

IgG1, IgG4 and IgE Antibody Reactivity to Mutant Forms of the Major Dust Mite Allergen Lep d 2 among Atopic and Nonatopic Subjects Naturally Exposed to *Lepidoglyphus destructor*

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Key Words

Allergen · Atopy · Disulfide · ELISA · Farmer · IgE · IgG subclass · Mutant · Hypoallergenic variants

Abstract

Background: *Lepidoglyphus destructor* is a common dust mite causing IgE-mediated sensitization. The major allergen, Lep d 2, has previously been cloned and expressed as several double Cys to Ser mutants with the purpose of producing hypoallergenic variants for immunotherapy. Our aim was to investigate the reactivity pattern of IgG1, IgG4 and IgE antibodies to wild-type (wt) rLep d 2 and four mutants among atopic and nonatopic subjects in relation to sensitization and exposure to *L. destructor*. **Methods:** Inhibition and sandwich ELISA were used to compare IgG1, IgG4 and IgE antibody reactivities to rLep d 2 variants in serum of 20 atopic and 18 nonatopic farmers naturally exposed to *L. destructor*. A group of 22 urban subjects served as controls. **Results:** Atopic farmers demonstrated correlating IgE and IgG4 levels to rLep d 2(wt) ($r_s = 0.70$; $p < 0.0001$) which were significantly ($p < 0.0001$) higher than those of nonatopic farmers and urban controls. No IgG4 antibodies were detected in nonatopic farmers despite chronic allergen exposure. A parallel reactivity pattern of IgE and IgG4 to all rLep d 2 mutants was observed. The mutant lacking all 3 disulfide bonds, rLep d 2.6Cys, demonstrated nei-

ther any IgE nor IgG4 reactivity. In contrast, IgG1 antibodies had a different reactivity pattern and were detected among most subjects irrespective of atopy, exposure to *L. destructor* or disulfide impairments in rLep d 2. Moreover, IgG1 levels to rLep d 2(wt) and rLep d 2.6Cys correlated ($n = 60$; $r_s = 0.65$; $p < 0.0001$). **Conclusions:** IgE/IgG4 Ab to rLep d 2 were restricted to atopic farmers and demonstrated parallel recognition patterns of conformational epitopes. In contrast, IgG1 antibodies are ubiquitously found and mainly recognize sequential structures. The observed isotypic difference and interindividual variation in antibody specificities among atopic and nonatopic subjects imply careful investigation of hypoallergenic variants destined for immunotherapy.

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Introduction

Allergy to dust mites is a problem in most parts of the world and many of the individual allergens have now been identified and characterized. It has been established that besides house dust mites also storage mites lead to sensitization and clinical symptoms [1]. In farming environments the storage mite *Lepidoglyphus destructor* is one of the dominating sources of allergens causing respiratory allergy [1, 2].

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Allergen-specific immunotherapy is classically used to treat allergic disorders such as hay fever and asthma. Although the underlying mechanisms are still largely unsolved clinical improvements have been associated with changes in T cell responses [3, 4], suppression of IgE antibody synthesis and induction of mainly IgG1/IgG4 blocking antibodies (Ab) [5–7]. Allergen-specific IgG Ab but at lower levels are also commonly found among untreated atopic subjects [5–7]. Modified recombinant (r) allergens having reduced IgE but preserved T cell reactivity are presently under investigation as potential tools for improved immunotherapy [8]. Modifications of entire allergens have an advantage over T cell-reactive peptides as a more complete epitope repertoire is represented. The risk of adverse reactions is reduced and induction of new B cell responses to various components present in whole extracts is avoided [9].

Oligomerization, fragmentation and site-specific mutagenesis have been used to produce several hypoallergenic recombinant variants of common major allergens, e.g. Bet v 1, Der p 2 and Der f 2 [8, 10–13]. One approach to produce hypoallergenic variants of the group 2 house dust mite allergens Der p 2 and Der f 2 has been to eliminate proper disulfide bridge formation by replacing cysteine (Cys) with other amino acid residues [10–13]. In vivo studies on mice and monkeys have shown that the mutant Der f 2 (C8/119S) can provide desensitization comparable to that of wild-type allergen [14, 15].

The major allergen of *L. destructor*, Lep d 2, has been cloned [16]. It demonstrates a 40% sequence identity with the group 2 allergens Der p 2 and Der f 2 and the six cysteine residues forming 3 disulfide bonds are highly conserved among this group of allergens [17]. With the purpose of producing hypoallergenic variants of rLep d 2 several mutants having Cys to Ser substitutions were previously expressed [18]. One mutant, rLep d 2.6Cys, lacking all three naturally occurring disulfide bonds, had no detectable in vitro IgE reactivity. Moreover, we recently demonstrated that most *L. destructor*-sensitized subjects tested in skin prick test were negative to this mutant while significant cellular responses were recorded [Kronqvist et al., Eriksson et al., unpubl. data].

