ORIGINAL ARTICLE

Helios-Negative Regulatory T Cells as a Key Factor of Immune Tolerance in Nonallergic Beekeepers

Ruiz-Leon B[1,2,3]*, Navas A[1,2]*, Serrano P[1,2,3], Espinazo M[1,3], Guler I[1], Alonso C[1,2,3], Jurado A[1,2,3], Moreno-Aguilar C[1,2,3]

[1]Maimonides Biomedical Research Institute of Cordoba (IMIBIC)/Reina Sofia University Hospital/University of Cordoba, Cordoba, Spain
[2]Department of Immunology and Allergy, Reina Sofia University Hospital, Cordoba, Spain
[3]National Network ARADyAL. Health Institute Carlos III, Madrid, Spain
*Both authors contributed equally

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Abstract

Background: Although exposure to stings has been identified as the leading risk factor for anaphylaxis due to Hymenoptera venom allergy, professional beekeepers receive hundreds of stings yearly without developing systemic reactions.
Objective: This study aims to analyze the mechanisms underlying bee venom tolerance in beekeepers.
Methods: A cross-sectional study was conducted. Participants were recruited and classified into 3 groups: allergic patients (APs), who experienced systemic reactions after bee stings, with a positive intradermal test and specific IgE (sIgE) to *Apis mellifera* venom (AmV); tolerant beekeepers (TBKs), who received ≥50 stings/year; and healthy nonexposed controls (HCs). We measured serum levels of sIgE and specific IgG4 (sIgG4) to AmV, rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5, and rApi m10, as well as AmV-induced basophil degranulation, percentage of T-cell subsets, regulatory T cells (Treg), and IL-10 production.
Results: Compared with TBKs, APs had high levels of sIgE to AmV and all its allergic components (*P*<.001), together with a high basophil activation rate (*P*<.001). Conversely, compared with APs, TBKs had higher levels of sIgG4 (*P*<.001) and IL-10 (*P*<.0001), as well as an enhanced CTLA-4+ Treg population (*P*=.001), expanded Helios$^-$ Treg (*P*<.003), and reduced type 1 helper T cells (T_H1) (*P*=.008), T_H2 (*P*=.004), and T_H17 (*P*=.007) subsets.
Conclusions: The profile of TBKs, which was strongly marked by Treg activity, differed from that of TBKs. This natural tolerance would be led by the expansion of inducible Helios$^-$ Treg cells at the peripheral level. The Helios$^-$ Treg population could be a novel candidate biomarker for monitoring tolerance.

Key words: Helios protein. Regulatory T cells. Immune tolerance. Bee venom allergy. Beekeeping.

Resumen

Antecedentes: Aunque la exposición a las picaduras ha sido identificada como el principal factor de riesgo en la anafilaxia debido a himenópteros, los apicultores profesionales sufren cientos de ellas al año sin desarrollar reacciones sistémicas.
Objetivo: Analizar los mecanismos de tolerancia al veneno de abeja en apicultores.
Métodos: Se realizó un estudio transversal. Los participantes se clasificaron en tres grupos: pacientes alérgicos (AP) que habían sufrido reacciones sistémicas tras la picadura de abeja, con pruebas cutáneas positivas e IgE específica (sIgE) frente al veneno de *Apis mellifera* (AmV); apicultores tolerantes (TBK) que hubiesen recibido ≥50 picaduras/año; y controles sanos no expuestos (HC). Se determinaron los niveles séricos de sIgE e IgG4 específica (sIgG4) frente a AmV, rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5 and rApi m10, así como la desgranulación de los basófilos inducida por AmV, el porcentaje de subpoblaciones de células T, células T reguladoras (Treg) y la producción de IL-10.
Resultados: En comparación con los TBKs, los APs presentaron niveles elevados de sIgE a AmV y todos sus componentes alergénicos (p<0,001), junto con una elevada tasa de activación de basófilos (p<0,001). Por el contrario, en comparación con los APs, los TBKs tenían niveles más elevados de sIgG4 (p<0,001) e IL-10 (p<0,001), así como aumento de las poblaciones Treg CTLA-4$^+$ (p=0,001), Treg Helios- (p<0,003), y una reducción de las subpoblaciones T-helper 1 (p=0,008), T-helper 2 (p=0,004) y T-helper 17 (p=0,007).
Conclusiones: En los TBKs se encontró un perfil diferente marcadamente caracterizado por la actividad T reguladora. Esta tolerancia natural podría conducir a la expansión de células Treg Helios- inducibles a nivel periférico. La población Treg Helios- podría constituir un novedoso biomarcador candidato, útil en la monitorización de la tolerancia.

Palabras clave: Proteína Helios. Células T reguladoras. Tolerancia inmunológica. Alergia al veneno de abeja. Apicultura.

