

BRIEF COMMUNICATION

Comparable profiles of serum histamine and IgG4 levels in allergic beekeepers

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Keywords

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Abstract

Growing evidence identifies histamine as a key player in allergic responses, but the reports relating serum histamine to tolerance are scarce and inconclusive. This study investigated the relationship of circulating histamine to the tolerant phenotype in allergic beekeepers. The results showed a positive correlation between the serum levels of histamine and specific immunoglobulin G4 (sIgG4) to honeybee venom, but not with total IgE or sIgE. Interestingly, both sIgG4 and histamine levels were negatively correlated with the time since the last bee sting. In contrast to total IgE and sIgE, serum sIgG4 and histamine levels followed comparable patterns, being higher in tolerant/recently stung individuals and lower in the least frequently stung subgroup. The data obtained in this pilot study associated, for the first time, serum histamine levels with allergen tolerance in allergic individuals and provided the lead for further considering the putative immunomodulatory properties of histamine in allergic responses.

Immunoglobulin G4 (IgG4) is considered as an indicator of tolerance in allergic individuals. Although specific IgG4 (sIgG4) and sIgE are frequently investigated to confirm a history of allergic reaction and to assess tolerance to allergen exposure, these factors have a poor overall predictive value (1). The complexity of mechanisms underlying tolerance in allergic individuals cannot be fully explained by the existing data, and additional and/or complementary components that may contribute to the protective phenotype have yet to be established. In this regard, growing evidence identifies histamine as a key player in the immune responses that drive chronic inflammation (2–5). Acting via four receptors, designated as H₁–H₄, histamine modulates immunocompetent cell activity and promotes peripheral tolerance in allergic and immune responses (2). However, the reports relating basal serum histamine levels to the protective phenotype are scarce and largely inconclusive (6).

Natural exposure of beekeepers to high doses of honeybee venom (HBV) represents a valuable model to understand the pathogenesis of allergic responses and to explore novel mechanisms of allergen tolerance in healthy individuals. Following sensitization and allergy to HBV, beekeepers may

subsequently outgrow their allergy (7). However, the underlying mechanisms remain largely elusive, and diagnostic biomarkers that would distinguish between tolerant and nontolerant individuals are not currently available.

In an effort to dissect the contribution of histamine to the tolerant phenotype and to consider its prognostic relevance, this pilot study sought to examine the association between serum levels of histamine, HBV sIgG4, and sIgE in tolerant and nontolerant allergic beekeepers.

Methods

Detailed experimental methods are given in Supporting Information. The demographic and clinical data of the study population are summarized in Table S1.

Results

Eight (73%) of the 11 beekeepers outgrew their allergy during beekeeping (tolerant beekeepers). The distribution of age, years of beekeeping, age of first reaction, period since the last bee sting (BS), and severity of the allergic response was identical

across subgroups (Table S2). Neither total IgE nor sIgE levels were significantly different between the subgroups of the study population (Table S3). Interestingly, serum histamine was positively correlated with HBV sIgG4 (Fig. 1A), but not with total IgE ($\rho = 0.219$, $P > 0.05$) or HBV sIgE ($\rho = 0.144$, $P > 0.05$). Moreover, serum sIgG4 (Fig. 1B) and histamine (Fig. 1C) levels were significantly higher in tolerant compared to nontolerant subjects and in the recent compared to the past BS subgroup

(Table S3). Both sIgG4 ($\rho = -0.818$, $P < 0.01$) and histamine ($\rho = -0.560$, $P = 0.073$) levels were negatively correlated with the period of time since the last BS. Furthermore, the profiles of both variables were similar, being higher in the tolerant/recent BS subgroup, followed by the tolerant/past BS and the nontolerant/past BS subgroups (Fig. 2A, B). Another factor that may have shaped the parallel increases in serum sIgG4 and histamine levels, and hence tolerance to HBV, would be the BS frequency.

