

Modified antigenic reactivity of anti-phospholipase A₂ IgG antibodies in patients allergic to bee venom: Conversion with immunotherapy and relation to subclass expression

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Background: We have previously reported that, in addition to modifying IgG levels and subclass distributions, wasp venom immunotherapy (VIT) rapidly changes IgG antibody specificity. **Objectives:** We investigated whether such a change can be documented in the IgG response to the major bee venom allergen, phospholipase A₂ (PLA₂), from patients allergic to bees treated with VIT; whether it is coupled to the shift in IgG subclass distribution (IgG₄ predominance) usually observed during VIT; and whether it restores the specificity displayed by IgG antibodies from nonallergic individuals.

Methods: Antibody specificity was evaluated in 17 patients allergic to bee venom in competitive ELISAs by using streptavidin biotin technology. Patients were tested before and during specific immunotherapy (at 15 days and 6 months) and compared with another group of 17 patients treated with venom injections for at least 2 years (VIT patients) and 30 healthy individuals.

Results: The capacity of individual sera to prevent PLA₂ binding of pooled IgG from allergic patients changed rapidly with mean percentage inhibitions falling from 84% ± 14% before starting VIT to 27% ± 13% and 28% ± 7% after 15 days and 6 months of treatment, respectively ($p < 0.001$ by one-way analysis of variance [ANOVA]). IgG titers were only slightly increased. The capacity of individual sera to prevent the binding of pooled IgG from patients receiving VIT changed rapidly with mean percentage inhibition increasing from 60% ± 12% before starting VIT to 85% ± 6% and 82% ± 6% after 15 days and 6 months of treatment, respectively ($p < 0.001$ by one-way ANOVA). Similar results were found regardless of whether pooled IgG₁ or pooled IgG₄ were used.

Conclusion: VIT results in a rapid change in the antigenic reactivity of anti-PLA₂ IgG antibody of human allergic sera, restoring, although not completely, the specificity peculiar to IgG from healthy individuals. This suggests that allergic status and immunoprotection correlate with the preferential expression of distinct IgG specificities, which appear equally distributed over the IgG₁ and IgG₄ antibody subclasses. It is, however, not known whether the shift in IgG specificity is one of the operative mechanisms of VIT. (*J Allergy Clin Immunol* 1998;102:118-26.)

Key words: Bee venom allergy, rush immunotherapy, IgG antibody, IgG subclasses, epitopes, immunodominance

Although its mechanism of action still remains unclear, venom immunotherapy (VIT) provides full protection against bee stings in 75% to 80% of patients allergic to bee venom.^{1,2} Evolution of the IgG response has been quite extensively investigated. Although it is widely accepted that the measurement of specific IgG has no predictive value in patients receiving VIT,³⁻⁷ several studies have documented a rise in IgG levels and a gradually increasing predominance of IgG₄ during prolonged VIT.⁸⁻¹⁰ Studying the evolution of the IgG response in patients allergic to wasp venom treated with venom injections, we recently reported an additional rapid change in antibody-epitope specificity.¹¹ However, we found quite significant overlaps in the specificities displayed by IgG antibodies from allergic patients and patients receiving VIT. The large number of epitopes contained in the unpurified whole venom extract used in the first set of experiments could have masked differences. Therefore we used the more purified bee venom phospholipase A₂ (PLA₂) to investigate whether an evolution in the specificity of anti-PLA₂ IgG antibodies from patients allergic to bee venom might be better documented during the course of VIT. In addition, we also studied whether this switch could be coupled to the change in subclass hierarchy (IgG₄ predominance) usually induced by VIT and whether the switch might restore the specificity displayed by IgG antibodies from nonallergic individuals.

METHODS

Patients

Serum samples were collected from three groups of subjects. Group 1 consisted of patients allergic to bee venom. Seventeen patients (mean age ± SD, 32 ± 14 years; eight women and nine men) were selected for VIT on the basis of exhibiting a systemic reaction to bee stings. Immediate sensitivity was confirmed by intradermal skin tests with bee venom (Pharmalgen; Pharmacia, Uppsala, Sweden) and by measurement of serum-specific IgE antibodies with RASTs (Cap System, Pharmacia). Reactions to bee stings were graded according to Müller's classification¹²: class 1, 4 patients; class 2, 3 patients; class 3, 7 patients; and class 4, 3 patients. Serum samples were collected before starting VIT ($n = 17$), which we refer to as the preimmunotherapy allergic group.

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Abbreviations used

ANOVA:	Analysis of variance
OD:	Optical density
PBS:	Phosphate-buffered saline
PLA ₂ :	Phospholipase A ₂
VIT:	Venom immunotherapy

To perform a kinetic study, serum samples were also collected after 15 days ($n = 15$) and 180 days ($n = 10$) of treatment.

Group 2 consisted of long-term desensitized patients (VIT patients). Seventeen patients allergic to bee venom (mean age $\pm$ SD, 36 ± 9 years; seven women and 10 men) were treated with bee venom injections (100 to 200 μ g venom per month) for at least 2 years. Within the remaining text, sera collected from long-term treated patients (≥ 2 years) are referred to as VIT sera.