Background

A single sting from a hymenopteran (bees and wasps) can induce an extremely severe and potentially life-threatening allergic reaction in sensitized individuals [1,2]. Around 0.3% to 8.9% of the population can experience systemic adverse events secondary to the allergic mechanisms triggered by a hymenopteran sting [3,4]. In contrast with observations in other allergic diseases, atopy is not a requirement for allergy to these insects, with the number of stings being one of the main risk factors identified [5]. Therefore, the unavoidable exposure to stings in some professions implies an element of susceptibility to systemic reactions, thus reducing the work opportunities of allergic individuals [6]. Beekeeping—a form of small livestock farming where several individuals, habitually from the same family, work together to perform highly qualified and seasonal tasks [7]—is undoubtedly the socioeconomic sector most affected by this health problem.

Many professional beekeepers receive hundreds of bee stings every year without developing systemic reactions [6]. As such, they represent a valuable human in vivo model for exploring the mechanisms of acquisition of tolerance to allergens, because of their natural exposure to high doses of bee venom. This particular type of exposure has been previously reported to naturally favor the appearance of high levels of specific IgG4 (sIgG4) to *Apis mellifera* venom (AmV) [8], although the underlying causes of naturally acquired immunity have not yet been completely clarified.

Allergen-specific immunotherapy has been shown to induce basophil and mast cell desensitization, regulation of IgE-IgG4 secretion and generation of regulatory T (Treg) cells, resulting in suppression of effector type 1 helper T cells (T_H1), T_H2, and T_H17, which, respectively, produce large amounts of INF-γ, IL-4, and IL-17 [9]. Additionally, the suppression of inflammatory cytokines from dendritic cells is thought to play a role in the constellation of changes derived from Treg enhancement [10].

The immune status of tolerant beekeepers resembles that of bee venom–allergic individuals who have achieved protection through *A mellifera* immunotherapy (AmIT). This similarity suggests that the immunological phenomena inherent to T-cell regulation, probably induced by AmIT, could also be involved in the natural tolerance acquired by beekeepers. Since it has recently been reported that there are different forms of AmV allergy according to the sensitization profile in terms of individual allergenic components present in the venom [11,12], the sensitization profile could be of interest when attempting to identify different patterns of protective immune response in the natural environment. In any case, there are no reliable biomarkers to identify the risk of systemic reactions after a bee sting or to assess the real level of protection of exposed individuals, given that the sIgG4 level is considered a biomarker of exposure only and not of a protective effect [9].

Considering this background, the aim of this study was to analyze tolerance and allergy based on classical parameters as well as unpublished ones in a group of beekeepers who do not experience systemic reactions and in a group of patients who develop anaphylaxis secondary to bee sting venom. We examined the possible immunological differences between the groups and the putative underlying immune tolerance mechanisms in healthy beekeepers exposed to bee stings to identify a reliable marker of protection that could prove suitable for translating research findings into clinical practice.

Methods

The Methods section of the supplementary material provides full details about the skin test procedure, total IgE levels, sIgE and sIgG4 levels, tryptase levels, the basophil activation test results, identification of $T_H1/T_H2/T_H17$ cell subpopulations, identification of regulatory T-cell subpopulations, IL-4 and IL-10 production, and statistics.

Study Design

A total of 54 individuals aged ≥18 years were included in this cross-sectional observational study at the Department of Immunology and Allergy at Reina Sofia University Hospital, Córdoba, Spain. Participants were stratified into 3 groups according to the following criteria:

- Allergic patients (APs, n=20): patients with (*a*) at least 1 episode of anaphylaxis after a bee sting, (*b*) a positive intradermal response to AmV at a concentration of ≤0.1 μg/mL, (*c*) levels of serum specific IgE (sIgE) to AmV >0.35 kU/L, (*d*) no previous AmIT, and (*e*) consecutive visits to our clinic between January and December 2016.
- Tolerant beekeepers (TBKs, n=17): active beekeepers reporting more than 50 stings per year for more than 10 years without experiencing extensive local or systemic reactions. These persons were recruited outside the beekeeping season.
- Healthy controls (HCs, n=17): nonallergic individuals not exposed to bee stings.

All study participants underwent a physical examination and were given a structured questionnaire to identify their geographic area and determine the number and severity of systemic episodes after stings [13].

This study was approved by the Ethics Committee of Reina Sofía Hospital (reference number FCO-VAC-2015-01). All the participants provided their written informed consent.

Results

Characteristics of the Study Population

Of the 54 individuals included, 35 (64.8%) were men and 19 (35.1%) were women. Patient ages ranged from 18 to 68 years (median 43). No patients had an immune disease. Distribution of the variables age, sex, geographic location, and total serum IgE level was non-Gaussian in all 3 groups (APs, TBKs, and HCs). We found a significantly higher proportion of males in the AP group (80.0%) and TBK group (82.4%) than in the HC group (29.4%; *P*=.0010). Therefore, a comparison of the variables analyzed in APs and TBKs stratified by sex did not reveal intragroup differences. Compared with HCs, APs and TBKs usually lived in rural locations (*P*=.00279). In the AP

group, 7 patients (35.0%) were diagnosed with grade 2 severity systemic allergic reactions, and 13 (65.0%) were diagnosed with grade 3 severity systemic allergic reactions. The mean (SD) baseline serum tryptase of APs was 4.65 (2.52) µg/L.