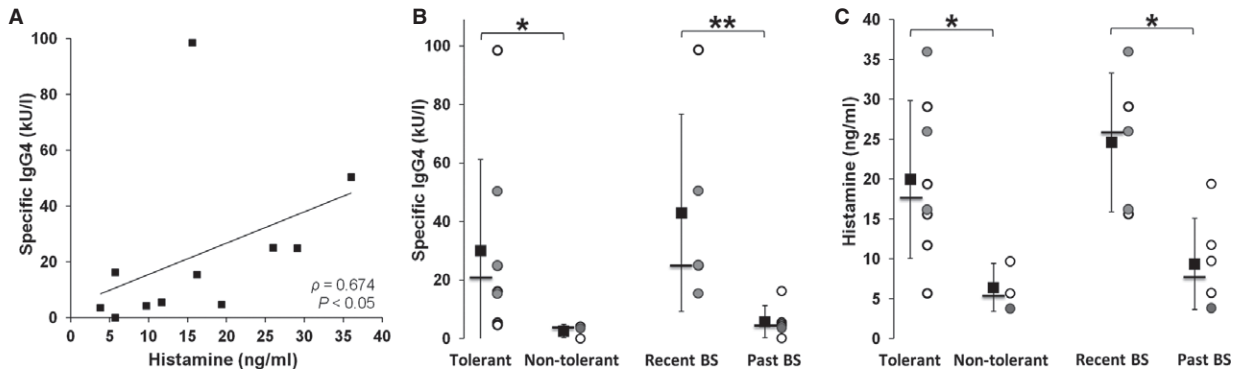


Figure 1 Specific IgG4 (sIgG4) to honeybee venom (HBV) and histamine levels in the serum of beekeepers. (A) Correlation between serum levels of sIgG4 and histamine. ρ , Spearman's rank correlation coefficient ($n = 11$). Distribution of serum levels of (B) sIgG4 to HBV and (C) histamine in tolerant ($n = 8$) and nontolerant ($n = 3$) and in recent ($n = 5$) and past ($n = 6$) bee stung (BS) subgroups of

beekeepers. Data are shown as mean $\pm$ SD (closed squares) and median (horizontal lines). Scattered points represent individual values in the serum of male (closed circles) and female (open circles) subjects. * $P < 0.05$, ** $P < 0.01$ between the indicated subgroups (Mann–Whitney U -test).

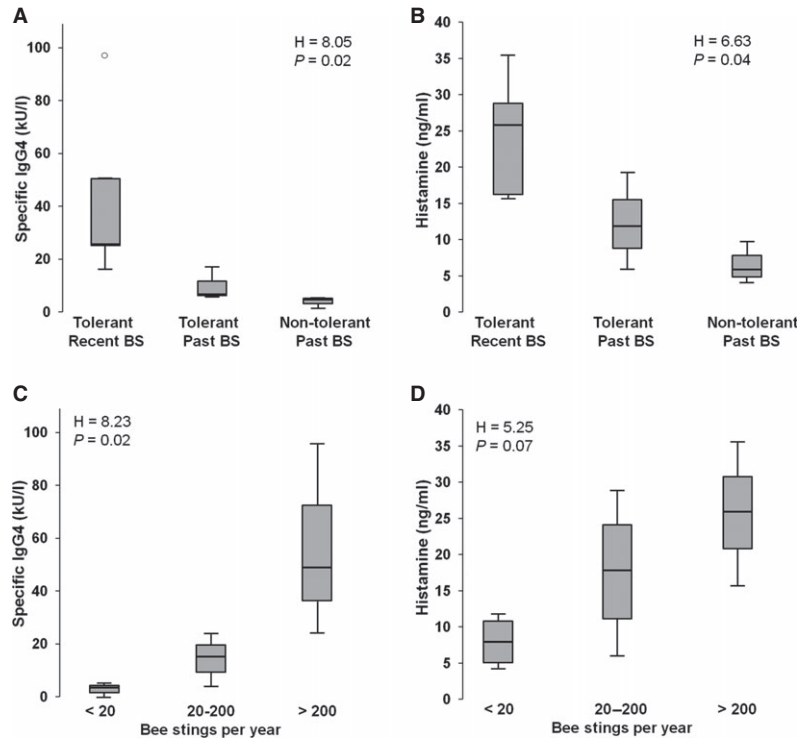


Figure 2 Boxplots showing the comparable profiles of specific IgG4 to honeybee venom and histamine in the serum of beekeeper subgroups. Subgroups were defined according to the (A, B) toler-

ant/nontolerant phenotype and the time since the last bee sting (BS), and (C, D) frequencies if annual BS. P , significance determined by the Kruskal–Wallis H -test (2 d.f.).

