

Short communication

IgE responses to *Dermatophagoides pteronyssinus* native major allergens Der p 1 and Der p 2 during long-term specific immunotherapy

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We investigated by ELISA the IgE response to whole extract of the house-dust mite *Dermatophagoides pteronyssinus* (Dp) and to the native major allergens, Der p 1 and Der p 2, in sera from 18 adult patients (group A) with Dp-allergic asthma before (t_0) and 1, 2, 3, and 4 (t_1 – t_4) years after subcutaneous specific immunotherapy (SIT). A qualitative reduction ($P=0.05$) of the IgE responses to Dp and Der p 2 was observed from t_1 to t_4 , but a highly statistical significant decrease appeared at t_3 ($P<0.01$). With regard to Der p 1 IgE values, the immunotherapy induced a significant decrease ($P<0.01$) at t_3 , but not before. In group A, the IgE responses to Der p 1 and Der p 2 were not correlated at t_0 ($r_s=0.31$; $P=0.21$) but were correlated at t_3 ($r_s=0.78$; $P=0.001$). We also examined sera from 14 adult patients (group B, same SIT schedule as group A) who were without respiratory symptoms at the end of the third year (t_3) of Dp SIT. At this time (t_3), there were no significant differences in Der p 1 and Der p 2 IgE levels between group A and group B.

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An emerging body of evidence suggests that the immunologic recognition of the major allergens of the house-dust mite *Dermatophagoides pteronyssinus* (Dp), Der p 1 and Der p 2, may differ in allergic and normal subjects. In fact, in a recent study on the physiologic basis of the T-cell response to Der p 1 and Der p 2 under standardized conditions (1), we confirmed an earlier report (2) that in normal subjects Der p 1 is not immunogenic and that Der p 2 exerts a powerful, not class II major histocompatibility complex (MHC)-restricted polyclonal mitogenic activity. This finding supports the view that Der p 2 could act as a coligand or mimic the ricin toxin costimulatory action (1). Moreover, in a study on the natural humoral response to the mite *Lepidoglyphus destructor*, Johansson et al. (3) reported the absence of a specific serologic

response in the allergen-exposed healthy group, and concluded that the “healthy” immune system seems to “ignore” inhaled allergens.

Therefore, we hazarded the hypothesis that in normal subjects no MHC-restricted response is elicitable by mite molecules, and, if any response develops, it must pass through IgE with a priming induced by particular molecules that could be more than simple allergens. In other words, we suggested that IgE could sometimes represent a physiologic step of the immunoresponse to certain antigen/allergen systems.

Thus, this study aimed to examine, according to this assumption, the different IgE response to Der p 1 and Der p 2 in the sera of two series of patients affected by respiratory allergy, before and during subcutaneous specific immunotherapy

(sc SIT), and to determine whether a causal relationship exists between the two responses.

Material and methods

Sera

Serum samples were obtained from two patient groups. Group A sera came from 18 adult subjects affected by Dp respiratory allergy (asthma), previously recruited for another study. For each patient, samples harvested at the time of the diagnosis (t_0) and at the end of the first (t_1), second (t_2), third (t_3), and fourth (t_4) years of sc SIT were available. Dp SIT had been performed with commercial extracts standardized in biologic units (4), according to the administration schedule suggested by the manufacturer (ALK-Abellò, Madrid, Spain).

Because we did not have complete clinical data for all group A subjects during the long-term SIT, we selected another, distinct series of patients. Group B sera came from 14 adult patients at the end of the third (t_3) year of Dp SIT (with the same administration schedule as group A), with SIT prescribed after a diagnosis of HDM-Dp-allergic asthma. The inclusion criterion was that all the patients must be in complete remission of the respiratory symptoms (clinical recovery).

Mite extract

D. pteronyssinus extract was prepared from whole-mite cultures (ALK-Abellò). Proteins were extracted with gentle shaking at room temperature for 1 h in 0.05 M ammonium bicarbonate buffer 1/10 (w/v) at pH 8. The homogenate was then centrifuged at 22 000 g for 30 min at 4°C, and the obtained supernatant was passed through a 0.2- μ m filter (Millipore, Bedford, MA, USA) after extensive dialysis against distilled water. The protein content was determined by the Lowry method (5).