Two patients were found to have a REMA score ≥2 and were diagnosed with indolent systemic mastocytosis. No statistically significant differences were found between the groups in terms of the other study characteristics shown in the Table. The raw data for all the variables analyzed for each study participant (HCs, APs, and TBKs) are shown in Supplementary Table 1.

Specific IgE and IgG4

sIgE and sIgG4 values for AmV, rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5, and rApi m 10 measured in the 3 groups are shown in Figure 1. Considering a cut-off of 0.35 kU/L, 70.6% of TBKs and 6% of HCs were sensitized to AmV. We found a significantly higher level of sIgE to AmV and its components in the AP group than in the HC group, except for rApi m 2 (*P*=.064; Figure 1C). Even though sIgE values to AmV, rApi m 1, and rApi m 2 were significantly higher in APs than in TBKs (Figure 1A-C), no significant differences were found between levels of sIgE to rApi m 3, Api m 4, rApi m 5, or rApi m 10 (Figure 1D-G). Sensitization to rApi m 1 was most prevalent among APs (75%), followed by sensitization to rApi m 10 (55%), rApi m 5 (50%), Api m 4 (30%), rApi m 2 (15%), and rApi m 3 (15%). We found 13 different sensitization profiles in the allergic population, the most frequent being monosensitization to rApi m 1, followed by sensitization to rApi m 1 + rApi m 5 + rApi m 10 (Figure 2).

Considering sIgG4 to whole AmV and its components, we found that the TBK group exhibited significantly higher levels than HCs and APs (Figure 1A-G). Comparison of APs and HCs revealed no differences except for sIgG4 to AmV and rApi m 1 (Figure 1A and B).

Table. Clinical and Demographic Characteristics of the Study Population

	Allergic patients (n=20)	Tolerant beekeepers (n=17)	Healthy controls (n=17)	*P* Value
Male sex, No. (%)	16 (80.0)	14 (82.4)	5 (29.4)	.0010
Median (max-min) age, y	40.5 (68-18)	48 (64-27)	42 (59-21)	.353
Rural location[a], No. (%)	8 (40.0)	7 (41.2)	0	.00279
Systemic allergic reactions[b]				
Severity grade 2, No. (%)	7 (35.0)	-	-	
Severity grade 3, No. (%)	13 (65.0)	-	-	
Median (max-min) total serum IgE, kU/L	159.8 (9897.5-1.5)	57.4 (1269.7-4.9)	21.5 (1149.2-1.5)	.454

[a]Rural location was considered as one with <10 000 inhabitants.
[b]EAACI 2018 grading [13].

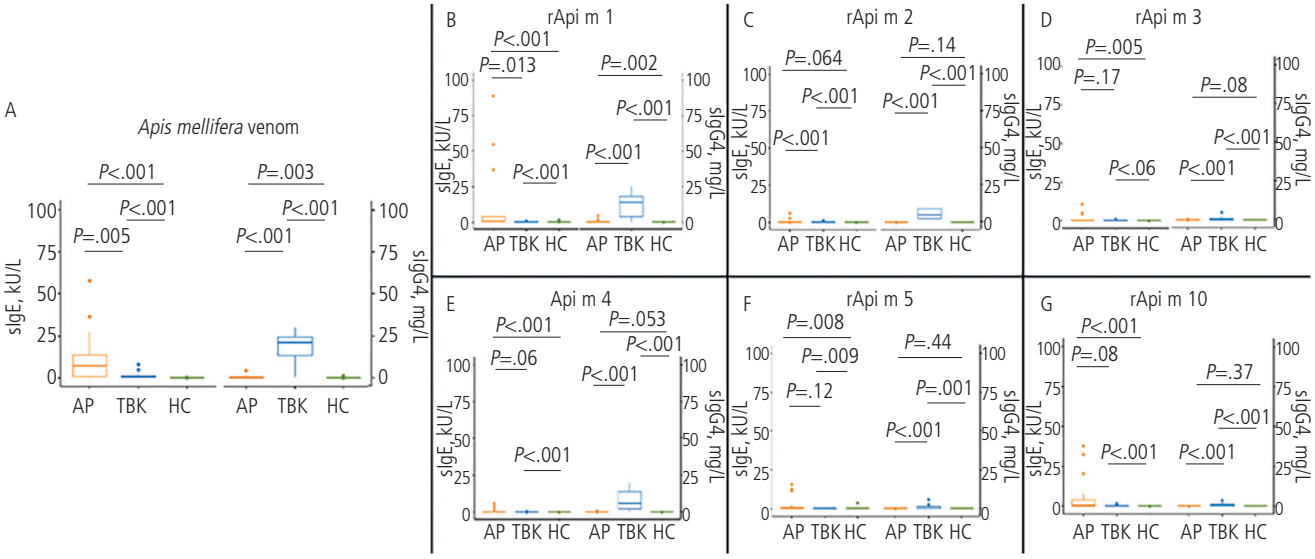

Figure 1. Boxplots for the serum specific IgE level (sIgE) and specific IgG4 level (sIgG4) to whole honeybee venom (*Apis mellifera*) and its components (rApi m1, rApi m2, rApi m3, Api m4, rApi m5, and rApi m10) (A-G) of allergic patients (AP), tolerant beekeepers (TBK), and healthy controls (HC).