Notably, both serum sIgG4 ($p = 0.905$, $P < 0.001$) and histamine ($p = 0.714$, $P < 0.05$) levels were positively correlated with the frequency of annual BS. Significant differences in serum sIgG4 levels (Fig. 2C) and a marginally significant difference in histamine levels (Fig. 2D) were observed among the subgroups that reported low, medium, or high frequencies of annual BS. On the contrary, no such differences were obtained for either sIgE or total IgE levels (Fig. S1).

Discussion

The data provide first evidence for the comparable profiles of histamine and HBV sIgG4 levels in the serum of allergic beekeepers, thus associating high basal circulating histamine with the tolerant phenotype and, ultimately, with the putative anti-inflammatory mechanisms that may provide protection against allergic responses.

Clinical tolerance involving increased IgG4 seems to develop upon high allergen exposure in beekeepers (8, 9). Interestingly, histamine levels were associated not only to serum HBV sIgG4 but also to the frequency of annual BS. The correlation between the protective HBV sIgG4 antibodies and BS frequency is in accordance with previous studies in beekeepers (10) and in patients undergoing allergen-specific immunotherapy (11). Considering that increases in sIgG4 and histamine levels may reflect protection against HBV, it can be suggested that, along with the tolerogenic IgG4, histamine-mediated pathways may drive the tolerant phenotype in individuals who had experienced an allergic reaction to HBV in the past and continue to be heavily exposed to the allergen.

Despite the recognized histamine-mediated pleiotropic actions, the mechanisms underlying its association with sIgG4 and tolerance are far from clear. Regarding HBV allergy, H_2 receptor up-regulation on specific Th2 cells was shown to suppress allergen-stimulated T cells and to increase IL-10 secretion upon high dose allergen exposure, thus promoting the development of peripheral tolerance and contributing to immunomodulation (9). Moreover, during venom immunotherapy, H_2 and H_4 receptor mRNA expression was up-regulated in basophils (12), whereas H_1 receptor antagonists enhanced the efficacy and prevented H_2 receptor up-regulation in allergen-specific T cells (11, 13). Although these reports provide evidence on the contribution of histamine in allergen-specific immune tolerance, decisive data on serum basal histamine levels in allergic and tolerant individuals are virtually lacking. Currently, it is hard to determine the source of the increased basal levels of circulating histamine in tolerant beekeepers. Without excluding the contribution of non-professional histamine-producing peripheral blood cells,

altered histamine metabolism and release from basophils and mast cells might be an obvious suggestion, yet not reasonably substantiated by the available literature (6, 14).

Despite the small sample size, this preliminary study associates for the first time serum histamine levels with tolerance to HBV, thus reflecting yet elusive complex immune interactions that may drive atopic diseases. Moreover, it provides the lead for further considering, in large cohort studies, the potential exploitation of serum histamine quantification as a prognostic indicator that would facilitate optimal allergic disease management.

Author contributions

C.C. designed and conducted the study, performed the data analysis and interpretation, drafted the manuscript, and approved the final version to be published. X.A. and M.M. performed clinical evaluations, revised the article, and approved the final version to be published. A.K. and D.R. revised the article and approved the final version to be published. E.T. conceived, designed and guided the study, the data analysis and interpretation, critically revised the manuscript, and approved the final version to be published.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Methods S1. Materials and methods.

Table S1. Demographic and clinical data of the study population.

Table S2. Distribution of the participants' characteristics across the studied population subgroups presented as P values for exact significance (Mann–Whitney U -test).

Table S3. Serum levels of total IgE, specific IgE and IgG4 to honey bee venom and histamine in beekeeper subgroups.

Figure S1. Boxplots showing the profiles of specific IgE to honey bee venom and total IgE in the serum of beekeeper subgroups.