Purification of Der p 1 and Der p 2

Der p 1 and Der p 2 major allergens were purified from aqueous extract of *D. pteronyssinus* culture by monoclonal antibody (mAb) affinity chromatography, as described elsewhere (1). Briefly, Der p 1 was eluted from mAb Pt 1.5.13 immunosorbent by using 100 mM glycine, pH 2.5. Der p 2 was eluted with the same buffer from mAb CLB@ DpX immunosorbent. Separate immunoaffinity columns were used for each allergen. Eluted fractions were pooled on the basis of protein content as detected by absorbance at 280 nm, dialyzed against distilled water, lyophilized, and frozen at -40°C until use. Allergen purity was assayed by SDS-PAGE.

ELISA

D. pteronyssinus (Dp)-, Der p 1-, and Der p 2-specific serum IgE were measured by ELISA. Polystyrene microtiter wells (Costar, Cambridge, MA, USA) were incubated at 37°C for 1 h with 50 μ l/well of Dp extract (40 μ g/ml) or purified allergens in coating solution (0.1 M bicarbonate buffer, pH 9.6) at 10 μ g/ml of concentration. After washing three times with PBS 0.1% Tween 20 (PBS-T), wells were saturated with 150 μ l of a PBS-BSA 1% solution for 15 min and washed again with PBS-T. Serum samples (50 μ l) were added to the allergen-coated wells and the wells were incubated for 2 h at room temperature. After washing four times with PBS-T, 50 μ l of a 1/1500 anti-human IgE mAb peroxidase-conjugated (6) PBS solution (BSA 1%, Tween-20 0.05%) was added to each well, and the wells were incubated at room temperature for 1 h. After washing as above, 50 μ l of the OPD substrate (*o*-phenylenediamine dihydrochloride 1 mg/ml in 50 mM citrate-phosphate buffer, 0.03% H₂O₂, pH 6) was added to the wells and incubated for 30 min, at room temperature, in the dark. After the reaction was stopped with 50 μ l of HCl 2 M, the color developed was read at 492 nm in an automated ELISA reader. The results, compared with a reference curve obtained by measuring in parallel standard sera of known concentrations, were expressed in RAST units/ml (RU/ml). Quantitative results were reproducible within a factor of 2.

Statistics

The two-way analysis of variance for randomized blocks was used to compare the mean values of Dp-, Der p 1-, and Der p 2-specific IgE, in group A, from t_0 to t_4 . In the same group, multiple comparison of mean values of the Dp-, Der p 1- and Der p 2-specific IgE assessed at t_1 , t_2 , t_3 and t_4 with the t_0 values, was performed with the Bonferroni *t*-test and the Duncan test. The comparison between group A and group B Der p 1- and Der p 2-specific IgE concentrations at t_3 was performed with modified Student's *t*-test for unequal variance. The correlations between Der p 1 and Der p 2 IgE responses in group A at t_0 and t_3 were evaluated with Spearman's nonparametric test.

Results

The analysis of variance applied to group A showed a significant difference ($P < 0.001$) between mean values of the Dp-, Der p 1-, and Der p 2-specific IgE, from t_0 onward. The same analysis

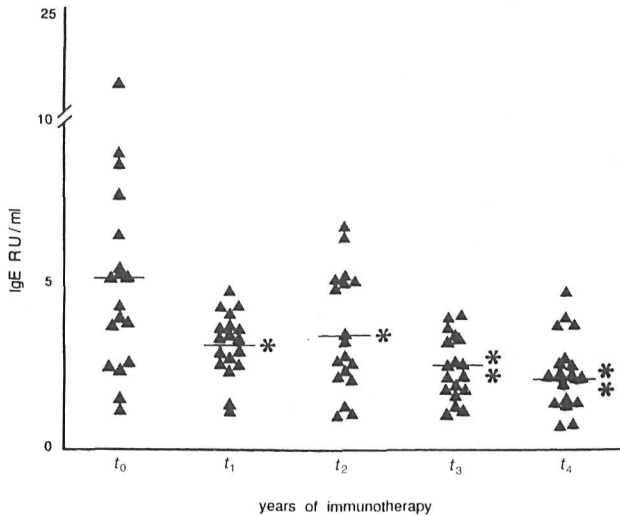


Fig. 1. Levels of specific IgE to *D. pteronyssinus* whole extract (triangles) in sera of patients (group A) with allergic asthma before starting specific immunotherapy (t_0) and at following years (t_1 – t_4) of treatment. Values are expressed in conventional RU/ml. Bars=means. * $P=0.05$; ** $P<0.01$.

showed a significant difference ($P<0.001$) between subject blocks.

