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Evaluation of IgG RAST® FEIA for the Assay of Venom-Specific IgG Antibodies during Venom Immunotherapy

Key Words

Insect venom allergy
Venom-specific IgG
IgG RAST FEIA
Pharmacia CAP system
Venom Immunotherapy

Abstract

Background: Successful venom immunotherapy (VIT) in Hymenoptera allergy is usually associated with a strong increase in venom-specific serum IgG antibodies (sIgG). **Methods:** We evaluated a new commercial test for the assay of sIgG (Pharmacia CAP System™ IgG RAST® FEIA; FEIA), in comparison with a conventional ELISA technique. Sera from 40 bee- and 40 *Vespula*-allergic patients were analyzed by FEIA and ELISA before and 3 months after starting VIT. **Results:** The correlation between sIgG obtained with the two methods was significant: $r = 0.862$, $p < 0.0001$ for bee venom (BV), $r = 0.861$, $p < 0.0001$ for *Vespula* venom (VV). The geometric mean values obtained with FEIA were higher for both venoms (BV $p = 0.03$; VV $p < 0.01$). A highly significant increase ($p < 0.0001$) was observed during VIT with both methods. This increase was concordant in 93% of VV- and 90% of BV-treated patients. Intra- and interassay relative coefficients of variation were below 10% for FEIA. **Conclusion:** IgG RAST FEIA is a reproducible and sensitive method for the assay of venom-specific sIgG.

Introduction

Between 2 and 19% of the adult population in Europe and the USA have a history of large local reactions, and between 0.8 and 5% a history of generalized allergic reactions to Hymenoptera stings [1–3]. In rare instances, these reactions may be fatal [3]. Hymenoptera venom (HV) allergy is associated with antibodies of the IgE isotype.

The role of venom-specific IgG antibodies (sIgG) is less clear. Aalberse et al. [4] observed high sIgG levels (especially IgG4) in highly exposed over long periods of times who tolerate bee stings often without even a local reaction. Others showed successful passive immunization of bee-allergic patients by beekeeper γ -globulin [5–7]. During venom immunotherapy (VIT), sIgG increases rapidly in the ini-

tial phase and remains elevated throughout treatment [8]. All these observations suggest a protective role for sIgG in HV allergy. However, prospective studies, where serum was investigated immediately before a sting challenge, showed no predictive value for sIgG or sIgG4 levels in untreated patients [9, 10], in patients on long-term VIT of more than 3 years duration, and in patients in whom VIT was stopped [11–14]. Moreover, there is a wide variation of sIgG, not only in patients on VIT, but also in untreated patients, beekeepers and even nonallergic controls [4, 7, 9, 10, 15].

Routine commercial tests to assay venom-specific IgG-antibodies have so far not been available. Pharmacia Upjohn has recently introduced such a test based on the Immuno CAP® technology, the Pharmacia CAP System™ IgG RAST® FEIA (FEIA). To evaluate this test, we have com-

Table 1. Results of sIgG determination by FEIA and ELISA before and during VIT

		BV VIT		VV VIT	
		FEIA	ELISA	FEIA	ELISA
sIgG geometric mean values, mg/l					
Total	(n = 80)	11.9	9.2	9.8	5.0
Before VIT	(n = 40)	10.3	9.7	12.3	5.7
After VIT	(n = 40)	21.9	19.7	20.7	13.3
Intraassay reproducibility					
rel. CV, % (mean $\pm$ SD)	(n = 80)	7.5 $\pm$ 17.7	3.9 $\pm$ 3.0	5.0 $\pm$ 8.3	5.6 $\pm$ 4.4
Interassay reproducibility					
rel. CV, % (mean $\pm$ SD)	(n = 80)	6.6 $\pm$ 1.1	16.8 $\pm$ 2.3	5.6 $\pm$ 3.3	10.5 $\pm$ 1.0

pared its performance in 40 patients with BV or *Vespula* venom (VV) allergy before and during VIT to that of an ELISA technique used in our laboratory for 15 years [8].