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Subjects and methods

Subjects

The study was conducted in Greek beekeepers engaged in apiculture professionally or amateurishly that were members of regional beekeepers' associations. The procedures were performed between 10.00 am to 13.00 pm from December to February, which is the low-exposure season for the Greek beekeepers. The participants completed a questionnaire providing data on the number of years engaged in beekeeping, stings per year, age of first reaction, period since last bee sting (BS), medication use and history of atopic or any other disease. The severity of the reported sting reaction was estimated by an allergist according to Müller grading system (1) based on the related information provided by the participants.

Out of 372 beekeepers that were questioned, 64 had a history of sting allergy. Eleven volunteers (mean age = 42 y, range = 29–54 y; 4 male and 7 female; 8 professional and 3 amateur; average beekeeping time = 19 y, range = 2–39 y) were selected according to the following inclusion criteria. They had a definite history of grade I to IV allergic reaction to BS (1), and they had either outgrown their allergy (tolerant beekeepers) or experienced an allergic reaction (non-tolerant beekeepers) after the last BS. Pregnant women and beekeepers experiencing a BS on the day of the procedure were excluded from the study to avoid any interference in the determination of basal histamine levels due to the induction of histamine release. The participants were medication-free, with no comorbid conditions and they came from northern (Thessaloniki, Drama, Xanthi) and southern Greece (Attica, Euboea, Thebes, Corinth) to ensure the most representative geographic distribution of the sample possible. All participants who fulfilled the inclusion criteria were included in this report. All procedures complied with the Greek ethical laws; they were performed with the written informed consent of the participants and approved by the local ethics committee.

The demographic and clinical data of the population are summarized in Table S1. All subjects had experienced an allergic reaction after a sting in the past. They all had negative skin prick tests (SPTs) to inhalant allergens and to HBV, whereas 4 (36%) participants had positive intradermal tests (IDs) with 0.1µg/ml HBV extract. sIgE to HBV was > 0.35kU/L in all beekeepers. Three (27%) subjects reported a history of atopic dermatitis in childhood, but the SPTs with major inhalant allergens and peanut were negative at the time of testing.

Eight (73%) subjects outgrew their allergy during beekeeping and they reported subsequent BS without experiencing a reaction (tolerant beekeepers). Three (37.5%) of the tolerant beekeepers reported a high frequency of > 200 annual BS, 4 (50%) medium (20–200 BS) frequency and 1 (12.5%) a low frequency of < 20 BS per year. The remaining 3 (27%) participants experienced an allergic reaction after the last BS (non-tolerant), all reporting a low frequency of < 20 annual BS. Five (45%) subjects had been stung over the last 5–40 days prior to blood sampling, whereas 6 (55%) subjects experienced the last BS at least 6 months before the procedure and they were assigned to recent BS

and past BS groups, respectively. All participants of the recent BS group were tolerant, whereas 3 out of 6 (50%) individuals in the past BS group were non-tolerant (Table S1). Non-tolerant beekeepers that were recently stung were not found in the study population.

Skin testing

Skin prick tests (SPTs) were performed with 10 µg/ml purified HBV extract of *Apis mellifera* (Alyostal[®], Stallergenes, Antony Cedex, France). Normal saline (N/S; 0.9% NaCl) and 10 mg/ml histamine hydrochloride (Stallergenes, Antony Cedex France) served as a negative and positive control, respectively. A wheal with mean diameter of at least 3 mm greater than the negative control was considered positive SPT. Subsequently, intradermal (ID) skin tests were performed with 0.05 ml of 0.01 and 0.1 µg/ml purified HBV extract, using 0.05 ml N/S as the negative control. A wheal 3 mm greater than the negative control that occurred within 20 min at the injection site was considered as positive ID test and revealed sensitization to HBV. To assess the allergic status, SPTs with the following common inhalant and food allergens were also performed: house dust mites (*Dermatophagoides pteronyssinus*, *D. farinae*), *Alternaria alternata*, *Parietaria officinalis*, Grass mix, *Cynodon dactylon*, *Olea europaea*, *Artemisia vulgaris*, *Aspergillus* sp. mix, cat, dog and peanut (Alyostal Prick[®] 100 IR-IC/ml, Stallergenes, Antony Cedex, France).