Finally, group 3 consisted of 30 healthy blood donors (mean age $\pm$ SD, 38 ± 9 years; 18 women and 12 men) with no history of systemic reaction to insect stings.

Immunotherapy protocol

An ultrarush immunotherapy protocol¹³ was used in this study. Ten incremental doses of venom were administered subcutaneously every 15 minutes. The initial dose was 0.01 μ g venom, and subsequent doses were increased until a cumulative dose of 68.5 μ g had been administered on day 1. The maximum dose (100 μ g) was reached on day 2, and the cumulative induction dose was approximately 180 μ g venom. The maintenance dose (100 μ g venom) was injected again on day 15 and thereafter once a month.

Antigen

PLA₂ was purchased from Sigma Chemical Co. (St. Louis, Mo.). Agar gel electrophoresis showed only one major oval band. Immunoprint performed with hyperimmune human pooled sera confirmed that antibody reactivity is directed toward this major band.

IgG binding to PLA₂

Specific IgG retention of native antigen was evaluated in a solid-phase ELISA. The wells of 96-well polystyrene microplates (Nunc-Immunoplates; Gibco, Merelbeke, Belgium) were passively coated with PLA₂ (1 μ g/ml in 10 mmol/L phosphate-buffered saline [PBS], pH 7.2), and additional nonspecific binding was prevented by saturation with PBS containing 1% wt/vol gelatin (Hemacel, Hoechst). After 30 minutes of incubation with ethylenediamine tetraacetic acid (10 mmol/L, pH 7.2), 100 μ l of each serum, diluted 1:25 (similar results were obtained with serum dilutions ranging from 1:12.5 to 1:100) in PBS containing 0.05% vol/vol Tween 20 (PBS-Tween) and 0.1% wt/vol gelatin, was added to separate wells and incubated overnight at room temperature. After several washes with PBS-Tween buffer, 100 μ l of a solution of peroxidase-conjugated protein A (Sigma Chemical Co.; 0.1% vol/vol in PBS-Tween) was added and incubated for 2 hours at 40° C. After a further wash, the substrate solution (0.15% vol/vol H₂O₂, 0.3% wt/vol o-phenylenediamine in 0.2 mol/L citric acid, and 0.4 mol/L sodium phosphate) was then added (100 μ l/well). The enzyme reaction was stopped by the addition of 100 μ l of 2 mol/L hydrochloric acid to each well. The binding activity of the antibodies of each serum was determined by reading the absorbance at 492 nm on a microtiter plate reader (Dynatech MR 5000; Dynatech Laboratories, Chantilly, Va.). Antibody titers were expressed in arbitrary units according to a calibration curve made with serial dilutions of hyperimmune pooled sera. The procedure, performed in duplicate, was repeated twice for each serum. Nonspecific binding was evaluated in parallel by performing the same assay with microplates previ-

ously coated with blocking buffer and no antigen. The zero control was evaluated by using antigen-coated wells and the diluent buffer in place of serum.

Competitive binding tests

IgG antibody purification. IgG fractions were separated from randomly pooled sera (12 sera from patients allergic to bee venom collected before immunotherapy and 12 sera from patients treated for at least 2 years with venom injections) by affinity chromatography on recombinant protein-G sepharose¹⁴ after IgG rheumatoid factor had been removed by adsorption on sheep red cells sensitized with rabbit hemolysins. The purified IgG fraction was then dialyzed against carbonate buffer (50 mmol/L, pH 9.5) to eliminate the glycine buffer (10 mmol/L, pH 2.0) used for elution in the purification process. The concentration of the IgG antibody solutions was evaluated by spectrophotometry.

IgG₁ and IgG₄ fractions were then separated from pooled IgG by chromatography on sheep anti-human IgG₁ or IgG₄ antibody (Behring Marburg) sepharose-CL₄B columns. IgG₁ and IgG₄ fractions were eluted from the column by using glycine buffer (10 mmol/L, pH 2.0) and immediately neutralized with tris-HCl buffer (10 mmol/L, pH 9.0). A further dialysis against the carbonate buffer was then performed. Control experiments indicated that purified IgG₁ and IgG₄ fractions were not contaminated with the other subclass. IgG₁ and IgG₄ fractions diluted 1:10 to 1:1280 in the described carbonate buffer were adsorbed passively on 96-well polystyrene microplates, and adsorption capacities were saturated with gelatin (Haemacel 1% wt/vol, Hoechst). After washing in PBS-Tween, 100 μ l of a solution of mouse anti-human IgG₁ or IgG₄ (Bioyeda) diluted 1:1000 in PBS-Tween was added. After a 2-hour incubation at 40° C and another washing, 100 μ l of the solution of goat anti-mouse IgG conjugated to peroxidase (Chemicon) diluted 1:1000 in PBS-Tween was added for a further 2-hour incubation at 40° C. Detection of binding activity was then performed as described above.

Biotinylation of IgG fractions. The pooled IgG, IgG₁, and IgG₄ fractions were incubated for 4 hours at room temperature with biotin (250 μ g/mg IgG antibody in the above described carbonate buffer). Excess biotin was then eliminated by further dialysis against PBS (10 mmol/L, pH 7.2).