The aim of this study was to compare the IgG1, IgG4 and IgE Ab reactivity patterns to wild-type and mutant forms of rLep d 2 among atopic and nonatopic subjects with an expected high (farmers) or low (urban population) natural exposure to *L. destructor*. The results provide information on isotypic and interindividual differences in the humoral immune response in relation to structure and usefulness of modified allergens in immunotherapy.

Material and Methods

Recombinant Allergens

Cloning, *Escherichia coli* expression and affinity purification of wild-type (wt) and mutant forms of rLep d 2 have been described [16, 18]. Briefly, mutants including a C-terminal 6 × His-tag were obtained by site-directed mutagenesis of the isoform Lep d 2.01 [16]. In 3 double mutants, C8/117S, C21/26S and C72/77S, two paired Cys positions were mutated to Ser resulting in the removal of one disulfide bond each. In one mutant, Lep d 2.6Cys, all 6 cysteines were mutated leading to removal of all 3 disulfide bonds in Lep d 2. The control allergen rMal f 1, from the yeast *Malassezia furfur*, was similarly produced (kindly provided by Dr. P. Whitley).

Serum Samples

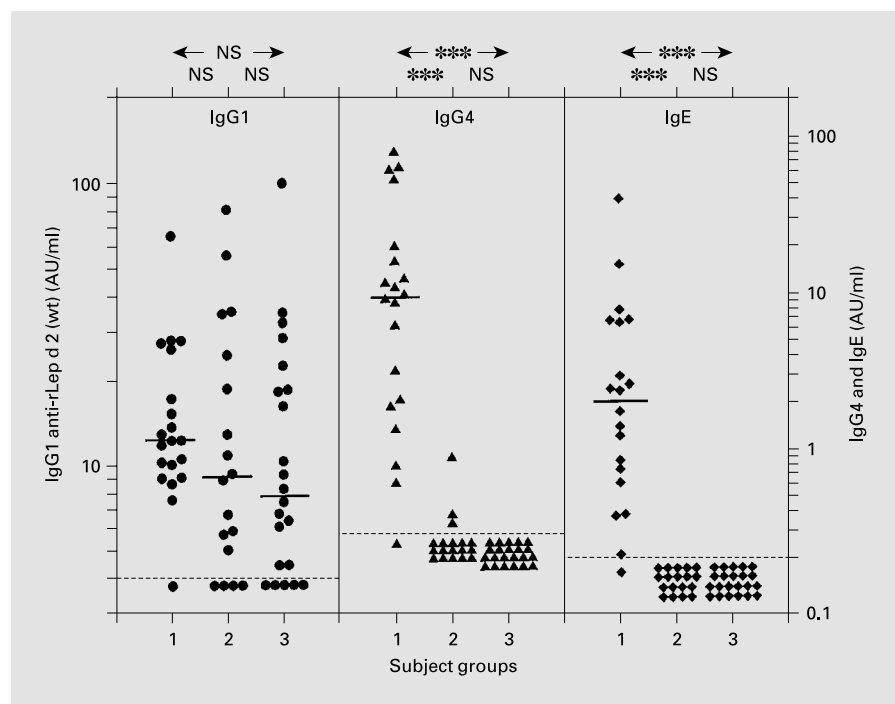
Serum samples from a total of 60 subjects were selected and divided into four groups according to IgE-CAP results (CAP System RAST FEIA, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) and degree of exposure to the dust mite *L. destructor*. Group 1 comprised 20 sera taken from atopic farmers suffering from asthma (n = 2), rhinoconjunctivitis (n = 4) or both (n = 14). These 20 farmers had a positive (≥ 0.35 kU/l) IgE-CAP to both *L. destructor* extract (median 2.7 kU/l; range 0.47–17) (d71; Pharmacia & Upjohn Diagnostics AB) and to rLep d 2 (median 4.5 kU/l; range 0.39–29) [19]. Group 2 included 18 sera from nonatopic farmers having a negative IgE-CAP to rLep d 2 and to 22 common allergens. Groups 3a and b represented 22 sera from an urban population including 11 atopic subjects positive in IgE-CAP to birch (t3; Pharmacia & Upjohn Diagnostics AB) but negative to *L. destructor* extract (d71), and 11 non-atopic subjects with a negative IgE-CAP to both *L. destructor* extract and Phadiatop (Pharmacia & Upjohn Diagnostics AB) screening test. The farmers from groups 1 and 2 are occupationally exposed to *L. destructor* [2] whereas the urban subjects from groups 3a and b are considered to have had a low natural exposure. None of the subjects had undergone *L. destructor*-specific immunotherapy.