Immunoglobulin and histamine quantification

Whole peripheral blood (8 ml) was collected before the skin testing and centrifuged using standard procedures. The sera were aliquoted and stored at -80 °C until assayed. Serum total IgE, HBV sIgE and sIgG4 antibody levels were measured using the ImmunoCAP system (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. HBV sIgE levels > 0.35 kU/l, were considered positive and revealed sensitization to HBV.

Serum histamine was successively extracted in *n*-butanol, sulphuric acid and *n*-heptane, any residual amounts of histidine being removed by washing in NaCl-saturated NaOH as previously described (2). Histamine levels were quantified fluorophotometrically in duplicate, at 360 nm excitation and 450 nm emission, after condensation with *o*-phthalaldehyde and expressed as ng/mL of serum (2).

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Simple descriptive statistics were used to describe the population. All statistical analyses were carried out using IBM SPSS Statistics version 20. Distribution of the participants' characteristics across groups and comparisons between two groups were conducted using the Wilcoxon-Mann-Whitney *U* test. Multiple comparisons were performed by the Kruskal-Wallis *H* test. Statistical dependence between variables was assessed by the non-

parametric Spearman's rank correlation coefficient (ρ). A P value < 0.05 was considered significant with 95% confidence interval.

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2. Tiligada E, Aslanis D, Delitheos A, Varonos D. Changes in histamine content following pharmacologically-induced mast cell degranulation in the rat conjunctiva. *Pharmacol Res* 2000;**41**:667–670.

Table S1 Demographic and clinical data of the study population.

Patient No	Gender	Age (years)	Region	Years of beekeeping	Professional BK	Stings per year	Last BS	Grade of AR	Tolerant BK	Atopy	Skin test* to HBV
1	F	29	South	25	Yes	<20	Past	I	No	No	Negative
2	F	45	South	26	No	<20	Past	III	Yes	No	Negative
3	F	49	South	19	No	20–200	Past	IV	Yes	No	Positive
4	F	39	North	2	Yes	<20	Past	III	No	Yes	Negative
5	M	33	South	10	Yes	<20	Past	II	No	No	Positive
6	F	40	North	4	Yes	20–200	Past	I	Yes	No	Positive
7	M	46	North	23	Yes	>200	Recent	I	Yes	No	Negative
8	F	54	South	30	Yes	>200	Recent	I	Yes	Yes	Negative
9	M	42	North	5	No	20–200	Recent	I	Yes	No	Negative
10	F	35	South	4	Yes	20–200	Recent	III	Yes	No	Positive
11	M	46	South	39	Yes	>200	Recent	I	Yes	Yes	Negative

AR, allergic reaction; atopy: reported history of atopic dermatitis in childhood; BK, beekeeper; BS, bee sting; HBV, honey bee venom; past: > 6 months since last BS; recent: < 40 days since last BS; grade according to Müller grading system; * both intradermal and skin prick tests were performed, when at least the intradermal test was positive, the skin test was considered as being positive

Table S2 Distribution of the participants' characteristics across the studied population subgroups presented as *P* values for exact significance (Mann Whitney *U* test).

	Gender	Region	Professional BK	Atopy	Skin test* to HBV
Age	0.927	0.927	0.376	0.279	0.527
Years of beekeeping	0.648	0.073	1.000	0.479	0.164
Age of first AR	0.571	1.000	0.095	1.000	0.400
Time since last BS	0.412	0.527	0.776	0.133	0.412
Severity of AR	0.230	0.412	0.279	0.776	0.230

AR, allergic reaction; BK, beekeeper; BS, bee sting; HBV, honey bee venom; * both intradermal and skin prick tests were performed, when at least the intradermal test was positive, the skin test was considered as being positive

Table S3 Serum levels of total IgE, specific IgE and IgG4 to honey bee venom and histamine in beekeeper subgroups.