Competition between biotinylated pooled IgG fractions and individual sera for binding to solid-phase-bound antigen

Polystyrene microplates (96-well) were passively coated with PLA₂ (0.1 μ g/ml in PBS), and additional nonspecific binding was prevented by saturation with PBS containing 1% wt/vol gelatin. One hundred microliters of solution containing individual sera (diluted 1:40) and biotinylated pooled IgG fraction diluted 10 μ g/ml and 5 μ g/ml for allergic and VIT fractions, respectively, in PBS-Tween containing ethylenediamine tetraacetic acid (10 mmol/L) and gelatin (1% wt/vol) were transferred, after vigorous mixing, to coated plates and incubated overnight at room temperature. After the samples had been washed three times with PBS-Tween buffer, 100 μ l of a solution of peroxidase-conjugated polystreptavidin (0.1% vol/vol in PBS-Tween) was added, and the plates were incubated for 3 hours at 40° C. After an additional washing step, the binding activity was determined as described above. The binding activities (optical density [OD]) displayed by each of the biotinylated pooled IgG fractions alone served as the reference values. The results are expressed as the percentage reduction of this (OD) value when individual sera are added to the biotinylated pooled IgG fractions. The percentage binding inhibitions were calculated as follows:

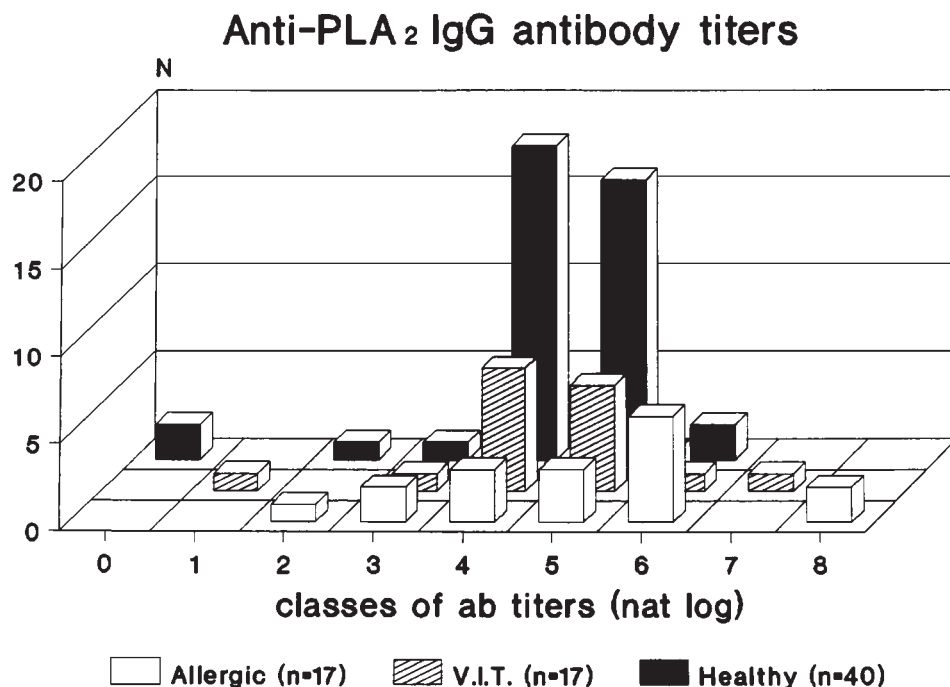


FIG. 1. Frequency histograms of specific IgG titers after natural logarithmic transformation for allergic (preimmunotherapy), VIT, and healthy control populations.

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\text{OD}_S - \text{OD}_{NS}}{\text{OD}_R - \text{OD}_{NS}} \right)$$

where OD_S , OD_R , and OD_{NS} represent OD_S in the presence of serum, OD_R in the absence of serum (reference value), and OD_{NS} corresponding to nonspecific binding. Nonspecific binding was evaluated in parallel by performing the inhibition assay exactly as described above (IgG fraction plus serum) with microplates previously coated with blocking buffer and no antigen. The zero control was measured by using coated plates and peroxidase-conjugated polystyrene. A similar trend of results was obtained with different conditions for the inhibition assays (antigen coating procedure, biotinylated IgG-antibody concentration, and serum dilutions), but the most appropriate ones were selected for the final experiments.

Competition between biotinylated pooled IgG₁ and pooled IgG₄ fractions and pooled sera

Similar competition experiments were performed with biotinylated pooled IgG₁ and IgG₄ fractions from each group (diluted 1:40 in the dilution buffer described above) and serial dilutions (1:20 to 1:320) of pooled sera from allergic patients before starting VIT and after 15 days of treatment, healthy individuals, and patients treated with VIT for 2 years. Results are expressed as binding inhibition curves.