Antibody Assays by ELISA

Allergen-specific IgG1, IgG4 and IgE Ab were determined by sandwich ELISA using monoclonal Ab essentially as previously described [20, 21]. Briefly, microtiter plate wells were coated with purified allergens (5 µg/ml). All serum samples were assayed at a final 50-fold (IgG1), 10-fold (IgG4) or 5-fold (IgE) dilution following preincubation with the corresponding allergen (10 µg/ml) or diluent alone. The net results in optical density at 405 nm were used to interpolate the concentrations of Ab from standard curves of chimeric IgG1, IgG4 and IgE anti-NIP hapten Ab run in parallel using BSA-NIP conjugate (10 µg/ml) as coating antigen [20]. Taking the serum dilution into account and considering a net optical density of 0.020 as cutoff, the serum sensitivity in these assays were 4.0 (IgG1), 0.30 (IgG4) and 0.20 (IgE) arbitrary units (AU)/ml. In the calculations undetectable levels were given half the value of the detection level, e.g. 0.10 AU/ml for IgE.

The inhibitory activity of the wild-type and mutant forms of rLep d 2 was compared in inhibition ELISA using selected sera containing elevated levels of IgG1, IgG4 and/or IgE Ab to wild-type rLep d 2. Six 5-fold (IgG4 and IgE) or three 10-fold (IgG1) dilutions of allergen starting with a concentration of 5 µg/ml and diluent alone were incubated overnight at room temperature with suitable dilutions of the serum samples. The residual antibody activity was determined and the results expressed in percentage of that obtained with diluent alone.

Fig. 1. Serum levels of IgG1, IgG4 and IgE Ab to rLep d 2(wt) in 20 atopic farmers sensitized to *L. destructor* (group 1), 22 non-atopic farmers (group 2) and 22 urban control subjects (group 3) as measured by ELISA. Horizontal bars indicate medians and dashed lines the sensitivity of the assays. The statistical differences between groups are indicated at the top. *** $p < 0.001$. NS = Not significant.



Similar coating efficiency and concentrations of the different rLep d 2 protein preparations were ascertained in sandwich ELISA using an anti-His-tag monoclonal Ab (clone BMG-His-1, Boehringer Mannheim, Stockholm, Sweden). Alkaline-phosphatase-conjugated rabbit anti-mouse Ig Ab [20] was used as secondary reagent to detect the response of 4-fold diluted allergen concentrations starting with 16 $\mu\text{g/ml}$.

Statistical Analysis

Medians between groups were compared by Mann-Whitney U test or Kruskal-Wallis test, and correlations by Spearman rank correlation. A two-tailed $p < 0.05$ was considered significant.

Ethics

The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm.

Results

Antibodies to rLep d 2(wt)

A highly significant correlation ($r_s = 0.94$; $p < 0.0001$; $n = 20$) was registered for the 20 atopic farmers between the IgE-CAP levels to rLep d 2(wt) and those obtained with IgE-ELISA (median 2.2 AU/ml; range <0.20 –42) (fig. 1). No positive IgE-ELISA (≥ 0.20 AU/ml) was found in the 18 nonatopic farmers or among the 22 urban subjects (fig. 1). As no significant differences in the IgE, IgG1

and IgG4 Ab levels to rLep d 2(wt) were observed between groups 3a and b (data not shown) the two were combined into one urban control group 3.

Also, IgG4 Ab to rLep d 2(wt) were almost exclusively detected among the atopic farmers whose levels ($n = 20$; median 10.3 AU/ml; range <0.30 –86 AU/ml) were significantly ($p < 0.0001$) higher compared to both those of the nonatopic farmers ($n = 18$; median <0.30 AU/ml; range <0.30 –0.95) and the urban controls ($n = 22$; all <0.30 AU/ml) (fig. 1).

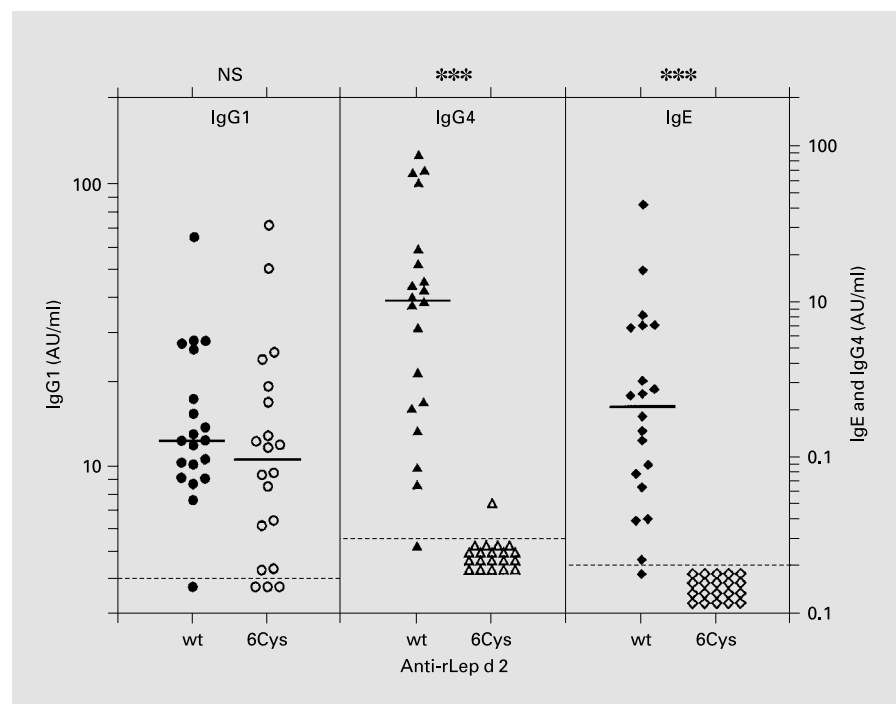
In contrast, most individuals (69–95%) in the three groups had detectable IgG1 Ab to rLep d 2(wt) with the highest proportion among the atopic farmers, but no significant differences in the median levels between the three groups were found (fig. 1).

