

Correspondence

Reverse-sandwich ELISA may underestimate antibody titers

To the Editor:

We would like to comment on the article by Miyazawa et al., "A reverse-sandwich ELISA for IgG antibody to a pollen allergen" (J ALLERGY CLIN IMMUNOL 1988;82:407-13.¹). A reverse-sandwich ELISA, with biotinylated allergen and streptavidin-conjugated β -D-galactosidase, was suggested as an alternative for an antigen-binding assay with radiolabeled allergen. It was claimed to detect a total activity of IgG, IgA, and IgE antibodies in the serum of patients with pollinosis.

In the course of hyposensitization therapy, the allergen-specific IgG-antibody population shifts from IgG1 to IgG4.² The IgG4 subclass differs from the other subclasses by functional monovalency.³ The reverse-sandwich ELISA is a two-side assay with immobilized allergen and biotinylated allergen. Only bivalent antibodies can be detected in such an assay. With the suggested ELISA, IgG titers will be underestimated, particularly when IgG4 is predominating.

This is illustrated by the results presented in Fig. 1. We selected 25 sera for IgG antibodies to *Lol p I* (Rye I), a major allergen from *Lolium perenne* pollen.⁴ IgG antibodies to *Lol p I* were detected in an antigen-binding assay with purified ¹²⁵I-labeled *Lol p I*⁵ and Sepharose-coupled protein A as IgG-capturing agent.⁶ The sera were tested in 1:40 dilutions (50 μ l per test). Under the same conditions, they were also tested with monoclonal antibody to IgG4 coupled to the Sepharose instead of protein A.⁶ As an equivalent of the reverse-sandwich ELISA, we tested the sera (in 1:8 dilutions) in a two-side assay with Sepharose-coupled *L. perenne*-pollen extract and ¹²⁵I-labeled *Lol p I*. The appropriate *Lol p I* density on the solid phase was achieved by coupling 0.5 mg of pollen extract to 100 mg of CNBr-activated Sepharose. All sera were also tested for IgA antibodies to *Lol p I* with monoclonal antibody to IgA coupled to Sepharose.

The 25 sera demonstrated percentages binding in the protein A test between 10% and 60%. In 12 cases, IgG4 was responsible for >75% of the allergen-binding capacity of the serum. For the remaining 13 sera, the contribution of IgG4 was <35%. The group with a high IgG4 contribution scored significantly lower in the two-side assay. The correlation between the two-side assay and the protein A test is low ($r = 0.19$). For IgG4 and protein A test, the correlation coefficient is 0.75; for protein A and a combination of two-side and IgG4 assay, the correlation coefficient of the multiple regression analysis is much higher ($r = 0.91$). It could be argued that the sera that were relatively high in the two-side assay had high contributions of IgA or IgE to the allergen-binding capacity. However, the antigen-binding activity of IgA was <3% of the binding activity measured in the protein A assay. To exclude significant contribution of isotypes not bound by protein A, we incubated three of the low IgG4 sera with Sepharose-coupled protein A and tested the supernatant in both protein A and two-side assay; >90% of the two-side assay activity was removed by the protein A Sepharose.

Predicting IgG antibody levels from results of a two-side assay is unreliable because IgG4 will not be detected. Our conclusion is that a reverse-sandwich ELISA by itself is not an appropriate method for monitoring the effect of immunotherapy.

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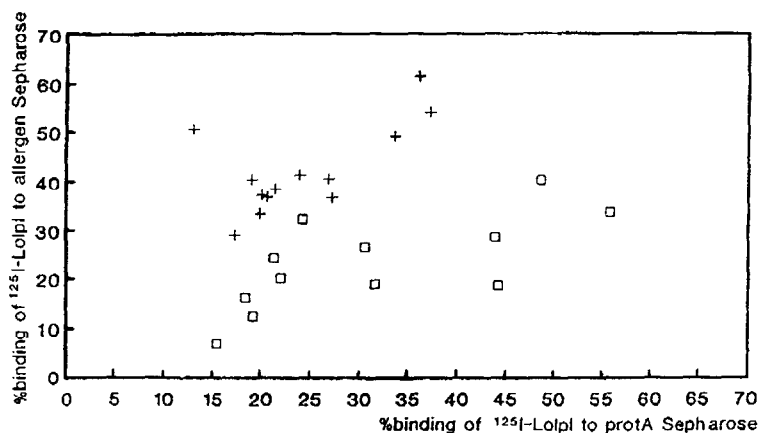


FIG. 1. Sera with high IgG4 levels are underestimated in the two-side assay; patients with a low IgG4 contribution (<35%) (+); patients with a high IgG4 contribution (>75%) (□).

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Reply

To the Editor:

We agree with the claim of van Ree et al. that the reverse-sandwich (RS) ELISA does not detect monovalent antibodies. After publishing our article, we have studied the relationship between antibody activities as determined by the RS ELISA after absorption of IgE from the sera and by an indirect ELISA for IgG4 antibody. In the latter assay, we incubated the sera in 1:10 dilutions for reaction with the solid-phase antigen and used a 1:1000 dilution of peroxidase-conjugated monoclonal antibody to IgG4 (Yamasa Shoyu Co., Chiba, Japan); there was no nonspecific reaction in control sera collected from individuals living in a pollen-free area. We found that there was a poor correlation between the above two activities; furthermore, some sera were positive only by the IgG4 ELISA (manuscript in preparation). These data suggest that IgG4 antibody, which may be functionally monovalent,¹ is not detectable by the RS assay and that IgG4 antibody is different from other non-IgE RS antibodies as to host's antibody response to the allergen.

However, we think that the RS assay is still useful for investigating the antibody response to allergens for the following reasons: First, we found that both IgG4 and RS antibody concentrations were significantly higher ($p < 0.05$) in the sera from hyposensitized patients

than from untreated patients with Japanese-cedar pollinosis; geometric mean concentrations were $10^{1.09 \pm 0.31}$ and $10^{1.46 \pm 0.46}$ arbitrary units per milliliter, respectively, in the former group ($n = 14$), whereas they were $10^{1.55 \pm 0.44}$ and $10^{1.05 \pm 0.26}$ units per milliliter, respectively, in the latter group ($n = 8$). Second, we have been experimenting pullulan-conjugated allergens for immunotherapy.²⁻⁵ When we injected the allergen-pullulan conjugate to antibody-negative volunteers, we found that some volunteers responded with high titers of RS antibody (probably IgG1) but without IgG4 and IgE antibodies, even after repeated injections (unpublished data). Third, we compared IgE, IgG4, and RS antibody-positive rates between forestry workers who had been working with Japanese-cedar trees and age-matched control individuals residing in urban Tokyo. As presented in Table I, positive rate of only RS antibody was higher in the forestry workers; IgG, excluding IgG4, antibody was produced more prevalently in individuals who had been exposed to the allergen for longer years.

In conclusion, for monitoring the IgG antibody response both to natural allergen exposure and to immunotherapy, we think that the assaying of not only IgG4 but also other subclass antibodies is important. Although relative antibody contents among different subclasses are not comparable, the combination of the RS ELISA and the indirect IgG4 ELISA is a simple and inexpensive procedure for the above purpose.

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TABLE I. Antibody-positive rates to Japanese cedar-pollen allergen (Cry j I)

Group	Average age (yr)	Antibody-positive rate (%)		
		IgE (RAST ≥ 2)	RS (≥ 10 U/ml)	IgG4 (≥ 3 U/ml)
Forestry workers (n = 59)	57.2	13.6	40.7	18.6
Urban residents (n = 50)	55.3	12.0	24.0	16.0

NS. Not significant.