In group A, the multiple comparison between mean values of the Dp- (Fig. 1), Der p 1-, and Der p 2-specific IgE (Fig. 2) at t_0 vs t_1 – t_4 showed a qualitative decrease ($P=0.05$) for Dp and Der p 2 since t_1 , but a highly statistically significant decrease ($P<0.01$) appeared at t_3 . With regard to Der p 1-specific IgE values, a significant decrease ($P<0.01$) was evident at t_3 , but not before (Fig. 2).

IgE responses to Der p 1 and Der p 2 in the second series of patients (group B), at the third year of SIT (t_3), were not statistically different from those of group A at the same SIT time (t_3), since the Der p 1 IgE mean value was 1.22 (SD 2.95) ($t_{15}=0.49$, $P=NS$), and the Der p 2 IgE mean value was 3.01 (SD 5.21) ($t=0.39$, $P=NS$) (Fig. 3).

The IgE responses to Der p 1 and Der p 2 in group A were not correlated at t_0 ($r_s=0.31$, $P=0.21$), but did correlate at t_3 ($r_s=0.78$, $P=0.001$).

Discussion

IgE levels in group A patients confirmed Der p 2 as the most immunologically active molecule with respect to Der p 1; in fact, the quantitative IgE response to the former allergen was always higher than to the latter. Furthermore, in the same group, the SIT-induced reduction of the Dp and Der p 2 IgE values throughout the study showed the Der p 2 response to be the relevant one among the others addressed to the whole extract.

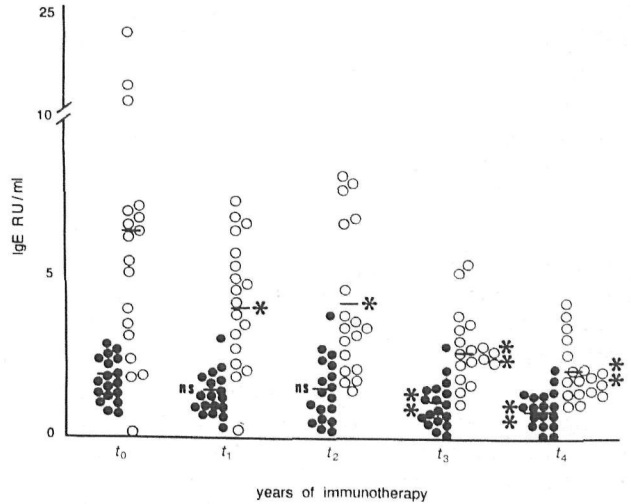


Fig. 2. Levels of specific IgE to native purified major allergens Der p 1 (closed circles) and Der p 2 (open circles) in sera of patients (group A) with allergic asthma before starting specific immunotherapy (t_0) and at following years (t_1 – t_4) of treatment. Values are expressed in conventional RU/ml. Bars=means. * $P=0.05$; ** $P<0.01$.

Nevertheless, even if Der p 1 IgE levels were very low from the beginning of the study, the significant reduction induced by SIT only at t_3 suggests caution in discounting the Der p 1 role in allergic asthma. Indeed, as a constituent of mite feces, it is readily airborne (7) with respect to the “body” allergen (8).

In the second series of patients (group B), which was comparable with group A for diagnosis and SIT, the Der p 2 IgE values at t_3 were not significantly different from the Der p 2 IgE assessed in group A sera at the same SIT time. Because the inclusion criterion in group B was “the complete remission of respiratory symptoms”, it is probable that in group B the clinical improvement induced by SIT was related to the reduction of Der p 2 IgE. This assumption coincides with the results obtained in 12 patients (Fig. 3) because Der p 2 IgE levels in their sera were low. However, the high levels of Der p 2 IgE found in the sera of two patients who were asymptomatic raise the question: why were these antibodies apparently not pathogenic in these subjects?