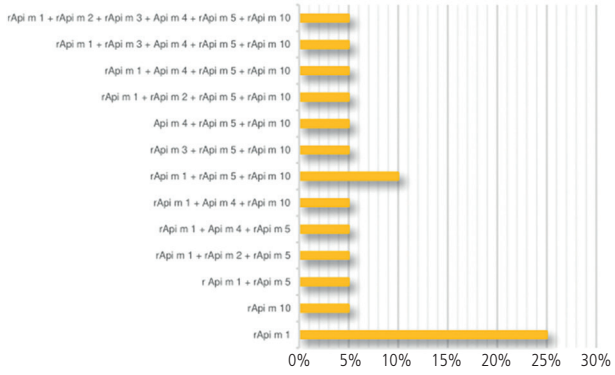

Figure 2. Sensitization profiles found in the allergic patient group according to the serum specific IgE level to each *Apis mellifera* venom component, considering 0.35 kU/L as the positive cut-off value. The proportion of allergic patients exhibiting the identified profiles is displayed.

Basophil Activation Test

When using AmV as a stimulus at a concentration of 0.1 µg/mL (Figure 3A), we found that the percentage of degranulated basophils was higher in the AP group than in the others, reaching statistical significance only in the TBK group (*P*=.024). No differences were found between TBKs and HCs (*P*=.417). However, when AmV was tested at a concentration of 1 µg/mL (Figure 3B), the AP group showed a significantly higher proportion of degranulated basophils than both TBKs (*P*<.001) and HCs (*P*<.001). The proportion of degranulated basophils in the TBK group was also significantly higher than in the HC group (*P*=.038). The correlation between the percentage of basophil degranulation in the TBK group and sIgG4 levels to AmV was significantly negative (*P*=.005), although positive with sIgE levels to AmV (*P*=.020) (Figure 4).

CD4⁺ Lymphocyte Subsets and IL-10 and IL-4 Production

A similar proportion of peripheral Treg cells, defined as $CD4^{+}CD25^{high}CD127^{low}$, was recorded (AP vs TBK, *P*=.93; AP vs HC, *P*=.42; and TBK vs HC, *P*=.58). However, the

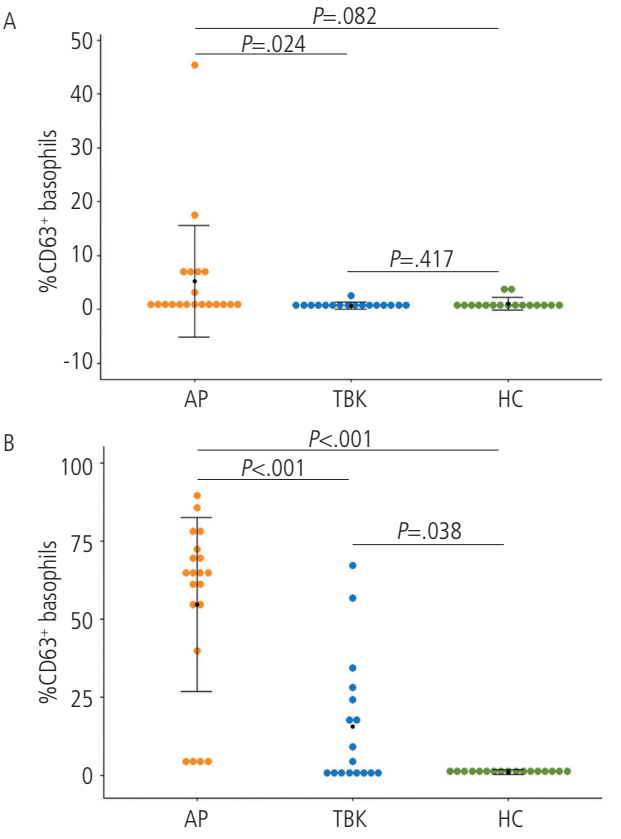

Figure 3. Percentage of degranulated basophils (%$CD63^{+}$) of allergic patients (AP), tolerant beekeepers (TBK), and healthy controls (HC) when using 0.1 µg/mL (A) and 1 µg/mL (B) of whole honeybee venom as a stimulus. Mean and standard error of the mean are shown.