The IgE and IgG4 Ab levels to rLep d 2(wt) correlated significantly among the atopic farmers ($n = 20$; $r_s = 0.70$; $p < 0.001$) whereas the corresponding IgE/IgG1 ($r_s = -0.17$) and IgG4/IgG1 ($r_s = -0.15$) Ab levels did not.

Antibodies to rLep d 2.6Cys

No IgE reactivity was detected to rLep d 2.6Cys in any subject in the three groups with sandwich ELISA. Similar results were observed for IgG4, where low levels only (0.37–0.56 AU/ml) were detected in 1 subject from each group. In contrast, most individuals (75–95%) in the three

Fig. 2. Serum levels of IgG1, IgG4 and IgE Ab to the wild-type and 6Cys mutant of rLep d 2 in 20 atopic farmers sensitized to *L. destructor* as measured by ELISA. Horizontal bars indicate medians and dashed lines the sensitivity of the assays. The statistical differences between groups are indicated at the top. *** $p < 0.001$. NS = Not significant.



groups had detectable levels of IgG1 Ab to rLep d 2.6Cys.

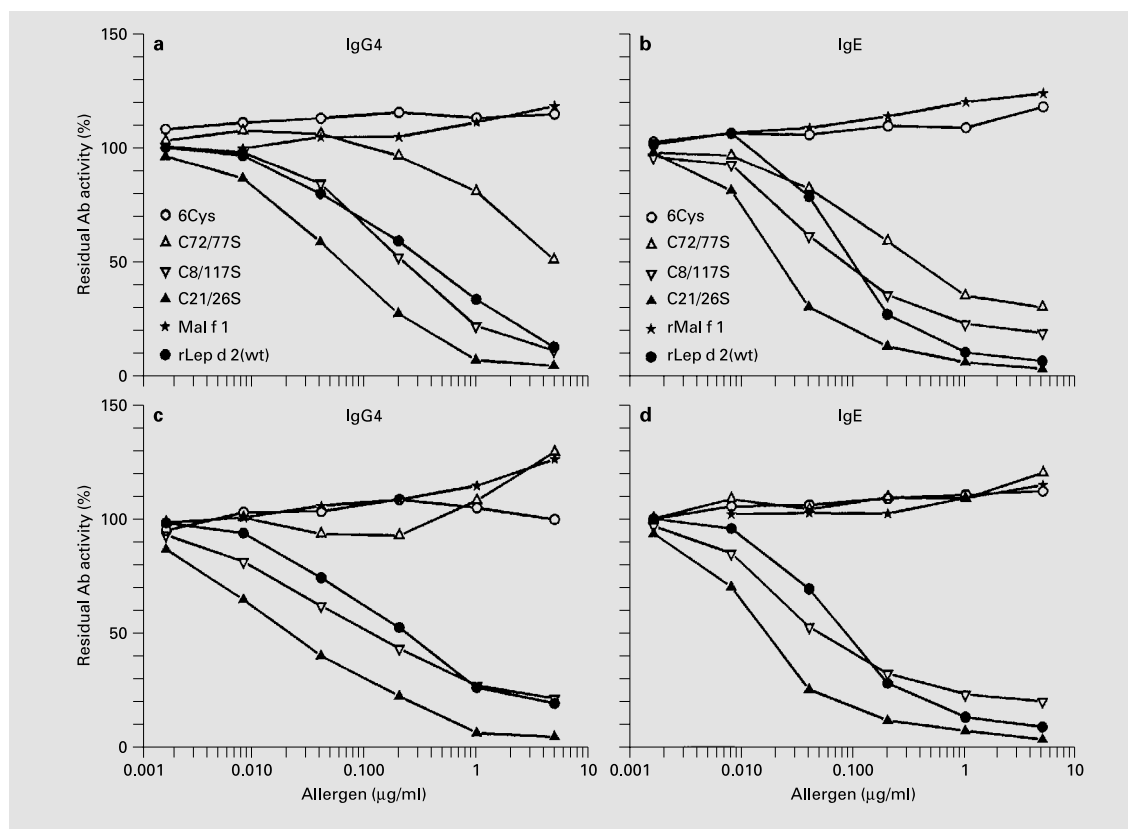
The differences in Ab levels to rLep d 2.6Cys and rLep d 2(wt) were highly significant ($p < 0.0001$) for IgE and IgG4, but not for IgG1, as shown for the 20 *L. destructor*-sensitized farmers (fig. 2). There were no significant differences in the medians of anti-Lep d 2.6Cys Ab levels for any isotype between the three groups (data not shown). Significant correlations were registered between the IgG1 levels to rLep d 2(wt) and rLep 2.6Cys in all three groups analyzed separately ($r_s = 0.58$ – 0.90 ; $p < 0.01$ – 0.001) or together ($n = 60$; $r_s = 0.65$; $p < 0.0001$).

