

The natural history of *Apis*-specific IgG and IgG4 in beekeepers

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Summary

Background Most published studies on changes of specific IgG or its subclasses as a response to stimuli by allergens have been performed on patients under immunotherapy. There are few reports analysing the response to immunoglobulins in patients exposed to allergens in their natural habitats.

Objective The aim of this work was to discover the natural history of *Apis* specific IgG (IgG-ap) and IgG4 (IgG4-ap) levels in beekeepers from the Canary Islands.

Methods We studied 242 beekeepers (Bks). We used a questionnaire and measured total IgE and seric *Apis* specific IgE (IgE-ap), IgG-ap and IgG4-ap against *Apis mellifera* venom.

Results All Bks had IgG-ap and IgG4-ap. IgE-ap was positive in 160 Bks (65.6 %), but only 92 (37.6 %) Bks were considered sensitized. IgG-ap and IgG4-ap showed significant correlation ($r = 0.84$); IgE did not correlate with IgG-ap or IgG4-ap. There was no seasonal variation in IgG-ap or IgG4-ap. The group of sensitized Bks had significantly lower IgG-ap and IgG4-ap levels ($P < 0.05$). The groups with longer beekeeping activity showed significantly higher levels of IgG-ap and IgG4-ap ($P < 0.001$). Bks with locals reactions had significantly higher IgG-ap and IgG4-ap than Bks who reported systemic reactions ($P < 0.05$).

Conclusion Our study showed that IgG-ap and IgG4-ap appear to increase in Bks, either according to their beekeeping experience or in subjects with local reactions after bee stings.

Keywords: *Apis*, allergy, bee, beekeepers, epidemiology, hymenoptera, IgE, IgG4, sensitization

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Introduction

Most published clinical studies on IgG and its subclasses are confusing and reveal contradictory results. Most tests were made on patients receiving immunotherapy, where the levels of antibodies in plasma have been analysed during treatment. In patients not subjected to immunotherapy, the number of studies is limited; the most common one being related to food allergens. A reduced number of studies have dealt with hymenoptera venom, mostly in patients under immunotherapy for systemic reactions to stings. However, in all of them it has been observed that the response to

allergens in the IgG subclasses seems to be limited to the IgG1 and IgG4 subtypes [1,2]. The significance of changes in the level of these immunoglobulins induced by immunotherapy or by exposure to allergens has not been explained, since there is not a clear distinction between their variations and clinical progression. Djurup and Malling observed that an increased IgG4 during immunotherapy correlated with a poor response to treatment [3], whereas Einarson *et al.* described the opposite effect [4]. Similar data have been reported for pollen-allergic patients following exposure to allergen [5]. Finally, in recent years there has been some investigation into the possible existence of regulatory mechanisms common to the synthesis of IgE and subclasses of IgG [6–8].

We believe that the available data are still insufficient. These antibodies warrant further study, especially in populations

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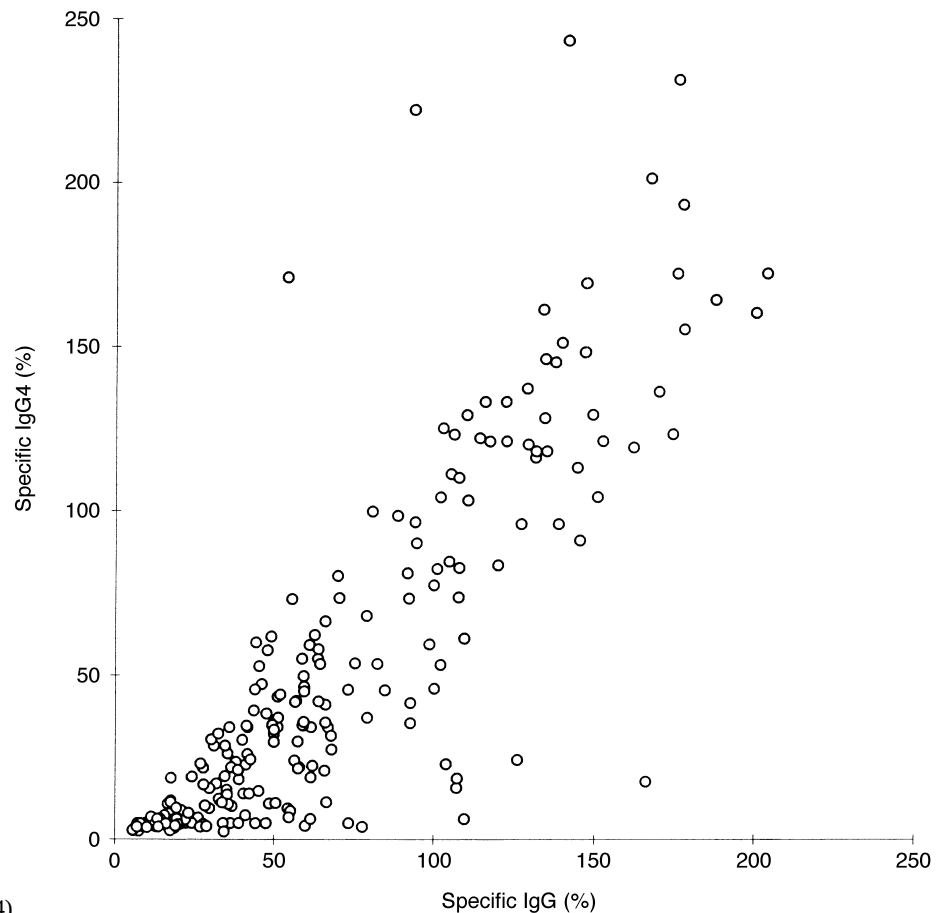