$Helios^{-}$ Treg population was significantly enhanced in the TBK group with respect to the AP group (*P*=.003) and the HC group (*P*=.010; Figure 5A). CTLA-4+ Treg cells (Figure 5B) were significantly increased in the TBK group with respect to APs (*P*<.001) and HCs (*P*=.007). When considering effector T-cells, all T_H1, T_H2, and T_H17 subsets (Figure 5C-E) were significantly reduced in the TBK group with respect to the AP group (T_H1, *P*=.008; T_H2, *P*=.004; and T_H17, *P*=.007) and HC group (T_H1,

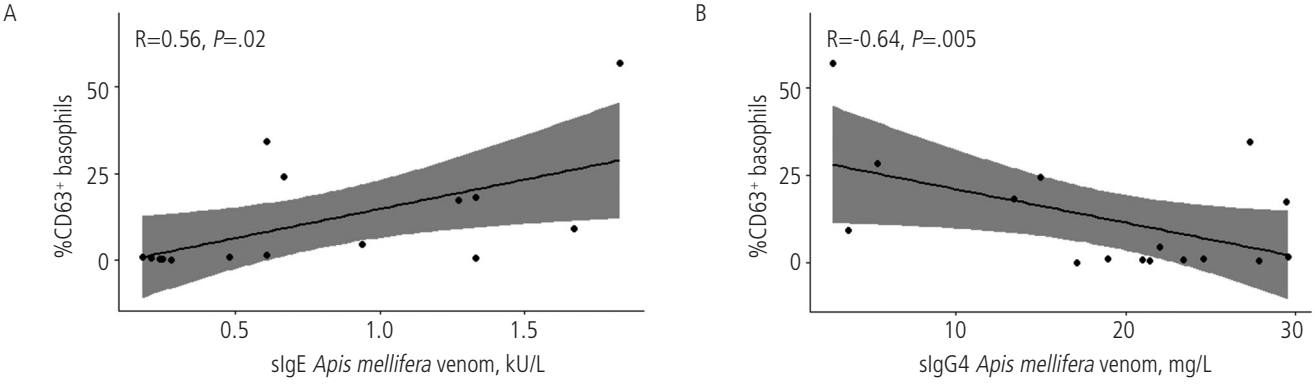

Figure 4. Correlation between the percentage of BAT positivity in tolerant beekeepers and levels of sIgG4 and sIgE. r indicates the Pearson correlation coefficient.

P=.001; T_H2, *P*=.004; and T_H17, *P*=.001). No differences were found for the remaining cell biomarkers studied.

IL-10 levels (Figure 6) were significantly higher in TBKs than in APs (*P*<.001) and tended to be slightly higher than in the HC group (*P*=.069). Similarly, IL-10 tended to be higher in the HC group than in the AP group (*P*=.079). Although IL-4 levels were higher in the AP group than in the TBK and HC groups, no significant differences were found between them (Figure 6).

Definition of Immunological Profiles

Variables identified as candidate biomarkers were classified as allergy-related (sIgE and BAT) and tolerance-related (sIgG4,