Inhibitory Reactivity of rLep d 2 Mutants with Anti-rLep d 2(wt) Ab

To further study the reactivity pattern of IgE and IgG4 Ab, the inhibitory capacities of rLep d 2(wt) and the four rLep d 2 mutants C8/117S, C21/26S, C72/77S and 6Cys were compared in inhibition ELISA. Four sera from *L. destructor*-sensitized farmers having both high IgE and IgG4 Ab levels to rLep d 2(wt) were used. IgE and IgG4 reacted in a parallel way within these 4 sera, and two reaction patterns, represented by 2 sera each, could be distinguished as exemplified (fig. 3a–d). In line with the results from the sandwich ELISA, rLep d 2.6Cys demonstrated neither any inhibitory activity with IgE nor with IgG4 Ab

at the highest concentration tested (5 μ g/ml) corresponding to at least a 100-fold reduction in activity compared to rLep d 2(wt). The C8/117S mutant had a similar inhibitory activity as rLep d 2(wt). Interestingly, the inhibitory activity of C72/77S could be divided into two patterns. In subject 2 (fig. 3c, d), it had no inhibitory activity whereas in subject 1 it reacted with both IgE and IgG4 Ab (fig. 3a, b), although the reactivity was much weaker than that of rLep d 2(wt). Furthermore, the C21/26S mutant demonstrated a 5- to 10-fold more efficient inhibitory capacity than the wild-type protein. The control allergen rMal f 1 did not exhibit any inhibitory activity (fig. 3a–d).

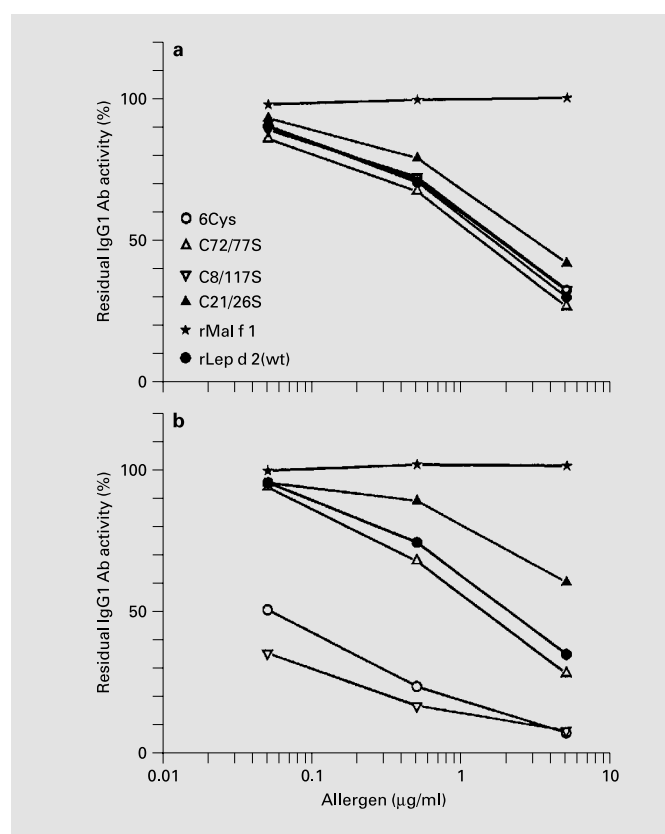
The reactivity pattern of IgG1 Ab to rLep d 2 mutants was also investigated by inhibition ELISA using 4 sera having elevated titers of IgG1 Ab to rLep d 2(wt). The results for 2 sera from atopic farmers, and 1 serum from a nonatopic farmer were similar and showed that all mutants including rLep d 2.6Cys and wild-type rLep d 2 had comparable inhibitory activities as exemplified with a serum from an atopic farmer (fig. 4a). Also in the serum from a nonatopic urban control subject (group 3) all Lep d 2 recombinants were inhibitory but interestingly the pattern was different in that two mutants, rLep d 2.6Cys and C8/117S, were substantially (>10 -fold) more efficient (fig. 4b). The similarly produced control rMal f 1 had no inhibitory activity (fig. 4a, b).