	Total IgE (kU/l)	P_1	HBV sIgE (kU/l)	P_2	HBV sIgG4 (kU/l)	P_3	Histamine (ng/ml)	P_4	n
Male	325.8 ± 561.7		3.2 ± 3.9		23.6 ± 19.9		20.5 ± 13.7		4
Female	152.3 ± 218.3	0.527	5.6 ± 11.8	0.788	22.0 ± 34.8	0.648	13.8 ± 8.4	0.527	7
Professional	280.9 ± 410.9		5.9 ± 11.0		27.8 ± 32.9		16.4 ± 12.4		8
Amateur	40.8 ± 27.2	0.376	1.5 ± .6	0.776	8.5 ± 5.9	0.630	15.8 ± 3.9	0.776	3
Atopic	119.8 ± 140.5		1.66 ± 1.3		41.2 ± 51.2		15.8 ± 10.1		3
Non-atopic	251.2 ± 419.5	> 0.9	5.9 ± 11.0	0.776	15.6 ± 16.0	0.630	16.4 ± 11.3	0.921	8
Tolerant	141.7 ± 203.9		5.3 ± 10.9		30.0 ± 31.2		20.0 ± 9.9		8
Non-tolerant	411.9 ± 655.3	> 0.9	3.2 ± 4.9	0.630	2.6 ± 2.2	0.012	6.4 ± 3.0	0.024	3
Recent BS	202.9 ± 244.9		7.6 ± 13.8		42.2 ± 33.7		24.6 ± 8.7		5
Past BS	225.8 ± 462.2	0.329	2.3 ± 3.3	0.662	5.6 ± 5.4	0.009	9.3 ± 5.7	0.017	6
Total	215.4 ± 361.8		4.7 ± 9.5		22.6 ± 29.1		16.3 ± 10.5		11

BS, bee sting; HBV, honey bee venom; Ig, immunoglobulin; n: number of subjects in the group; P_1 , P_2 , P_3 , P_4 , exact significance between subgroups for total IgE, HBV sIgE, HBV sIgG4 and histamine values, respectively, determined by the non-parametric Mann Whitney *U* test (95% confidence interval); sIg, specific Ig to HBV.