Specificity control

We investigated whether immunotherapy with bee venom induces a change in the specificity of IgG antibodies directed toward wasp venom antigens and toward the ubiquitous unrelated allergen Der p 1 (from the house dust mite *Dermatophagoides pteronyssinus*). The capacities of sera from patients allergic to wasp venom or mites, healthy individuals, patients allergic to bee venom, and patients receiving VIT to prevent the binding of biotinylated pooled IgG frac-

tions from sera of patients allergic to wasp venom to wasp antigens (Allergon Angelholm) and from sera of patients allergic to mites to Der p 1 (Bencard Rixensart) were compared in similar competitive ELISAs. These models were selected because we have recently documented clear-cut differences in the epitope dominance of specific IgG antibodies from different populations of sera.^{11,15}

Statistics

The data were entered into a personal computer and analyzed with the aid of the SPSS statistical package (Leuven, Belgium). Group comparisons were made by parametric tests where appropriate (one-way ANOVA with Scheffe's correction for multiple comparisons) and intragroup paired data were compared with paired *t* tests.

Spearman's correlation coefficients were calculated and tested for statistical significance to evaluate the relationship between clinical variables and serum IgG titers or inhibition capacities.

RESULTS

IgG binding to PLA₂

Fig. 1 shows the frequency histograms of anti-PLA₂ IgG antibody titers after natural logarithmic transformation for the three populations under study. There are extensive overlaps between distributions, with that of the control group being slightly to the left of those of the two other groups, indicating the higher IgG levels exhibited by the latter ($p < 0.01$ by one-way ANOVA). We found no significant difference between IgG titers displayed by the allergic and VIT groups ($p > 0.05$ by one-way ANOVA). Spearman's correlation analysis did not show any influence between age, sex, and grade of reaction and the antibody titers ($p > 0.1$).

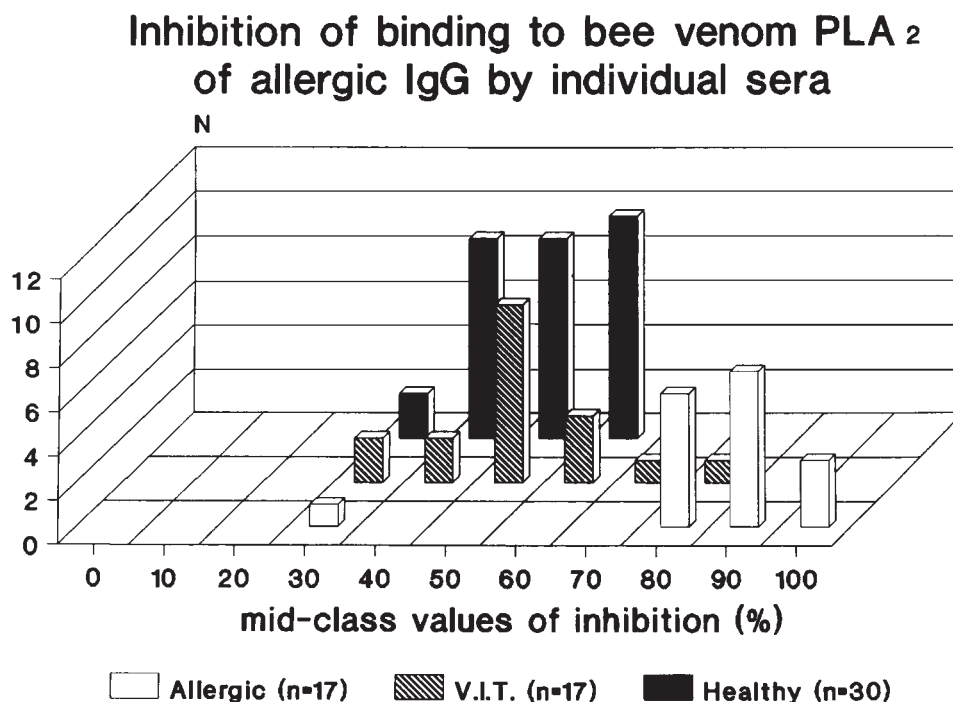


FIG. 2. Distribution of percentage inhibitions of allergic pooled IgG binding to PLA₂ by individual sera from allergic (preimmunotherapy), VIT, and healthy control groups.

Inhibition of the binding of biotinylated pooled IgG fractions

Figs. 2 and 3 show the frequency histograms of percentage binding inhibitions obtained with individual sera from the VIT, allergic, and control groups. Using the antigen-binding capacity of the biotinylated pooled IgG fraction from preimmunotherapy allergic sera as a reference (Fig. 2), we observed clearly separate distributions for preimmunotherapy allergic sera, with percentage inhibitions ($\pm$ SD) averaging $84\% \pm 14\%$, and VIT and control sera, with average percentage inhibitions of $50\% \pm 12\%$ and $49\% \pm 9\%$, respectively ($p < 0.0001$ by one-way ANOVA, reaching the significant level of $p < 0.05$ after Scheffe's correction for multiple comparisons). Using the antigen-binding capacity of the biotinylated pooled IgG fraction from VIT sera as a reference, we observed separate distributions, with percentage inhibitions ($\pm$ SD) averaging $85\% \pm 4\%$ for patients receiving VIT and mean percentage inhibitions of $60\% \pm 12\%$ and $73\% \pm 8\%$ for preimmunotherapy allergic patients and control subjects, respectively ($p < 0.0001$ by one-way ANOVA, reaching the significant level of $p < 0.05$ after Scheffe's correction for multiple comparisons) (Fig. 3).