Materials and Methods

Patients

Sera from 40 patients with a history of recent type I systemic allergic reactions to honey bee or *Vespula* stings and positive diagnostic tests with BV or VV were analyzed by FEIA and ELISA before and 3 months after starting VIT. All sIgG assays were done in duplicates. Intracutaneous skin test end point concentration, the lowest concentration resulting in a wheal of a least 5 mm in diameter with erythema, was 10^{-4} g/l or lower, and venom-specific IgE by Phadezym[®] RAST (Pharmacia-Upjohn Diagnostics, Uppsala, Sweden) was >0.35 Phadebas RAST units in all patients before starting VIT. VIT was started with gradually increasing venom doses up to a maintenance dose of 100 μ g of either venom and then continued with injections of this dose every 4 weeks as described previously [8].

Pharmacia CAP System[™] IgG RAST[®] FEIA

FEIA is based on Immuno CAP technology, well known for estimation of specific IgE antibodies. For the detection of sIgG, a monoclonal, murine, enzyme-labeled (β -galactosidase) anti-IgG-antibody is used. After addition of a developing agent (4-methylumbelliferyl- β -D-galactoside) fluorescence is measured in FluoroCount 96 and sIgG levels are calculated by comparison with a dilution series of standard IgG. The standards are calibrated against the WHO International Reference Preparation 67/86 for Human Serum Immunoglobulins A, G and M. Results are given in milligrams/liter.

ELISA

The ELISA technique used has been described in detail previously [8]: polystyrene tubes pretreated with 0.2% glutaraldehyde and thereafter coated with the allergen are used as the allergosorbent. After thorough washing with NaCl-Tween, 1 ml of test serum dilution is added to each tube followed by incubation overnight. After another three washes with NaCl-Tween, 1 ml of marker solution diluted 1:1,000 is added to each tube and incubated for 1 h at 25°C. The marker to detect IgG antibodies is a conjugate of staphylococcal protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) and alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo., USA). After wash-

ing again with NaCl-Tween, 1 ml of substrate (*p*-nitro-phenyl-phosphate 1 g/l in carbonate-bicarbonate buffer 0.05 mol/l pH 9.8, containing 10^{-3} mol/l $MgCl_2$) is added and the tubes are incubated for another 45 min at 25°C. The reaction is then stopped with 0.1 ml of NaOH, 2 mol/l. sIgG levels (mg/l) are calculated by comparison with a dilution series of a beekeeper serum pool or a pool of sera from *Vespula*-allergic patients on VIT with known contents of sIgG. The amount of venom-specific IgG in both pools was kindly determined by R. G. Hamilton, PhD, Baltimore, Md., USA.

Statistics

To analyze the correlation between sIgG measured by FEIA and ELISA, Spearman's rank correlation coefficient was calculated. Comparison of absolute sIgG levels obtained by FEIA and ELISA was done by the Wilcoxon signed rank test. The significance of an increase of mean sIgG after 3 months of VIT was also analyzed by the Wilcoxon signed rank test.

Intraassay reproducibility was assessed by calculating the relative coefficient of variation (CV) from duplicates in 80 sera each, assayed by both FEIA and ELISA for sIgG to BV and VV. If the individual CV of one test serum exceeded 20%, the test was repeated in duplicate.

Interassay reproducibility was assessed by calculating CV of the same serum (mean of duplicates) on 5 different days. Three different sera each with high, intermediate and low levels of sIgG were studied in this way by FEIA and ELISA for both BV and VV sIgG.

Results

Venom-Specific IgG Levels Before and During VIT

Mean values of sIgG against BV and VV before and during VIT as determined by FEIA and ELISA are given in table 1. As shown in figure 1a, b, a wide variation of individual sIgG levels to both venoms was observed with both methods, especially before VIT. Absolute sIgG levels measured by FEIA were significantly higher than by ELISA (BV: $p = 0.03$; VV: $p < 0.01$). This was more obvious for VV and low levels of sIgG.

With both methods, a highly significant increase in mean sIgG levels was found after 3 months of VIT (BV: FEIA $p < 0.0001$, ELISA $p < 0.0001$; VV: FEIA $p < 0.0001$,