To exclude the possibility that the values obtained in these patients could be due to a measurement error or to an atypic member of the studied population, we repeated the test and verified the recruiting criteria for group B. The results confirmed the previous findings.

The question of the exact contribution of the carbohydrate moiety to the overall reaction of an allergen (9) was first raised when allergens started to be characterized in the 1960s (10). In 1981,

Aalberse et al. (11) described a periodate-sensitive, heat-stable antigen recognized by IgE from human sera and present not only in many unrelated foods but also in pollen and often in vespid venom (cross-reacting carbohydrate determinant [CCD]). However, it was only recently that van Ree et al. (12), in a study of the purification of the novel major allergen Lol p XI from *Lolium perenne*, recognized the existence of sera with a predominantly anticarbohydrate IgE response, raising the question of the role of these anticarbohydrate antibodies in allergy.

Since the sugar epitope described in Lol p XI is highly reactive with the mAb Dp6, obtained after the immunization of Balb/c mice with *D. pteronyssinus* extract and specific for a carbohydrate determinant (12), it is possible that the immunotherapy could have already acted on such a component of the response, leaving the anti-peptide IgE as memory antibodies.

In this debate, the study of the correlation between Der p 1 and Der p 2 responses is intriguing. In fact, in patients suffering from asthma (group A, t_0), the IgE responses to the major allergens investigated were not related ($P=0.21$), but there was a significant correlation in the same patients at t_3 ($P=0.001$).

This finding suggests that SIT may induce a synchronization between the two serologic responses. In other words, such a coordinated IgE response could be regarded more as a physiologic than pathologic mechanism.

The fine mechanisms of antigen-allergen T and B restriction in normal and allergic subjects have yet to be clarified. In fact, it is being debated whether the linear-immunodominance model (continuous epitopes) applies to the restriction of Der p 1 and Der p 2 by the T-cell receptor and MHC in normal subjects (1). Similarly, it is being debated whether the currently accepted model of restriction applies to the B-cell receptor (Ig) recognition of mite antigens in normal subjects.

The absence of an IgE and IgG response in normal subjects described by Johansson et al. (3) strongly suggests a lack of serologic immunogenicity of the *L. destructor* antigens in the physiologic system. Moreover, Freudemberger et al. (14) reported that none of the normal subjects investigated were positive for specific serum IgG antibodies to the dust mite *D. farinae*.

The report by Oshika et al. (15) that 20% of controls were positive for Der p 2-specific IgG raises the question of whether the investigated controls were actually healthy "nonallergic" or healthy "postallergic" subjects.

In conclusion, in our study, conventional immunotherapy induced a highly significant reduc-

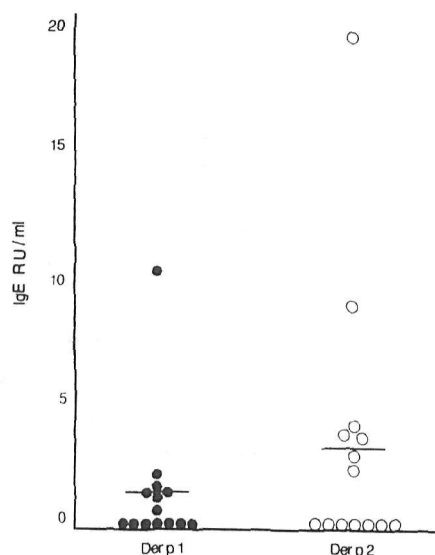


Fig. 3. Levels of specific IgE to native, purified major allergens Der p 1 (closed circles) and Der p 2 (open circles) in another series of patients (group B) with complete remission of respiratory symptoms at third year of specific immunotherapy. Bars=means.

tion of the Der p 1 and the Der p 2 IgE response only after the third year of treatment. This suggests that one must be cautious in generalizing the results of controlled clinical trials lasting less than 3 years (16, 17). Furthermore, in addition to the changes usually obtained with SIT (18), long-term treatment resulted in a significant correlation between the IgE responses to the two major allergens investigated.

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