Figure 1. Correlation between *Apis* specific IgG specific and IgG4 ($r = 0.84$)

receiving antigen stimuli in their natural habitat. We conducted the following study with the aim of finding out the natural history of *Apis*-specific immunoglobulin levels in beekeepers.

Material and methods

Subjects

We contacted the Provincial Societies of Beekeepers and Agricultural Extension Offices, through whom we arranged meetings with beekeepers (Bks) and their relatives in different municipal areas. The meetings were held in health centres or in specially prepared precincts with the subjects and our research team. Informed consent was obtained from every subject in the study.

We studied 246 beekeepers (the total number of registered beekeepers in the island is 584); 238 completed the study. The age (mean $\pm$ sd) was 41.8 ± 15.37 yr. There were 218 males and 28 females. All Bks were regularly stung by bees. The longest time between the last sting and the moment of the study was 5 months. None of them had

previously sought medical attention for sting reaction, neither had they received immunotherapy with Hymenoptera venom.

A questionnaire was conducted by an allergist which included questions about their relationship with beekeeping, length of involvement in beekeeping during their lifetimes and the most important reactions developed after being stung by bees. Subjects who had more than a local reaction at some time in their lives were asked to specify the type of reaction caused by subsequent stings.

Allergy tests

Skin tests with bee venom were carried out and in addition blood samples were taken to determine total IgE, IgE-ap, IgG-ap and IgG4-ap. All *in vitro* tests were performed following the instructions of the manufacturer (CAP-SYSTEM, Kabi-Pharmacia, Uppsalla, Sweden). The positive cut-off limit for IgE-ap was ≥ 0.35 kU/L. Bks were considered sensitized when they had presented a systemic reaction and skin test or IgE-ap were positive. The results of IgG-ap and IgG4-ap are expressed as a percentage, in comparison with a

Table 1. Comparison of *Apis* specific IgE, IgG and IgG4 levels among beekeepers sensitized and non-sensitized to *Apis* venom

	Specific IgE to Apis					Specific IgG to Apis					Specific IgG4 to Apis					
	<i>n</i>	Mean	Standard deviation	Median	Range*	<i>P</i> -value	Mean	Standard deviation	Median	Range	<i>P</i> -value	Mean	Standard deviation	Median	Range	<i>P</i> -value
Sensitized	92	6.6	10.2	2.6	1.4-9.9	0.000	51.4	38.18	38	19.1-68.4		37.23	42.34	18.8	6.1-51	
Non-sensitized	145	1.6	3.3		<0.35-1	0.000	72.29	50	56	35-105	0.0025	54.8	56.48	34.2	8.5-87.6	0.02

*Range runs from 25th to 75th percentile of data.

reference response (binding of a serum pool with a high birch IgG level to birch-coated tubes). The anti IgG reagent reacts with all four IgG subclasses and it is not possible to determine the individual contribution of any subclass.

Data collection and blood tests were performed between March and October 1992, thus covering three seasons. This study was approved by the Scientific Review Board of the Hospital de la Candelaria.

Statistics

The data obtained were analysed using the Statistical Package for Social Sciences (SPSS). Since none of the numerical values, except age, conformed to normal distributions using the Kolmogorov–Smirnov test, we used the non-parametrical test. Differences were considered significant when $P < 0.05$.