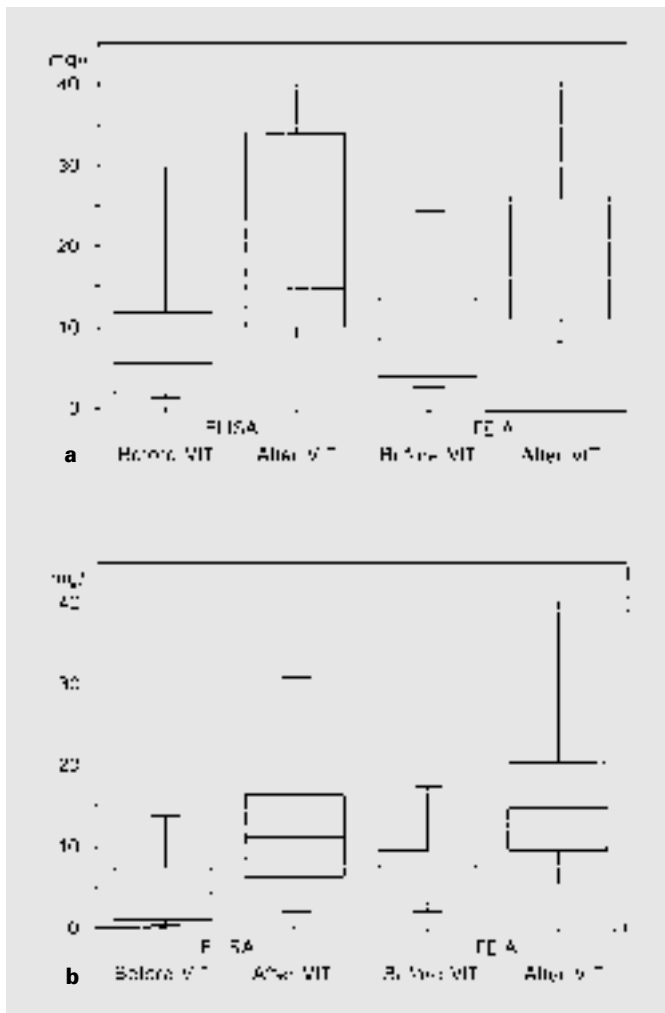


Fig. 1. Specific IgG to BV (a) and VV (b) before and after VIT (n = 80). This box plot displays the 10th, 25th, 50th, 75th and 90th percentile of the measured sIgG serum levels before and after VIT for FEIA and ELISA. A highly significant increase of sIgG was observed with both methods ($p < 0.0001$) by the Wilcoxon signed rank test.

ELISA $p < 0.0001$) (fig. 1a, b). This increase was concordant in 90% (36 of 40) of BV and 93% (37 of 40) of VV treated patients. With VV, all patients showed an increase in ELISA but 3 showed a decrease in FEIA. With BV, all patients showed an increase in FEIA but 4 did not show any change in ELISA. These 4 patients all had very high levels of sIgG, lying above the highest standard concentration (> 40 mg/l) in ELISA, already before VIT. As shown in figures 2a, b, sIgG values for both BV and VV obtained with FEIA and ELISA were closely correlated. Coefficients of correlation were highly significant for BV ($r = 0.862$, $p < 0.0001$) and for VV ($r = 0.861$, $p < 0.0001$).