Clinical variables such as age, sex, and severity of reaction did not influence inhibition capacities of sera ($p > 0.1$ by Spearman's correlation analysis). A negative correlation was documented when Spearman's correlation coefficients were calculated and tested for statistical significance to evaluate the relationship between IgG titers and serum inhibition capacities ($p < 0.05$ in all cases).

Kinetic of change in binding inhibition

Fig. 4 shows the development of inhibition capacity during VIT. The capacity of sera to prevent the binding to PLA₂ of the pooled allergic IgG changed, with the mean percentage inhibition ($\pm$ SD) decreasing from $84\% \pm 14\%$ before VIT to $27\% \pm 13\%$ after 15 days of treatment ($p < 0.0001$ by Student's *t* test for paired data). No further significant change was observed after 180 days of VIT, with percentage inhibitions at this time averaging $28\% \pm 7\%$ ($p > 0.1$ by Student's *t* test for paired data). However, the mean inhibition capacity of sera to prevent the binding of the pooled IgG from patients receiving VIT increased from $60\% \pm 12\%$ before VIT to $85\% \pm 6\%$ after 15 days ($p < 0.001$ by Student's *t* test for paired data) and did not change further (mean percentage inhibition, $82\% \pm 6\%$) after 180 days ($p > 0.1$ by Student's *t* test for paired data).

Specificity controls

When using the Der p 1 binding capacity of the biotinylated pooled IgG fraction from patients allergic to mites as a reference, we observed a clearly separate distribution for sera from patients allergic to mites, with percentage inhibitions averaging $90\% \pm 5\%$. Sera from the other groups exhibited percentage inhibitions averaging $37\% \pm 16\%$, significantly very different from those of the group allergic to mites ($p < 0.0001$ by one-way ANOVA, reaching the significant level of $p < 0.05$ after Scheffe's correction for multiple comparisons). No significant difference was observed in the behavior of preimmunotherapy sera from patients allergic to bee venom and sera from patients receiving VIT, with per-

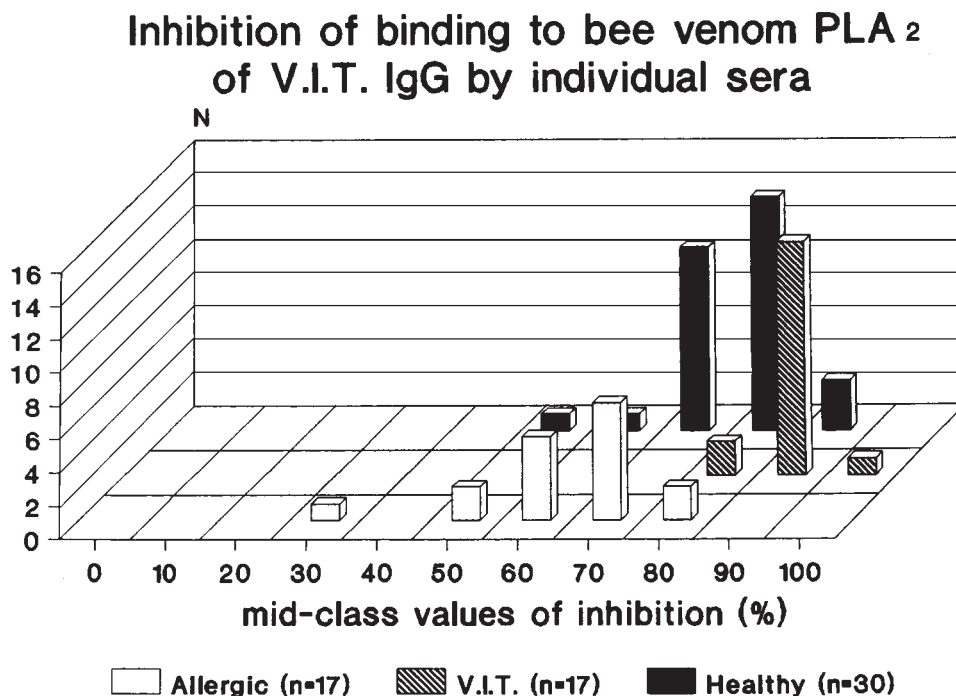


FIG. 3. Distribution of percentage inhibitions of VIT-pooled IgG binding to PLA₂ by individual sera from allergic (preimmunotherapy), VIT, and healthy control groups.

centage inhibitions averaging $32\% \pm 9\%$ and $37\% \pm 10\%$, respectively ($p > 0.1$ by one-way ANOVA; data not shown).

We then used the wasp-antigen binding capacity of biotinylated pooled IgG from patients allergic to venom as a reference. In this case a clearly separate distribution was observed for sera from patients allergic to wasp venom, with a mean percentage inhibition ($\pm$ SD) of $84\% \pm 12\%$ compared with sera from other groups whose percentage inhibitions averaged $41\% \pm 9\%$ ($p < 0.0001$ by one-way ANOVA, reaching the significant level of $p < 0.05$ after Scheffe's correction for multiple comparisons). Again, there was no significant difference between the percentage inhibitions recorded for preimmunotherapy sera from patients allergic to bee venom and sera from patients receiving VIT, with averages of $38\% \pm 7\%$ and $41\% \pm 8\%$, respectively ($p > 0.1$ by one-way ANOVA; data not shown).