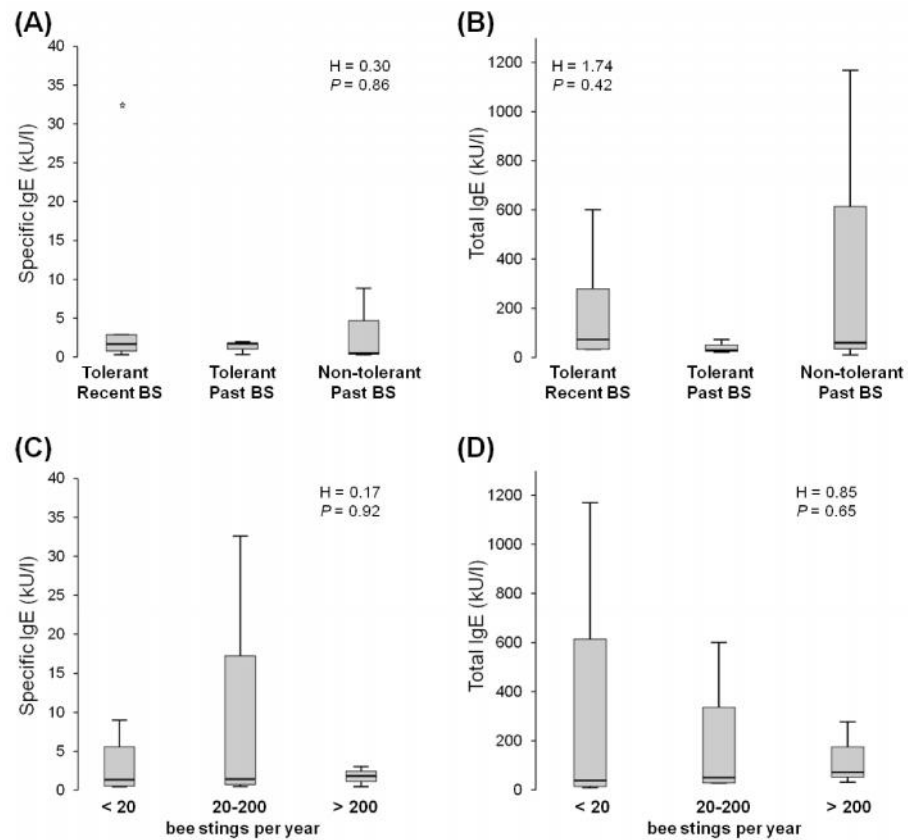


Figure S1 Boxplots showing the profiles of specific IgE to honey bee venom and total IgE in the serum of beekeeper subgroups. Subgroups were defined according to the (A, B) tolerant/non-tolerant phenotype and the time since the last bee sting (BS), and (C, D) frequencies of annual BS. P , significance determined by the Kruskal-Wallis H test (2 d.f.).

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Table S1 Demographic and clinical data of the study population.

Patient No	Gender	Age (years)	Region	Years of beekeeping	Professional BK	Stings per year	Last BS	Grade of AR	Tolerant BK	Atopy	Skin test* to HBV
1	F	29	South	25	Yes	<20	Past	I	No	No	Negative
2	F	45	South	26	No	<20	Past	III	Yes	No	Negative
3	F	49	South	19	No	20–200	Past	IV	Yes	No	Positive
4	F	39	North	2	Yes	<20	Past	III	No	Yes	Negative
5	M	33	South	10	Yes	<20	Past	II	No	No	Positive
6	F	40	North	4	Yes	20–200	Past	I	Yes	No	Positive
7	M	46	North	23	Yes	>200	Recent	I	Yes	No	Negative
8	F	54	South	30	Yes	>200	Recent	I	Yes	Yes	Negative
9	M	42	North	5	No	20–200	Recent	I	Yes	No	Negative
10	F	35	South	4	Yes	20–200	Recent	III	Yes	No	Positive
11	M	46	South	39	Yes	>200	Recent	I	Yes	Yes	Negative

AR, allergic reaction; atopy: reported history of atopic dermatitis in childhood; BK, beekeeper; BS, bee sting; HBV, honey bee venom; past: > 6 months since last BS; recent: < 40 days since last BS; grade according to Müller grading system; * both intradermal and skin prick tests were performed, when at least the intradermal test was positive, the skin test was considered as being positive

Table S2 Distribution of the participants' characteristics across the studied population subgroups presented as *P* values for exact significance (Mann Whitney *U* test).

	Gender	Region	Professional BK	Atopy	Skin test* to HBV
Age	0.927	0.927	0.376	0.279	0.527
Years of beekeeping	0.648	0.073	1.000	0.479	0.164
Age of first AR	0.571	1.000	0.095	1.000	0.400
Time since last BS	0.412	0.527	0.776	0.133	0.412
Severity of AR	0.230	0.412	0.279	0.776	0.230

AR, allergic reaction; BK, beekeeper; BS, bee sting; HBV, honey bee venom; * both intradermal and skin prick tests were performed, when at least the intradermal test was positive, the skin test was considered as being positive

Table S3 Serum levels of total IgE, specific IgE and IgG4 to honey bee venom and histamine in beekeeper subgroups.

	Total IgE (kU/l)	P_1	HBV sIgE (kU/l)	P_2	HBV sIgG4 (kU/l)	P_3	Histamine (ng/ml)	P_4	n
Male	325.8 ± 561.7		3.2 ± 3.9		23.6 ± 19.9		20.5 ± 13.7		4
Female	152.3 ± 218.3	0.527	5.6 ± 11.8	0.788	22.0 ± 34.8	0.648	13.8 ± 8.4	0.527	7
Professional	280.9 ± 410.9		5.9 ± 11.0		27.8 ± 32.9		16.4 ± 12.4		8
Amateur	40.8 ± 27.2	0.376	1.5 ± .6	0.776	8.5 ± 5.9	0.630	15.8 ± 3.9	0.776	3
Atopic	119.8 ± 140.5		1.66 ± 1.3		41.2 ± 51.2		15.8 ± 10.1		3
Non-atopic	251.2 ± 419.5	> 0.9	5.9 ± 11.0	0.776	15.6 ± 16.0	0.630	16.4 ± 11.3	0.921	8
Tolerant	141.7 ± 203.9		5.3 ± 10.9		30.0 ± 31.2		20.0 ± 9.9		8
Non-tolerant	411.9 ± 655.3	> 0.9	3.2 ± 4.9	0.630	2.6 ± 2.2	0.012	6.4 ± 3.0	0.024	3
Recent BS	202.9 ± 244.9		7.6 ± 13.8		42.2 ± 33.7		24.6 ± 8.7		5
Past BS	225.8 ± 462.2	0.329	2.3 ± 3.3	0.662	5.6 ± 5.4	0.009	9.3 ± 5.7	0.017	6
Total	215.4 ± 361.8		4.7 ± 9.5		22.6 ± 29.1		16.3 ± 10.5		11