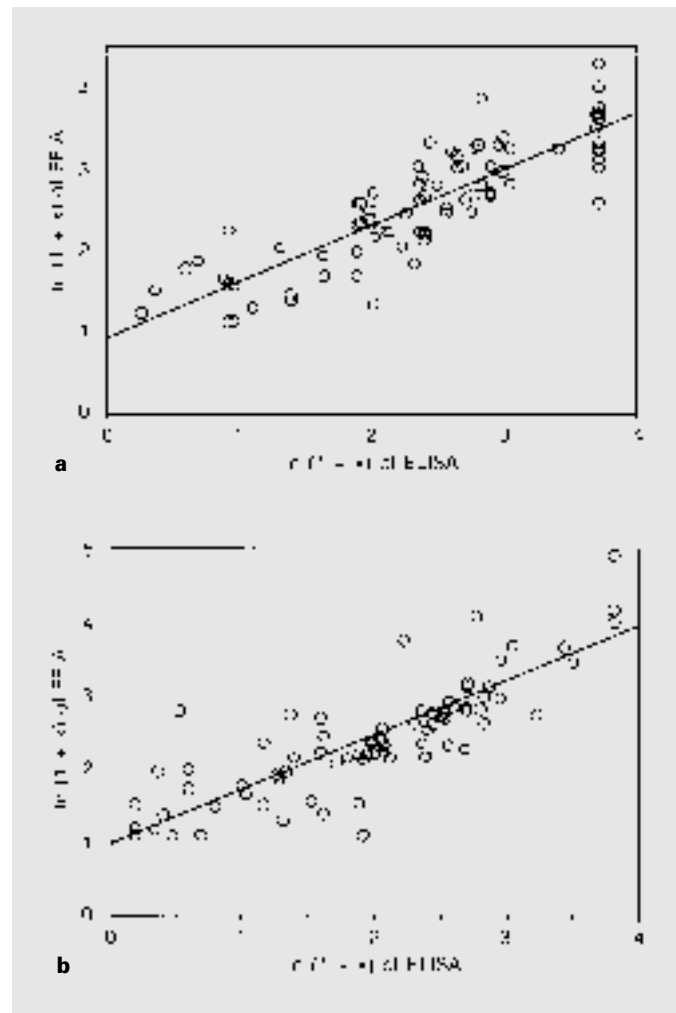


Fig. 2. Correlation of sIgG to BV (a) and VV (b) obtained by FEIA and ELISA. A highly significant correlation is observed, but FEIA values, especially in the low range, are significantly higher than those obtained by ELISA.

Reproducibility

The mean values of the relative coefficients of variation (CV) with standard deviations are given in table 1.

Intraassay reproducibility was judged from the mean values of CVs obtained from 80 individual double estimations for each venom in both tests. Intraassay reproducibility was comparable for FEIA and ELISA with mean CVs between 3.9 and 7.5%. As figures 3a, b show, very high individual CVs of $> 50\%$ were, however, exceptionally observed in FEIA with both venoms (in 4 of 160 double estimations = 2.5%).

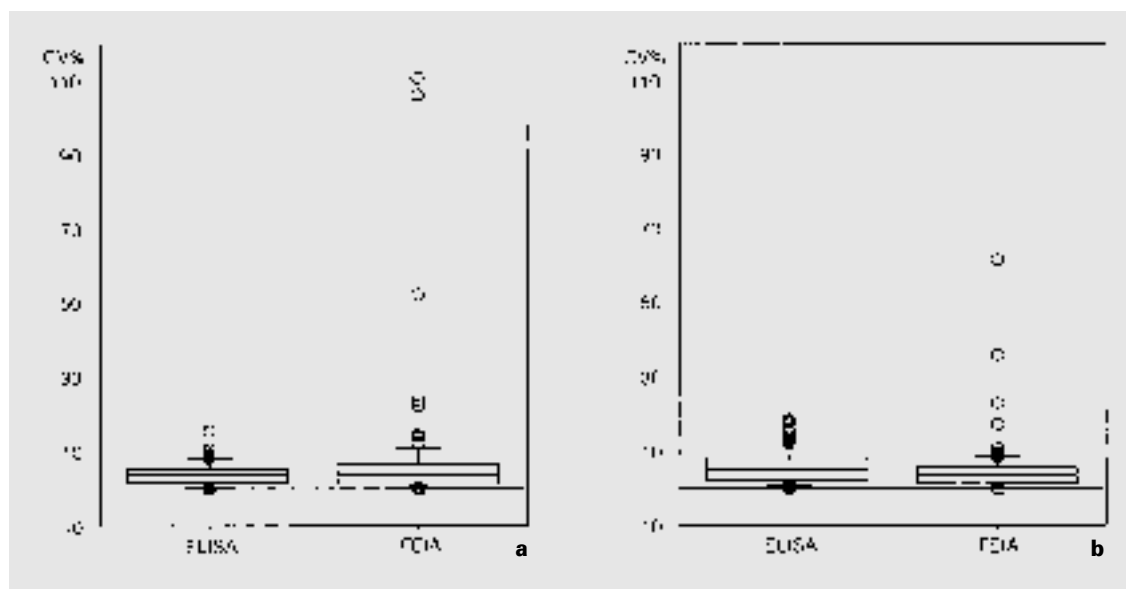


Fig. 3. Intraassay reproducibility of BV (a) and VV (b) sIgG with ELISA and FEIA. The box plot displays the 10th, 25th, 50th, 75th and 90th percentile of calculated relative CVs. The open circles show extreme values. The mean relative CV of less than 10% indicates a good reproducibility of both tests. Occasional very high individual relative CVs are however observed for FEIA.

The *interassay reproducibility* was judged from the mean values of duplicates of sera tested on five different days. As table 1 shows, interassay reproducibility with mean CVs of 5.6–6.6% was clearly better in FEIA than in ELISA with mean CVs of 10.5–16.8%.