Inhibition of the binding of biotinylated pooled IgG₁ and IgG₄ fractions by pooled sera

Fig. 5, A and B shows again that the antigen binding of allergic pooled IgG fraction, whatever the subclass (IgG₁ or IgG₄), is best inhibited by the pooled sera from its own clinical group. Pooled sera from other clinical groups frequently failed to completely inhibit binding when tested under the same experimental conditions and showed steeper decreases in inhibition with increasing dilution. This is particularly well illustrated in Fig. 5, A in which the inhibition capacity of the preimmunotherapy pooled allergic sera diluted 1:20 exceeded 100% and decreased only slightly with further dilution, whereas pooled sera from

the other groups only exhibited 70% to 80% inhibition and were strongly influenced by serum dilution. The inhibition values of over 100% recorded in several experiments were probably due to a matrix effect, reducing the OD to slightly under that of the blank control (dilution buffer only) in cases in which less-diluted sera added to the biotinylated IgG fraction were exerting a maximum inhibitory effect.

DISCUSSION

Specific immunotherapy is a standard clinical procedure for protecting individuals allergic to Hymenoptera venom from further insect stings.^{1,2,16-18} However, the mechanism behind its effectiveness remains unclear.

At the humoral level, it has been suggested that IgG could interfere with IgE antigen binding (*blocking activity*). This hypothesis is based on a rise in specific IgG titers (especially IgG₄ antibodies⁷⁻¹⁰) often observed during the course of VIT, which can provide passive protection.^{19,20} However, the lack of correlation between protection and IgG levels³⁻⁷ suggests that if protection is indeed IgG mediated, it cannot be monitored by the quantification of IgG titers only. Therefore IgG titer measurement is no longer a recommended procedure in the routine follow-up of patients receiving VIT.²¹

However, confirming preliminary data observed in the wasp allergy model,¹¹ this study shows that the relative stability of titers during VIT masks a significant change in antibody specificity. PLA₂, the major immunogenic and allergenic component of bee venom,^{22,23} is a 14 to 16 kd glycoprotein²³ with several distinct epitopes.²⁵⁻²⁸ Dif-

Evolution of inhibition capacities of sera during V.I.T. with bee venom

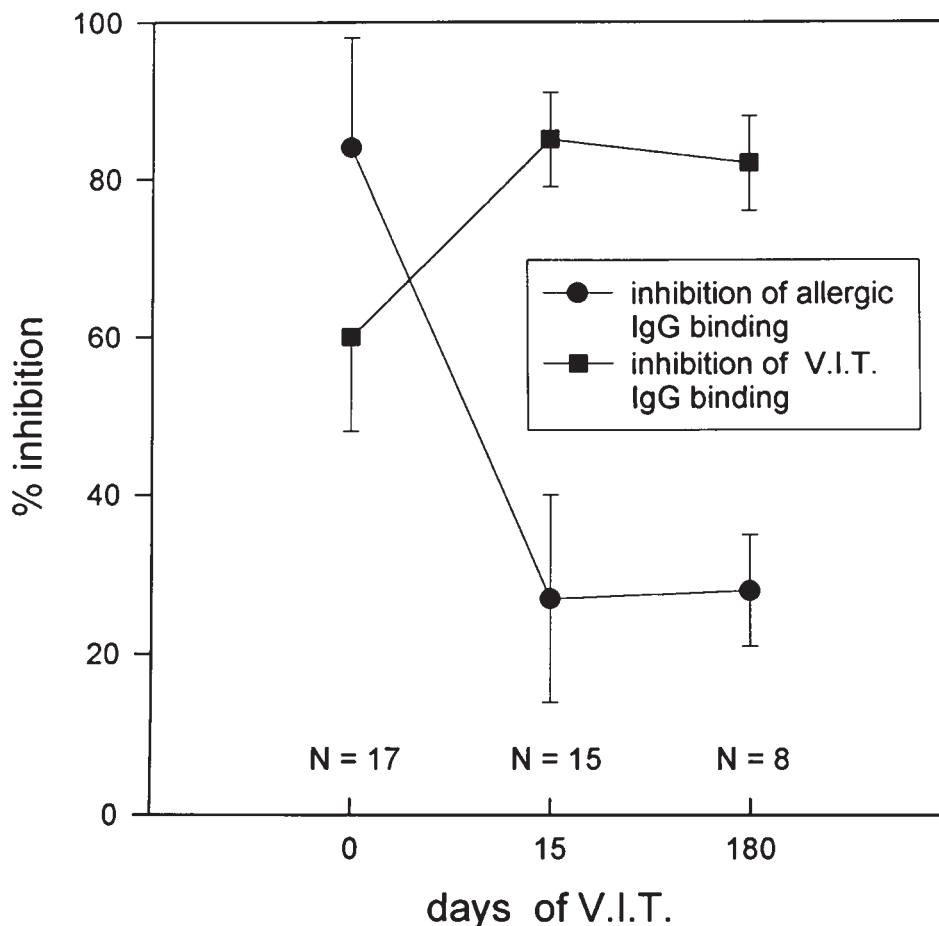


FIG. 4. Evolution of mean inhibition capacity of sera from allergic patients during VIT (binding inhibition of allergic [preimmunotherapy] and VIT-pooled IgG fraction).