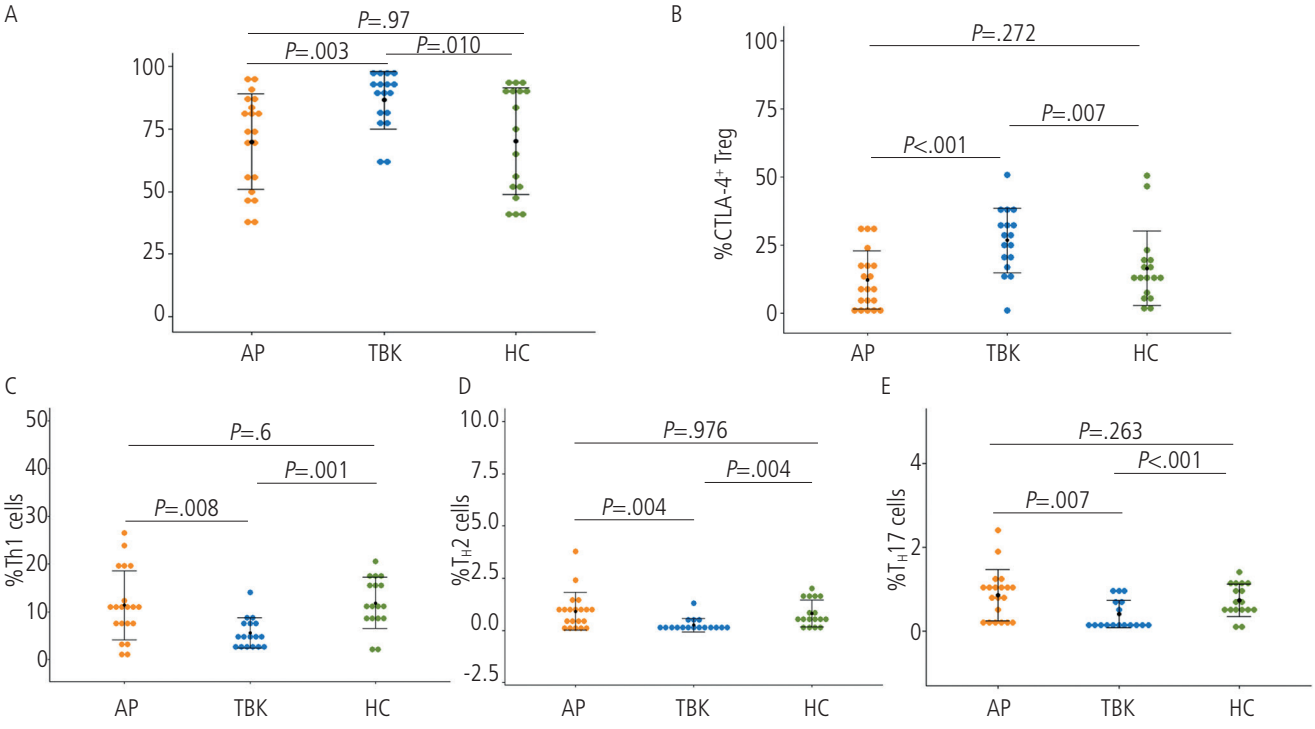

Figure 5. Percentage of Helios– Treg cells (A), CTLA-4+ Treg cells (B), T_H1 (C), T_H2 (D), and T_H17 (E) subsets of allergic patients (AP), tolerant beekeepers (TBK), and healthy controls (HC). Mean and standard error of the mean are displayed.

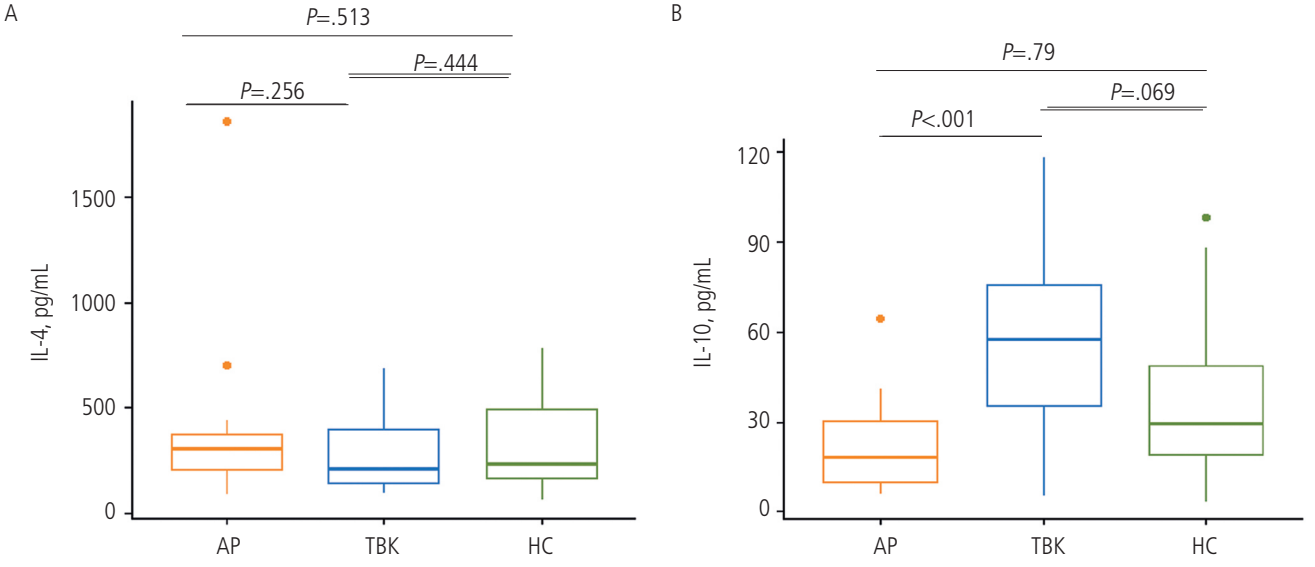

Figure 6. Boxplots for IL-10 and IL-4 levels (pg/mL) quantitated in culture supernatant of allergic patients (AP), tolerant beekeepers (TBK), and healthy controls (HC).

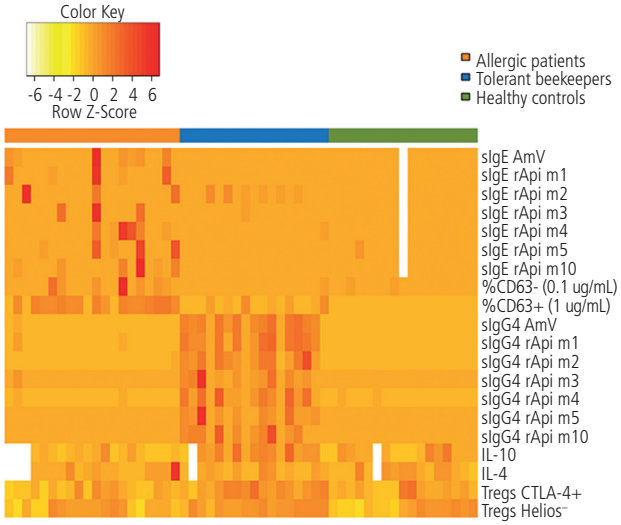

Figure 7. Heatmap including study parameters identified as biomarkers of tolerance. The color scale ranges from red for higher expressions to yellow for lower expressions. Annotations at the top of the heatmap show the study groups. The dendrogram shows clustering of samples (rows), which is based on hierarchical clustering with the Euclidean distance metric and average linkage.