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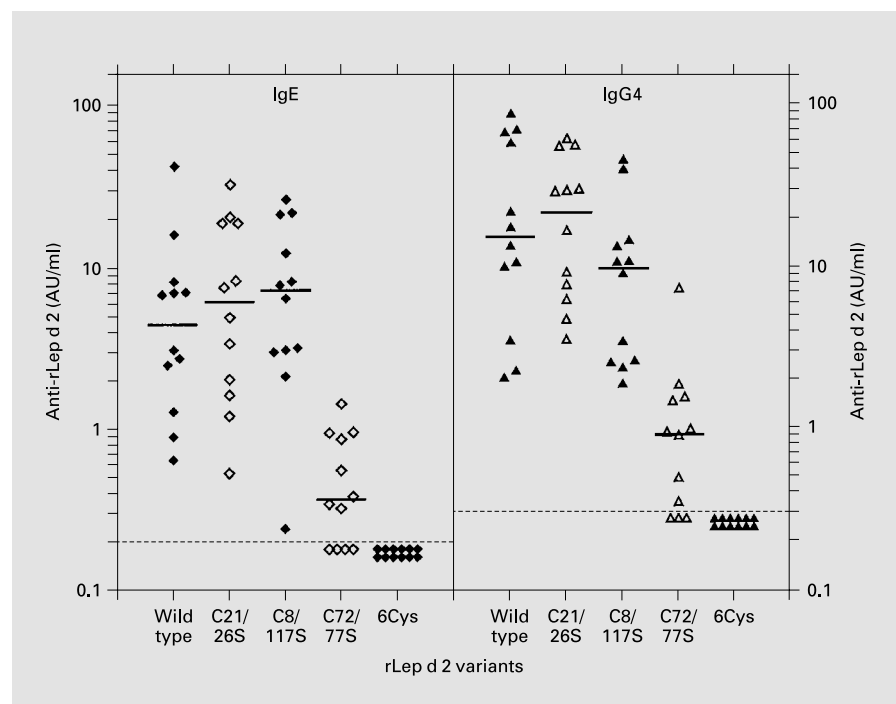
Fig. 3. Inhibitory profiles of rLep d 2 allergen variants with IgG4 and IgE anti-rLep d 2(wt) Ab in 2 atopic farmers with different reactivity patterns: patient 1 (**a, b**) and patient 2 (**c, d**). Serial dilutions of recombinant proteins were preincubated with serum and the residual antibody activity determined with ELISA.

Fig. 4. Inhibitory profiles of rLep d 2 allergen variants with IgG1 anti-rLep d 2(wt) Ab in serum from an atopic farmer patient 3 (**a**) and a nonatopic urban control subject 4 (**b**). Serial dilutions of recombinant proteins were preincubated with serum and the residual antibody activity determined with ELISA.



4

Fig. 5. IgE and IgG4 Ab levels to five rLep d 2 allergen variants in 12 atopic farmers sensitized to *L. destructor* as measured by ELISA. Horizontal bars indicate medians and dashed lines the threshold of sensitivity. Four nonatopic farmers and 4 urban controls tested had undetectable Ab levels to all rLep d 2 variants (data not shown).



IgG4 and IgE Ab Reactivity to C21/26S, C72/77S and C8/117S Mutants

As the inhibition ELISA pointed to differences in reactivity patterns among mutants, additional experiments were performed with IgE and IgG4 sandwich ELISA using the C21/26S, C8/117S and C72/77S mutants as solid phase antigen. Twelve sera from atopic farmers, 4 from nonatopic farmers and 4 from the urban control group were tested. No IgE or IgG4 antibody activity was registered to any of the mutants among the 8 sera from nonatopic farmers and urban controls (data not shown). As shown for the 12 atopic farmers the reactivity towards C72/77S was significantly reduced ($p < 0.0001$), whereas those to C21/26S and C8/117S were indistinguishable from the one to rLep d 2(wt) for both isotypes (fig. 5). A further significant ($p < 0.001$) loss in reactivity for both isotypes was registered between C72/77S and rLep d 2.6Cys (fig. 5). These results were mirrored by the significant correlations ($p < 0.001$) for both the IgG4 and IgE Ab levels to rLep d 2(wt) as compared to C21/26S ($r_s = 0.92-0.96$) and C8/117S ($r_s = 0.80-0.96$) but not to C72/77S ($r_s = 0.39-0.44$). The lack of correlation for C72/77S suggests diversity in the polyclonal IgE and IgG4 specificity.

Discussion

In this study we have used recombinant wild-type and several mutant forms of the major allergen Lep d 2 to demonstrate differences in reactivity pattern of IgG1, IgG4 and IgE Ab among atopic and nonatopic subjects expected to have different levels of natural exposure to the dust mite *L. destructor*.

There are very few studies on the humoral response in subjects naturally exposed to aeroallergens as compared to the vast literature associated with immunotherapy [5–7]. Moreover, most studies have been performed with whole allergen extracts as opposed to the few with recombinant wild-type or modified allergens. It is considered that immunotherapy gives a rise in mainly the IgG1 and IgG4 subclasses and that the magnitude, the ratio or kinetic appearance between the two isotypes may predict the outcome of treatment [5, 22]. The few studies on natural exposures suggest a similar pattern [23, 24] or perhaps more IgG4-restricted response to aeroallergen extracts among atopics as compared to nonatopics [25–28]. Our data with recombinant Lep d 2(wt) are in agreement with the latter studies and the levels of IgG4 and IgE clearly correlated as previously reported in some studies [28] but not others [25]. It is noteworthy that among the farmers it is only those sensitized to *L. destructor* who develop IgG4

Ab despite an expected similar level of exposure to dust mite allergen among all farmers [2]. Thus, chronic exposure does not seem sufficient for induction of IgG4, implicating also other factors, e.g. degree of exposure, sensitization route and adjuvants [7].