ferences in epitope specificity were demonstrated through parallel experiments in which competition for antigen binding occurred between pooled IgG from each reference group and individual sera. Binding to PLA₂ of the biotinylated IgG fraction from allergic patients was more strongly inhibited by allergic sera than by VIT and control sera. Similarly, the antigen binding of pooled IgG from VIT patients was suppressed to a greater degree by VIT sera than by the two other categories of sera, the inhibitory capacities of which were nevertheless different. These results cannot be explained by direct competition for binding to the same epitopes because the IgG titers were very similar in the three populations. Furthermore, our data document a negative correlation between IgG titers and inhibition capacities of sera, which we cannot yet explain. Finally, the rise in antibody avidity occurring during VIT²⁹ is also unlikely to be responsible for the evolution of inhibition capacities of sera. Indeed, in this case the inhibitory power of the VIT sera should

be superior to that from any other sera in all situations, including competition experiments performed with allergic pooled IgG in which it was not observed. Therefore if not positively related to antibody titers and affinity, the differences in the inhibitory capacities of each group of sera must reflect antibody populations (involving at least IgG antibodies) with different specificities. This distinction appears to be antigen specific because sera from patients allergic to bee venom and sera from patients receiving VIT demonstrate similar capacities to prevent the binding of pooled IgG from patients allergic to mites and wasp stings to Der p 1 and wasp venom, respectively. The possible interference in our competitive ELISA of autologous anti-idiotypic antibodies, which are usually restored in the serum of allergic patients by VIT treatment,^{30,31} has been excluded previously.^{11,15}

Several points emerging from this study require further discussion. First, although the inhibition capacities of control and VIT sera were similar in the cases illustrat-