IL-10, Helios⁻ Treg, and CTLA-4+ Treg) and included in a heatmap (Figure 7), where different expression levels were adjusted according to a color scale to display individual behaviors and analyze 2 profiles. In general, TBKs exhibited a high level of sIgG4 to AmV and its components, a significantly expanded CTLA-4⁺ Treg population, expanded Helios⁻ Treg cells, and high levels of IL-10. Conversely, APs showed high levels of sIgE, together with high rates of basophil activation. Individual number 37 must be highlighted as an outlier, owing to the high sIgE/IgE ratio, very low IL-10 levels, and a strongly expanded (98.6%) Helios⁻ Treg subset.

Discussion

Biomarkers of both allergic and immune tolerance responses were evaluated in 2 study populations highly exposed to bee stings: an AP group, who experience anaphylaxis, and TBKs, who receive hundreds of stings each year with no reactions. A nonexposed group of HCs was also analyzed.

The results showed a well-defined regulatory-suppressor profile for the TBK group. This was characterized by expanded proportions of CTLA-4⁺ Treg and Helios⁻ Treg cell populations and ex vivo high rates of basophil activation without clinical symptoms. Additionally, as previously described, TBKs were characterized by increased production of IL-10, increased amounts of sIgG4 to AmV and all its components (rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5, and rApi m 10), reduced numbers of effector T-cell populations (T_H1, T_H2, and T_H17), as well as lower levels of sIgE to bee venom and its allergenic components than APs. None of these findings was present in APs or HCs.

The Helios⁻ Treg count was higher in the TBK group than in the APs and HCs. Helios is a member of the Ikaros transcription factor family and is preferentially expressed at the mRNA level by Treg. It has been shown to identify thymic-derived Treg cells (tTreg), which mediate tolerance to self-antigens. Conversely, peripheral or induced Helios⁻ Treg cells (iTreg) target external antigens [19,20]. Therefore, Helios expression could enable the identification of phenotypically and functionally different Treg populations, with unique nonredundant TCR repertoires aimed at the detection of self- versus non–self-antigens [20-22]. This protein has been proposed as a marker for the distinction between centrally and peripherally induced Treg cells [21]. Our findings are consistent with this hypothesis and suggest that TBKs develop an expanded extrathymic Helios⁻ Treg cell (iTreg) subset because of high antigen exposure. Moreover, the number of stings (hundreds per year in the TBK group) could play a crucial role in developing allergenic tolerance driven by changes in Treg activity in and out of the beekeeping season [23]. The Helios⁻ Treg population could represent the effector subset responsible for suppressing the initially observed allergic response to bee venom, as demonstrated in the case of aeroallergens [24] and food allergens [25]. However, this possibility contrasts with the recently described increase in Treg Helios+ cells due to the immunotherapy against Der p 1 [26]. Furthermore, it has been reported that B-cell regulatory activity is also enhanced owing to the high dose of venom received by beekeepers during the beekeeping season [27].

Almost half of the tolerant beekeepers exhibited high rates of basophil activation, which is consistent with the detection of sIgE levels in this group but raises questions about the mechanisms underlying the absence of symptoms. Interestingly, the basophil activation index in TBKs correlated negatively with sIgG4 levels to AmV (Figure 4). Several of the characteristic protagonists in the TBK group regulatory-suppressor profile may help explain this exciting finding. Indeed, it has been reported that elevated sIgG4 levels in TBKs could impair basophil activation in vivo through cell surface Fcγ receptors [28]; alternatively, a vast repertoire of IgE in the AP group based on different concentrations, affinities, and clonalities could powerfully stimulate basophils [29]. This finding could also be due to a combination of both mechanisms. In any case, the utility of BAT has been widely evaluated using different types of allergens, including bee venom, for monitoring the achievement of tolerance. Despite the decrease with respect to baseline BAT reactivity during immunotherapy, some authors did not report any differences once tolerance had been reached. Thus, Kucera et al [30] found that 56.3% of allergic nonreactors to sting challenge after venom immunotherapy had positive results in BAT. The identification of TBKs who did not exhibit systemic reactions but had positive BAT results is in line with Kucera et al, supporting that the degranulation of basophils is not the best method for evaluating the tolerance acquired either by venom immunotherapy or naturally through high amounts of bee venom exposure.

Patients from the TBK group had high levels of sIgG4 to bee venom and its allergenic components. Similar results have been reported among beekeepers, although details of the underlying mechanism and its origins are not well known [31]. Nonetheless, this elevation is particularly significant in beekeepers tolerant to stings [8,32]. IgG4 plays a role as a