It has previously been shown for the group 2 dust mite allergens Der p 2 and Der f 2 [10–13] as well as Lep d 2 [18] that the 3 disulfide bonds are of differential importance for IgE reactivity. We show here that the disulfide bonds in rLep d 2 follows the same order of importance for IgG4 reactivity as that for IgE, i.e. Cys72-Cys77 >> Cys8-Cys117 > Cys21-Cys26. Moreover, the rLep d 2.6Cys mutant lacking all 3 disulfides was devoid of both IgE and IgG4 reactivity, confirming and extending our previous IgE results with immunoblotting and RAST inhibition [18].

Thus, among farmers sensitized to *L. destructor*, IgG4 and IgE, but not IgG1, presented parallel and quite similar reactivity patterns to all recombinant variants of Lep d 2. To our knowledge there are no comparative studies of IgE/IgG4/IgG1 specificity to modified allergens among atopic and nonatopic subjects following a natural allergen exposure. Some serological studies have indicated different epitope recognition for IgE and IgG4 to Bet v 1 [7], Fel d 1 [7, 29] and rDer p 2 [30], but also similar specificities may be envisaged from a transcriptional level [31]. Our results suggest overlapping, if not identical, epitope recognition for IgG4 and IgE in line with a sequential switch from IgM to IgG4/IgE [31, 32]. Moreover, the repertoire of IgE/IgG4 Ab varied between individual atopic subjects pointing to the necessity of using many individual sera as well as several assay approaches in the evaluation of modified recombinant allergens.

One of the suggested mechanisms behind successful immunotherapy is the quantitative increase in IgG1/IgG4 acting as blocking Ab [5–7]. As our results suggest that IgG4 and IgE are directed against similar conformational epitopes, the outcome of allergen vaccination with modified derivatives might be less efficient than anticipated. However, it was recently reported that unfolded allergens could give alternative IgG4 immune responses in vitro depending on antigen-presenting cells [33]. The in vivo induction of such IgG4 as well as IgG1 may give overlapping specificities, which would be beneficial for blocking [9].

Although our inhibition results are based on a few patients, it seems clear, also from the sandwich ELISA with rLep d 2.6Cys, that the IgG1 epitopes are different from those of IgE and IgG4. One may assume that the IgE and IgG4 epitopes are mainly conformational (which

agrees with the lack of IgE binding to Lep d 2-derived peptides [34]) whereas the IgG1 epitopes appear mostly linear. With another methodological approach, recent studies have suggested differences in the fine specificity of IgG Ab to Der p 1 and bovine β -lactoglobulin between allergics and healthy controls [35, 36]. In line with our IgG4 findings allergic subjects had IgG Ab preferentially directed to conformational epitopes. Moreover, although the substantially increased inhibitory capacity of rLep d 2.6Cys and C8/117S that we observed in an urban control could be circumstantial it might suggest some kind of difference in IgG1 Ab specificity also between farmers and urban subjects.

We found similar levels of IgG1 Ab to both rLep d 2(wt) and rLep d 2.6Cys in farmers and urban subjects irrespective of sensitization and degree of allergen exposure. Such findings have also been reported with *L. destructor* allergen extract [27]. Others have reported similar IgG1 levels to rDer p 2 in children regardless of atopic status [23, 25]. Although the levels were lower in children with a documented low exposure to *Dermatophagoides* there was still no atopy-related difference [23]. However, also increased levels of allergen-specific IgG1 Ab among atopics have been registered by ELISA [24] and immunoblotting [27]. The nature of allergen, epitope presentation, affinity of Ab, methodological principle or population selected may partly explain these discrepancies [5, 26]. Nonetheless the IgG1 results should be viewed in relation to the high frequency of allergen-specific T cell responses observed also among nonatopic subjects [37] resulting from either cross-reacting epitopes or some natural exposure.

In summary, our findings show that farmers sensitized to *L. destructor* mount parallel IgE and IgG4 responses to Lep d 2. The reactivity patterns of mutant variants suggest that the three disulfide bonds in rLep d 2 can be ranked in the same order of importance Cys72-Cys77 >> Cys8-Cys117 > Cys21-Cys26 for both IgE and IgG4 reactivity. However, omission of all 3 bonds, as in rLep d 2.6Cys, seems necessary to get an allergen variant practically devoid of IgE and IgG4 reactivity. The lack of IgG4 response among nonatopic farmers implicates that factors besides chronic allergen exposure are needed to induce this isotype. In contrast IgG1 reactivity was detected in most subjects irrespective of disulfide bond impairment in rLep d 2, sensitization or degree of exposure to *L. destructor*. Thus, our results suggest similar specificity among IgE/IgG4 Ab clearly different from that of IgG1 Ab in line with conformational Lep d 2-specific and linear epitopes, respectively. The observation of interindividual

variation in the antibody specificity to modified allergens among different techniques emphasizes the need for careful in vitro as well as in vivo evaluation before selecting hypoallergenic candidates for immunotherapy.

