting, and therefore subjects could potentially react to the next sting. The relevance of these unclear sensitizations has not been elucidated. Therefore we aimed to conduct a prospective study to clarify the effect of detectable sIgE to Hymenoptera

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venoms for the next sting. For this purpose, deliberate sting challenges with living bees and wasps were performed. Several years ago, we found that patients with low tIgE levels (<50 kU/L) had a higher risk for severe reactions.[8] We also observed that high tIgE levels (>250 kU/L) were associated with a higher frequency of AS.[5] Therefore we initially aimed to evaluate whether subjects with low tIgE levels were predisposed to (severe) SSRs or LLRs. Then we correlated test results of available diagnostic tools, such as the skin prick test, the intradermal test (IDT), sIgE determination, component-resolved diagnosis (CRD), and the basophil activation test (BAT) with the outcome of the sting challenge. We also monitored sIgE levels against the respective venom after sting challenges. In this context we tested the hypothesis of sIgE "consumption" because of exposure to venom allergens after a sting. Because of increased sIgE levels after the first challenge, we performed a second sting challenge in randomly selected subjects to investigate whether this increase was associated with the conversion of a clinically irrelevant sensitization into relevant hypersensitivity.

METHODS

Subjects

Subjects who tolerated previous Hymenoptera field stings without an SSR were initially screened for sIgE to Hymenoptera venom. Subjects with detectable sIgE to at least 1 Hymenoptera venom who met all other inclusion and exclusion criteria (see Table E1 in this article's Online Repository at www.jacionline.org) were asked to participate in the study. Finally, 110 subjects were enrolled. After a complete health check, including physical examinations, laboratory tests, spirometry, and electrocardiography, diagnostic tests for Hymenoptera venom allergy were performed. An atopic disposition was considered if there was at least 1 positive reaction to an allergen in a skin prick test panel of common aeroallergens. Forty-one were stung by a bee, 16 by a wasp, and 37 by both a bee and a wasp. In subjects with double sensitization, 2 sting challenges were performed on the same day. If the first sting was tolerated, another challenge was performed after 3 hours. Sting challenges were followed by repeated serologic tests (Fig 1). This study was approved by the ethics committee of the Medical University of Graz (approval no. 18-046).

Classification of sting reactions

According to the modified classification of Ring and Messmer,[2] generalized skin symptoms, such as flush, urticaria, and angioedema, were classified as grade I reactions. Mild-to-moderate respiratory, cardiovascular, or gastrointestinal symptoms were rated as grade II reactions. Bronchoconstriction, emesis, anaphylactic shock, and loss of consciousness were classified as grade III reactions. An LLR was defined as swelling exceeding a diameter of 10 cm that lasts longer than 24 hours.

Skin tests

The nature of sensitization was confirmed by means of standardized end point titration skin prick tests (10, 100, and 300 μg/mL) and IDTs (0.02 mL of 0.01, 0.1, and 1 μg/mL) with purified honeybee and vespid venom extracts (ALK-Abelló, Hørsholm, Denmark). Skin prick test and IDT results were considered positive in the presence of wheals 3 and 5 mm larger in diameter and erythema, respectively.

Determination of sIgE and tIgE levels

Specific and tIgE antibody levels in the patients' sera were measured by using ImmunoCAP 1000 (Thermo Fisher Scientific, Waltham, Mass), Immulite 2000 (Siemens, Tarrytown, NY), and ADVIA Centaur (Siemens), according to the manufacturer's instructions. CRD with rApi m 1 and rVes v 1 and 5 was done on the ImmunoCAP 1000. Diagnosis with nApi m 1, nVes v 1, and nVes v 5 was done on the ADVIA Centaur platform at the Department of I+D (ALK-Abelló, Madrid, Spain). For some analyses, patients were grouped according to low (<50 kU/L), intermediate (50-250 kU/L), and high (>250 kU/L) tIgE levels.

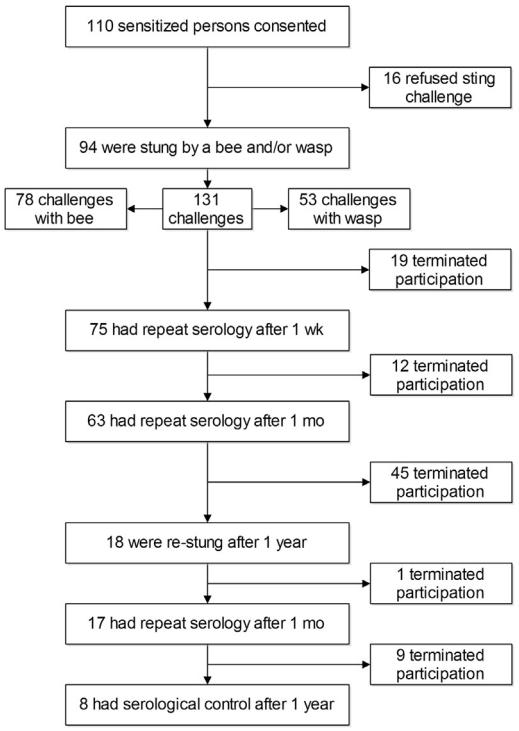

FIG 1. Screening, sting challenges, and follow-up. Initially, 110 sensitized subjects consented to participate in the study. Control visits were optional, and therefore some subjects discontinued the study prematurely.

BAT

BATs were performed, as previously described.[9,10] In brief, cell samples were analyzed by using 3-color flow cytometry (FC 500; Beckman Coulter, Fullerton, Calif). Basophils were identified as a single population of cells that stained positively for CD123 (FL-2) and negatively for HLA-DR (FL-4). Upregulation of CD63 expression was indicated by an increase in fluorescence in the FL-1 channel. Acquisition was terminated after 500 basophil target events. A 2.5-fold increase in the number of activated basophils (>25%) compared with the negative control (10%) at any of the test concentrations of the allergen was considered a positive response.

Deliberate sting challenge test

The insect sting challenge test was carried out with a definitely identified living bee (*Apis mellifera*) and/or wasp (*Vespula germanica* and *Vespula vulgaris*) supplied by the Institute of Zoology, Graz, Austria. The choice of the insect or insects was based on detectable sIgE with the CAP system. The challenge was performed on the upper forearm under partial inpatient conditions with intensive medical stand-by and a continuous infusion; it was considered valid if a wheal of 5 mm or greater in diameter and erythema after 15 minutes at the site of the sting occurred.

Data analysis

All data are expressed as medians (25% to 75% percentiles) on the raw scale, unless otherwise indicated. Data were tested for normality by using the Kolmogorov-Smirnov test. Continuous variables were analyzed by using the Kruskal-Wallis test; categorical variables were compared by using χ^2 or Fisher exact tests. The Cohen κ coefficient was calculated to check agreement