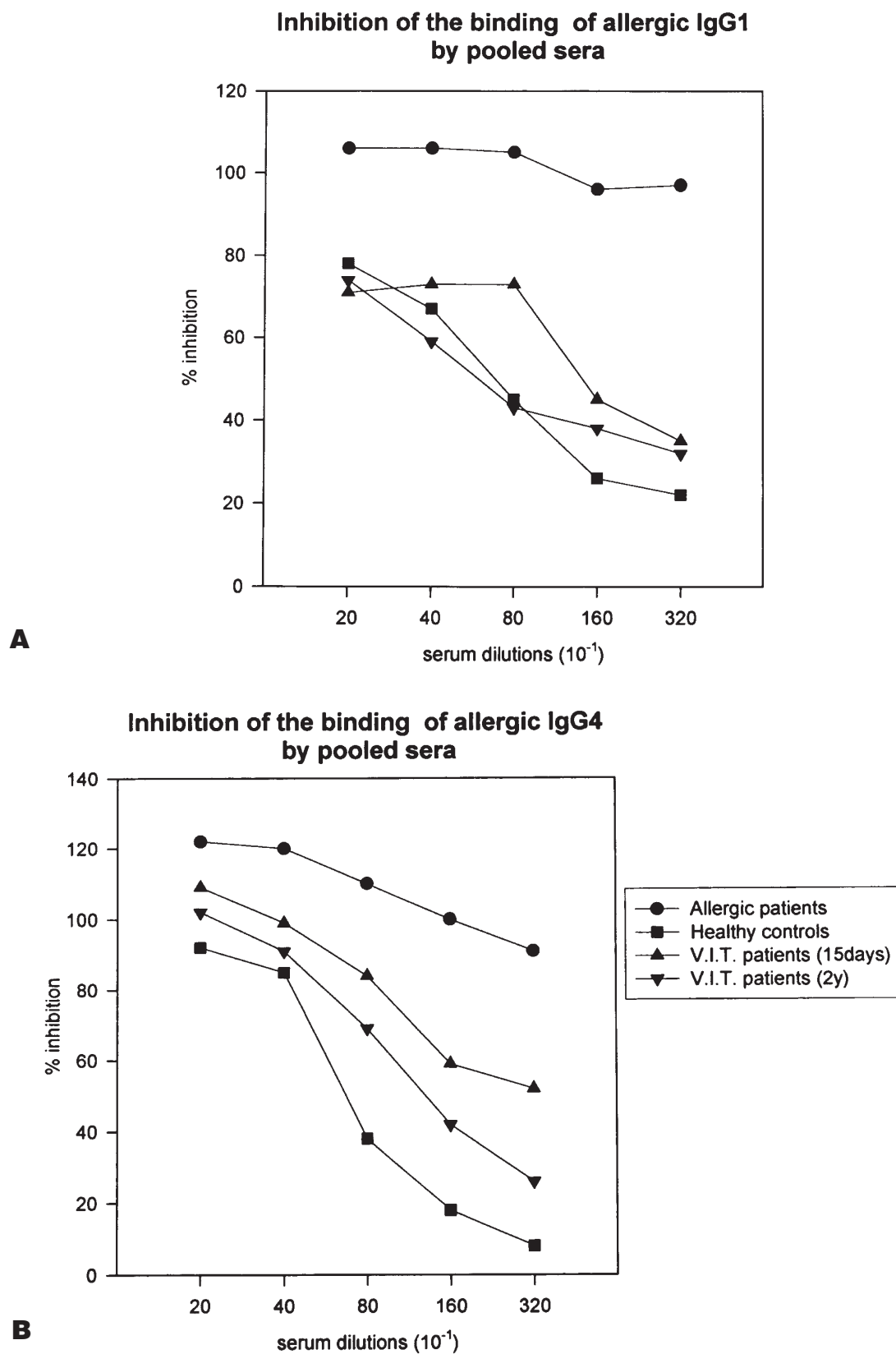


FIG. 5. Inhibition of binding of allergic pooled IgG₁ (**A**) and IgG₄ (**B**) by pooled sera from allergic patients, healthy individuals, and patients receiving VIT for 15 days and 2 years.

ed, they were not identical. This suggests that, although VIT went most of the way, it failed to completely restore the specificities peculiar to IgG from nonallergic (i.e., also protected) individuals.

Second, the serum from one allergic patient was shown to behave somewhat unusually in terms of inhibition capacities. It should be remembered that although the biotinylated pooled IgG fractions used in our experiments represent a public library of the human IgG epitopes recognized on PLA₂, this may not always include the pattern recognized by every individual. Furthermore, this particular patient may also have been sensitive to bee venom allergens other than the PLA₂ used in this study, such as hyaluronidase and mellitin.^{32,33}

Third, it is widely accepted that IgG antibodies to PLA₂ from honeybee venom are mainly restricted to IgG₁ and IgG₄ subclasses, with IgG₁ predominating in allergic patients and IgG₄ becoming increasingly predominant during prolonged VIT.^{8-10,29} It was therefore tempting to speculate that there is some link between the usual switch in IgG subclass and the change in IgG specificity. However, our results do not support this hypothesis because the new pattern of epitope dominance appears to be equally distributed over the IgG₁ and IgG₄ responses. In addition, the kinetics of these changes do not coincide. Indeed, the shift in dominance from IgG₁:IgG₄ antibody occurs slowly over months of chronic antigenic stimulation,³⁴ whereas the change in IgG specificity reported in this study occurs largely within the first 15 days, which coincides much better with the time scale needed for clinical protection. This rapid change could theoretically be due to the presence of serum antigen-antibody complex that would interfere with the inhibition ELISA. However, this possibility is unlikely because PLA₂-antibody complex should interfere similarly in the parallel experiments with purified VIT and allergic IgG. This was not observed. With regard to the mechanism responsible for the change in IgG reactivity, the large amount of antigen administered initially (180 µg of venom in 2 days) in the ultrarush VIT protocol could favor the dominance of new B-cell clones. Indeed, Kolbe et al.³⁵ have recently reported a shift in the immunodominant IgG epitopes recognized on PLA₂ when mice are immunized with high, as opposed to low, immunogen doses. Their findings also suggest that the IgG antibodies generated by a high-dose regimen display a specificity spectrum close to that of IgE and consequently exert a blocking effect on IgE activity. Such a functional relevance of the change in IgG antibody specificity (i.e., to control antigen recognition by IgE) remains to be investigated in the human model of VIT. If documented, this would indicate that, contrary to usual belief,^{25,36} immunoprotection is not a function specifically restricted to IgG₄ antibodies. Also, it is still unknown whether VIT changes IgE specificity as has been shown in grass pollen immunotherapy, although this was at the major allergen level rather than epitope level.³⁷ Some preliminary data seem to support this hypothesis in the bee-venom allergy model.³⁸

Finally, the antigen dose, as discussed above, can also

modulate cytokine production by CD4⁺ T-helper cells,³⁹ switching off the harmful T_{H2} allergic response.⁴⁰⁻⁴⁷ Because antibody production is regulated by T-cell derived factors, it is tempting to speculate on a link between the changes in T-cell reactivity and IgG antibody specificity, both of which occur early during the course of VIT. Newly generated T cells, although continuing to promote B-cell antibody production⁴⁵ with a shift in isotypic hierarchy,⁴⁸ would cause a change in the relative dominance of different B-cell clones. This also remains to be investigated by *in vitro* experiments.

In conclusion, this study demonstrates that patients allergic to bee venom display a specificity spectrum of anti-PLA₂ IgG antibodies distinct from that of individuals protected either naturally (i.e., healthy control subjects) or by successful immunotherapy. VIT restores, although not completely, the immunodominant pattern exhibited by the IgG of healthy individuals, even though the clinical relevance of this change is presently unknown. This rapidly occurring change involves both IgG₁ and IgG₄ responses and is thus not directly related to the more slowly developing change in subclass hierarchy usually observed during VIT. Together our results define, in addition to the usual isotypic distinctions (i.e., IgE:IgG₄ ratio), a new kind of humoral signature in the immune response to bee venom. Allergic status and immunoprotection correlate with the preferential expression of distinct dominant IgG specificities.

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