BS, bee sting; HBV, honey bee venom; Ig, immunoglobulin; n: number of subjects in the group; P_1 , P_2 , P_3 , P_4 , exact significance between subgroups for total IgE, HBV sIgE, HBV sIgG4 and histamine values, respectively, determined by the non-parametric Mann Whitney *U* test (95% confidence interval); sIg, specific Ig to HBV.

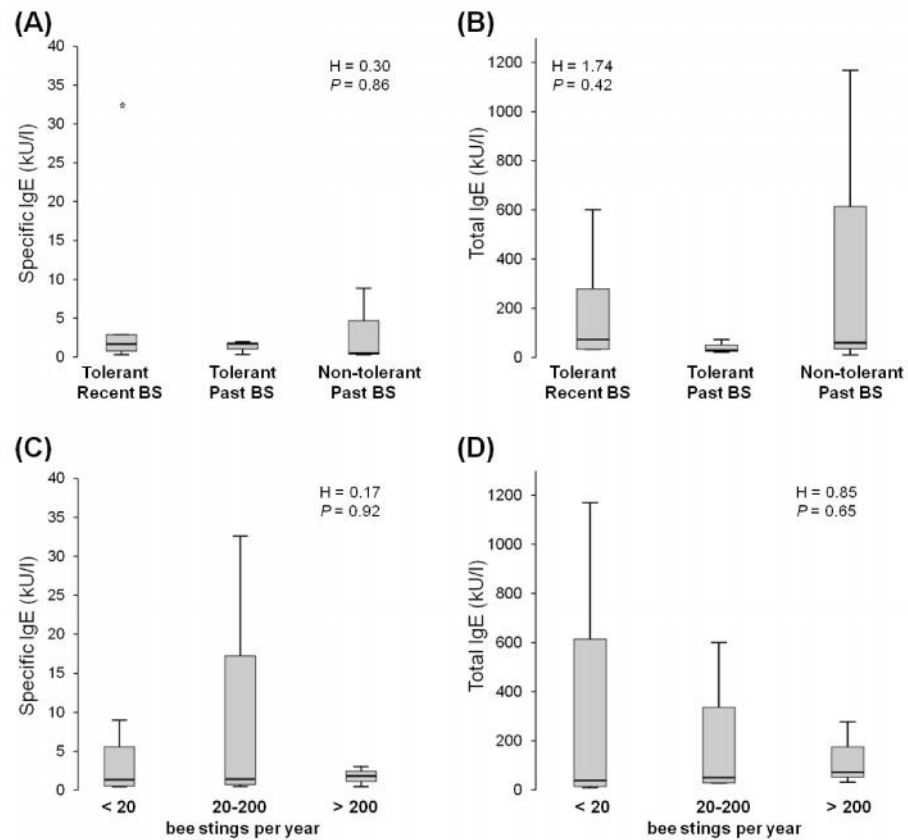


Figure S1 Boxplots showing the profiles of specific IgE to honey bee venom and total IgE in the serum of beekeeper subgroups. Subgroups were defined according to the (A, B) tolerant/non-tolerant phenotype and the time since the last bee sting (BS), and (C, D) frequencies of annual BS. P , significance determined by the Kruskal-Wallis H test (2 d.f.).