Results

IgE-ap was positive in 160 Bks (65.6%), but only 92 (37.6%) Bks were considered sensitized (they had presented a systemic reaction and showed skin test or IgE-ap positive). All Bks had IgG-ap and IgG4-ap. IgG-ap was 64.3 ± 46.6 % and IgG4-ap was 48.2 ± 52.1 %. Figure 1 shows the correlation coefficient between them. It was statistically significant, $r = 0.84$ ($P < 0.001$). No correlation was observed between either IgG-ap or IgG4-ap and total IgE. No significant differences were found in the IgG-ap and IgG4-ap values determined at different times during the year of study.

Specific IgG or IgG4 and sensitization to Apis

Table 1 contains the comparative dates of IgG-ap, IgG4-ap and IgE-ap among Bks who were sensitized and the negative group. The sensitized group showed significantly lower IgG-ap (51.4 ± 38.18 vs 72.04 ± 49.9) and IgG4 levels (37.24 ± 42.35 vs 54.83 ± 56.48) ($P < 0.05$ in both cases).

Using the total IgE, IgG-ap and IgG4-ap values, we performed a discriminate analysis to determine whether we could predict the which of the groups of Bks were sensitized or not sensitized against *Apis*. The most important variables in this respect were IgG-ap and total IgE. With these two variables it was possible to correctly classify only 143 (60 %) Bks. But if with this test we considered as sensitized only Bks who presented IgE-ap ≥ 0.7 kU/L, independently of the result of skin test or previous reaction, it was possible to correctly classify 161 Bks (68.5%). Notably, in the non-sensitized group the percentage of accuracy increased to 80 %.

Table 2. Comparison of *Apis* specific IgG and IgG4 levels among three subgroups of beekeepers according to beekeeping activity. T1 includes those BKs with less than 5 years of beekeeping activity; T2 includes Bks with 5–20 years of activity, and T3 for those with more than 20 years in beekeeping

	<i>n</i>	Specific IgG to <i>Apis</i>					Specific IgG4 to <i>Apis</i>				
		Mean	Standard deviation	Median	Range	<i>P</i> -value	Mean	Standard deviation	Median	Range	<i>P</i> -value
T1	108	53.04	43.07	41.4	18–73.2	0.0008	34.13	46.3	10.1	4.8–43.5	0.0002
T2	88	66.6	43.7	56.6	34.67–95.17		52.08	520.4	32.8	17.6–74.9	
T3	50	83.6	53.2	65.5	43.7–119.2		70.3	55.5	51.5	28.8–114	

* Range runs from 25th to 75th percentile of data.

Specific IgG or IgG4 and bee-keeping activity

Mean duration of BK activity was 10.6 ± 11.3 yr. To analyse the data we divided the duration of activity into three time groups: T1 ($n = 108$) included those Bks with less than 5 yr of beekeeping activity; T2 ($n = 88$) had 5 to 20 yr of beekeeping activity, and T3 ($n = 50$) had more than 20 yr in beekeeping. IgG-ap was $52.2 \pm 42.2\%$ in group T1; $69.7 \pm 45.8\%$ in group T2 and $80 \pm 50.8\%$ in group T3. IgG4-ap was $34 \pm 46\%$ in the group T1, $54 \pm 53.1\%$ in the group T2, and $68.5 \pm 54.6\%$ in the group T3. IgG-ap and IgG4-ap levels were also significantly higher in the two longer duration groups ($P < 0.001$).

Specific IgG or IgG4 and previous reactions to bee stings

From the whole population of Bks, 114 subjects reported they had had only local reactions, whereas the remaining 128 had had more intense reactions, including large local reactions and anaphylaxis. However, the majority of them (83.5%) had had reactions of less intensity when they were stung thereafter (data not shown). Table 3 contains the comparative data on IgG-ap, IgG4-ap and IgE-ap among subgroups according to reactions. IgE-ap was significantly higher in the systemic reaction group while IgG-ap and IgG4-ap were significantly higher in the local reaction group ($P < 0.05$).

Discussion

We studied a population of Bks with variable periods of BK activity and types of bee sting reactions. None of the subjects had previously received immunotherapy with Hymenoptera venom. The study was performed over a three-season period with a variable degrees of exposure. Although Bks in our setting have constant contact with their hives all year around, there is more intense activity during the spring and summer. In this study we did not find any significant differences in *Apis* specific IgG and IgG4 values

during any season, in contrast to Pastorello *et al.* [9] who reported different values before and after the beekeeping season.