Discussion

On the whole, the venom-specific IgG levels found by FEIA were in good agreement with those obtained with an ELISA technique, which has been used in our laboratory for many years: individual sIgG values correlated closely in both BV- and VV-allergic patients before as well during VIT. Moreover, with both methods, a highly significant and concordant increase of sIgG was observed during VIT.

The sIgG levels found with the FEIA were however usually somewhat higher than with ELISA, especially so with low sIgG levels and more pronounced with VV. The different methods of insolubilizing allergens – covalent coupling vs. adsorption to the allergosorbent – and different standardization systems used may well explain these findings. On rare occasions, discordant sIgG results were observed with the two methods. This is not surprising as FEIA and ELISA use different assays for the detection of sIgG: FEIA

uses a monoclonal murine anti-IgG antibody, whereas ELISA uses protein A from *Staphylococcus aureus*. While the former binds well to all IgG subclasses, the latter binds primarily to IgG1, IgG2 and IgG4, much less to IgG3 [15].

Intraassay reproducibility was very good for both FEIA and ELISA. Very rarely, high individual intraassay CVs were observed with the FEIA. Misinterpretations due to this can be avoided by the routine use of double assays. Interassay reproducibility was excellent for FEIA and acceptable for ELISA.

The recommendations for the assay of sIgG to HV are still controversial [11–13, 17, 18]. On the one hand, there are strong arguments in favor of a protective role of sIgG based on studies in beekeepers [4, 16, 19] and on trials using passive immunotherapy with beekeeper γ -globulins [5–7]. Also, sIgG increase rapidly in the first months of VIT and usually remain elevated during prolonged treatment, more or less parallel to the protection induced by VIT. Golden et al. [11, 18] and Lawrence et al. [12] found a good correlation between an sIgG level of >3 mg/l and protection in the first years of VIT in mostly VV-sensitive patients, with significantly higher mean sIgG in fully protected patients than in partial treatment failures. However, 2% of patients with high sIgG (>3 mg/l) still reacted on reexposure, while 78% of those with a low level (<3 mg/l) were

fully protected. We could not confirm these findings in BV-sensitive patients who were mostly reexposed during long-term VIT [13]. It is quite obvious that sIgG are of no predictive value in untreated patients [9, 10], in the first 2 weeks of VIT [20], in patients on protracted VIT [13, 21] and in patients who stopped VIT [14, 21]. Moreover, there is a wide variation of sIgG within groups such as untreated patients, patients during VIT, beekeepers or even nonallergic individuals [4, 7, 9, 10, 15], which makes determination of sensitivity and specificity of sIgG difficult. On the contrary, a strong increase of sIgG is almost invariably observed during the first months of VIT and is thus a sensitive indicator of an immunologically effective treatment. In clinical practice, a more than twofold increase of sIgG will usually be considered as significant. In occasional patients with very high pretreatment levels (>15 mg/l), a lower or even absent increase may be compatible with effective immunotherapy. On the other hand, in patients with very low (<3 mg/l) or unmeasurable pretreatment sIgG, a more than twofold increase in the order of >5 – 10 mg/l will be strived for.

More recent studies suggest that besides sIgG other protective mechanisms are induced by VIT. In the very early phase, desensitization of basophils may be involved [22], while during protracted VIT, a change in the cellular immune response [23, 24] seems more important.

Based on available data, some authors [3, 18, 21] recommend sIgG determination during early VIT. A lack of in-

crease of sIgG, after verification of the correct species and dose of venom applied, is taken as an indication to increase the maintenance dose to $200\text{ }\mu\text{g}$. Others [17] contest the value of this test for monitoring of VIT. Determination of specific IgG to major allergens to which the patient has IgG antibodies and is thus sensitized might correlate better with the outcome of VIT as judged by a sting challenge or a field sting. Unfortunately, major individual allergens are not commercially available in highly purified or recombinant form. Venom sIgG may occasionally be helpful, both in and outside VIT, for the identification of an insect that was responsible for a sting, because sIgG usually increases rapidly following such an event, irrespective of whether it caused an allergic reaction or not.

In conclusion, we found the Pharmacia CAP SystemTM IgG RAST[®] FEIA to be a well-reproducible and sensitive commercial test for the determination of venom-specific IgG-antibodies. To judge its reliability as a predictor of clinical protection, more extensive studies in both BV- and VV-sensitive patients in direct relation to protection, as indicated by the result of a sting challenge during VIT, are needed.

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