biomarker of exposure, but sIgG4 levels are not a reliable marker of individual tolerance, and simultaneous elevation of sIgE and sIgG4 to AmV may also occur [33]. However, the protective role of sIgG4 in allergic diseases by inhibiting mast cell degranulation has been demonstrated [34]. In addition to FcεRI, basophils and mast cells express the FcγRII receptor [35]. Of all the IgG subclasses, IgG4 has the highest affinity for the FcγRIIb inhibitor receptor [36]. Coaggregation of FcγRIIa induces basophil degranulation, but coaggregation of FcεRI and FcγRIIb through binding to IgE and IgG4 immune complexes can inhibit mast cell degranulation [37]. Additionally, IgG4 can inhibit the degranulation of mast cells and basophils by behaving as a blocking antibody, competing with IgE for allergen binding [35]. Grass pollen–specific IgG4 antibodies from a patient who had received immunotherapy inhibited the activation of basophils by blocking the interaction between the allergen and IgE [38]. Similarly, serum from peanut-allergic patients who had received immunotherapy containing specific IgG4 antibodies against peanut allergens inhibited the ability of sIgE to activate basophils and mast cells [34]. More recently, the ability of subcutaneous immunotherapy against Der p to inhibit the BAT has been evaluated [39]. The authors postulate that the increase in specific IgG4, rather than the reduction in specific IgE, correlates with BAT inhibition in patients receiving immunotherapy. To this end, the authors propose a mechanism by which specific IgG4 would compete with the ability of specific IgE to bind the allergen.

IgG4 exhibits another intriguing property, namely, its ability to develop a process called Fab-arm exchange in vivo, which gives rise to bispecific antibodies. This characteristic and the limited ability of IgG4 to form immune complexes may enhance the blocking property [35,40].

The production of cytokines such as IL-10 is associated with peripheral T-cell tolerance and the presence of Tregs [10]. Both IL-10 levels and the percentage of CTLA-4+ Treg cells were elevated in the TBK group. These findings could be associated with a tolerogenic phenotype of antigen-presenting cells. IL-10 inhibits the expression of molecules involved in antigen presentation (HLA and B7), thereby influencing activation of T_H1, T_H2, and T_H17 [41]. All 3 effector subsets were consistently reduced in TBKs, thus providing further evidence of the robust regulatory activity in this group. Although a typically T_H2 to T_H1 shift has been described after induction of hymenopteran venom tolerance, with an increase in INF-γ levels and a decrease in secretion of IL-4 and IL-13 [42,43], an in vivo expansion of IL-10–producing allergen-specific cells at the expense of both T_H1 and T_H2 subsets has also been described [44]. Indeed, peripheral T-cell tolerance is characterized by a decrease in T_H2 and T_H1 cells [45]. Moreover, in a "modified" T_H2 response, IL-10 production in the presence of IL-4 drives class-switching to IgG4 without IgE production [35].

High sIgE levels play a critical role in the development of severe reactions to bee venom following insect stings. To our knowledge, this is the first report in which such a thorough analysis of molecular components of bee venom was performed. Thus, after testing the 6 allergenic components belonging to AmV in the 3 populations of this study, our results for the TBK group, whose sIgE levels to AmV and its major allergens Api m 1 and Api m 2 were significantly lower than those of APs, were consistent with those of another study comparing the same 3 groups [8]. However, they differed from the findings of a recent study comparing asymptomatically sensitized individuals (without specifying whether they were beekeepers) [46], although both studies only analyzed Api m 1. When comparing beekeepers with nonexposed HCs, Matysiak et al [33] found significant differences regarding sIgE to bee venom (P=.038), but not to Api m 1 (P=.055). In contrast, we found significant differences in sIgE to all molecular components analyzed, except rApi m 3. The different results of both studies could be explained by the clinical characteristics of the individuals recruited (Matysiak et al examined 30 beekeepers, 2 of whom continued to experience systemic reactions), although both show that TBKs usually have detectable sIgE levels. Among APs, there was a predominance of allergic response markers defined by markedly elevated levels of sIgE to bee venom and its allergenic components (rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5, and rApi m 10), as well as a high rate of basophil activation to AmV (see above).

Interestingly, considering paradoxical behaviors, among the 17 TBKs, basophils degranulated in 9 individuals (52.9%), but in none of the HCs. Furthermore, 1 individual belonging to the TBK group was characterized by a marked basophil activation rate and a lower sIgG4 response. TBK number 37 (shown in the heatmap) must be highlighted because of the strong presence of an allergic profile and markers of weak tolerance. Taken together, these findings might represent a particular type of beekeeper risk profile, ie, a person who is eventually susceptible to a higher risk of systemic reactions after further bee stings.

The main limitation of this study is that it was conducted in a single center with a small sample. The diversity of sensitization profiles highlights the need for a higher number of patients from different geographical origins to ensure robust data. Moreover, it would be desirable to reduce the technical complexity of the methods used to translate them into daily clinical practice. Despite these drawbacks, the Helios$^-$ Treg population seems to be a novel candidate biomarker, which, together with the well-known CTLA4 and IL-10, will enable us to monitor the tolerance process from bench to bedside. Future studies should thoroughly address regulatory suppressor activity and its underlying mechanisms during venom immunotherapy.

Conclusions

A well-defined regulatory suppressor profile, which is strongly marked by Treg activity, was found for TBKs. This natural tolerance would be led by the expansion of inducible Helios$^-$ Treg cells at the peripheral level. Treg function remains the grounds on which tolerance is based, even in conditions of extreme exposure.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Aurora Jurado Roger

Department of Immunology and Allergy
Reina Sofía University Hospital
Avda. Menéndez Pidal, s/n
14004 Córdoba, Spain
E-mail: aurora.jurado.sspa@juntadeandalucia.es