Different types of response of IgG-ap and IgG4-ap have been reported in patients treated with immunotherapy [10]. It has been observed, in general, that IgG-ap increases markedly in the first few months of treatment; high levels continue for some time and gradually fall to pre-treatment levels after the fifth year. The increase in IgG4-ap follows a similar pattern, but if the stimulation persists the higher level may be maintained over a longer period of time. Thus, IgG4 is considered to act as a marker of prolonged antigenic exposure. These results, however, are mostly from limited follow-up studies of from 3 to 5 yr maximum, and there is very little information on more prolonged periods of time.

In the method which we used to determine IgG-ap and IgG4-ap, the anti IgG reagent reacted with all four IgG subclasses with similar efficiency. For this reason it was not possible to discover the individual contribution of any subclass to total specific IgG. But if we take into account the coefficient correlation found between them (0.84) (Fig. 1), we can deduce that a large part of IgG-ap could correspond to IgG4-ap.

In our study we did not find a linear correlation between duration of BK activity and IgG-ap or IgG4-ap. However, we found striking differences in antibody levels in the three subgroups (T1–T3), with significantly higher levels for the longer duration groups of beekeeping activity. This discovery was intriguing, since the distribution of values is not homogeneous. There are, for instance, Bks from group T1 with high levels of IgG-ap or IgG4-ap and others with relatively low values in group T2 or T3. To our knowledge there are no published reports to compare with our results. Müller *et al.* and Bousquet *et al.* [11,12] found a correlation between IgG values and number of stings received. It may be possible that our finding was due to the greater number of stings presumably received over longer periods of uninterrupted beekeeping.

Table 3. Comparison of Apis specific IgE, IgG and IgG4 levels among beekeepers who had had only local reactions after bee stings and Bks who had presented others systemic reactions

	Specific IgE to Apis					Specific IgG to Apis					Specific IgG4 to Apis					
	<i>n</i>	Mean	Standard deviation	Median	Range*	<i>P</i> -value	Mean	Standard deviation	Median	Range	<i>P</i> -value	Mean	Standard deviation	Median	Range	<i>P</i> -value
Systemic	145	4.7	9	1.7	<0.35–5.6		53.7	38.5	40.9	20.5–73.4		37.6	42	19	6.1–52	
Local reaction	114	2.1	3.8	0.55	<0.35–1.8	0.0011	76.9	52.6	58	39.1–109.6	0.011	60.5	60	38.3	11–94.4	0.033

* Range runs from 25th to 75th percentile of data.

The protective function of these antibodies is unclear. Bousquet *et al.* [13] reported significantly higher levels of IgG-ap in Bks than in healthy blood donors or sensitized patients, but this finding did not enable them to differentiate allergic from non-allergic subjects. In a latter study they did find that IgG-ap levels correlated with the number of stings received and reaction types, which enabled them to differentiate between Bks who might present allergic reactions if their values were lower or higher than 150 U/mL [13]. Other authors have noted that low levels of specific yellow jacket venom IgG following immunotherapy are associated with a greater degree of reactions [14], or that the administration of gamma globulin, from a pool of Bks, to sensitized subjects allowed them to tolerate up to 200 µg of venom [15,16]. On other hand, Müller and Ewan reported that even though these antibodies increased with immunotherapy, they do not have predictive value, whether from provoked or spontaneous stings [17,18].

In our study it is difficult to make comparisons between the IgG-ap or IgG4-ap and the date obtained from the clinical records, because the reactions reported by patients date from the whole period of their beekeeping and, although some of the reactions were recent, others would have occurred years before. In our study most subjects who reported important reactions to stings at some point, also reported less intense reactions to subsequent stings. On the other hand, the IgG-ap and IgG4-ap determinations were recent and only reflect values at a particular moment of their lives. Despite all the above, it remains surprising that subjects who have only presented local reactions should have significantly higher levels of both immunoglobulins than other subjects who reported more severe reactions. It could be suggested that the response of a specific class of IgG antibodies could have influence on the clinical response. Furthermore there might be other poorly understood factors involved, such as anti-idiotypic antibodies [19,20]. The renin angiotensin system could also be implicated; it was observed that it was significantly lower in patients with anaphylaxis induced by Hymenoptera venom [21,22].

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