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References

- Kronqvist M, Johansson E, Pershagen G, Johansson SGO, van Hage-Hamsten M: Increasing prevalence of asthma over 12 years among dairy farmers on Gotland: Storage mites remain dominant allergens. *Clin Exp Allergy* 1999;29/1:35–41.
- Boström S, Johansson E, Härfast B, Lundqvist L, Bäckman I, von Rosen E, van Hage-Hamsten M: Characterisation of the mite fauna (Acari) in Swedish barn dust. *Intern J Acarol* 1997;23:127–132.
- Secrist H, Chelen CJ, Wen Y, Marshall JD, Umetsu DT: Allergen immunotherapy decreases interleukin 4 production in CD4+ T cells from allergic individuals. *J Exp Med* 1993; 178:2123–2130.
- Ebner C, Siemann U, Bohle B, Willheim M, Wiedermann U, Schenk S, Klotz F, Ebner H, Kraft D, Scheiner O: Immunological changes during specific immunotherapy of grass pollen allergy: Reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997;27: 1007–1015.
- Djurup R: The subclass nature and clinical significance of the IgG antibody response in patients undergoing allergen-specific immunotherapy. *Allergy* 1985;40:469–486.
- Nakagawa T: The role of IgG subclass antibodies in the clinical response to immunotherapy in allergic disease. *Clin Exp Allergy* 1991; 21:289–296.
- Aalberse RC, van Milligen F, Tan KY, Stapel SO: Allergen-specific IgG4 in atopic disease. *Allergy* 1993;4:559–569.
- Valenta R, Vrtala S, Focke-Tejkl M, Bugajska-Schretter A, Ball T, Twardosz A, Spitzauer S, Grönlund H, Kraft D: Genetically engineered and synthetic allergen derivatives: Candidates for vaccination against type I allergy. *Biol Chem* 1999;380:815–824.
- Ball T, Sperr WR, Valent P, Lidholm J, Spitzauer S, Ebner C, Kraft D, Valenta R: Induction of antibody responses to new B cell epitopes indicates vaccination character of allergen immunotherapy. *Eur J Immunol* 1999;29:2026–2036.
- Nishiyama C, Fukada M, Usui Y, Iwamoto N, Yuuki T, Okumura Y, Okudaira H: Analysis of the IgE-epitope of Der f 2, a major mite allergen, by in vitro mutagenesis. *Mol Immunol* 1995;32:1021–1029.
- Smith AM, Chapman MD: Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: Contribution of disulphide bonds to the antigenic structure of the major house dust mite allergen Der p 2. *Mol Immunol* 1996;33:399–405.
- Smith AM, Chapman MD, Taketomi EA, Platts-Mills TAE, Sung SJ: Recombinant allergens for immunotherapy: A Der p 2 variant with reduced IgE reactivity retains T-cell epitopes. *J Allergy Clin Immunol* 1998;101:423–425.
- Noguchi E, Shibasaki M, Nishiyama C, Okumura Y, Takita H: IgE responsiveness to *Dermatophagoides farinae* in young asthmatic children: IgE binding study using recombinant allergens of Der f 1, Der f 2 and mutant proteins of Der f 2. *Int Arch Allergy Immunol* 1996;110: 380–387.
- Yasue M, Yokota T, Fukada M, Takai T, Suko M, Okudaira H, Okumura Y: Hyposensitization to allergic reaction in rDer f 2-sensitized mice by the intranasal administration of a mutant of rDer f 2, C8/119S. *Clin Exp Immunol* 1998;113:1–9.
- Yasue M, Yokota T, Okudaira H, Okumura Y: Specific immunotherapy in rDer f 2-sensitized monkeys by administration of a mutant of rDer f 2, C8/119S. *J Allergy Clin Immunol* 1999; 103/1(part 2):S141.
- Olsson S, van Hage-Hamsten M, Whitley P, Johansson E, Hoffman DR, Gafvelin G, Schmidt M: Expression of two isoforms of Lep d 2, the major allergen of *Lepidoglyphus destructor*, in both prokaryotic and eukaryotic systems. *Clin Exp Allergy* 1998;28:984–991.
- Ichikawa S, Hatanaka H, Yuuki T, Iwamoto N, Kojima S, Nishiyama C, Ogura K, Okumura Y, Inagaki F: Solution structure of Der f 2, the major mite allergen for atopic diseases. *J Biol Chem* 1998;273:356–360.
- Olsson S, van Hage-Hamsten M, Whitley P: Contribution of disulphide bonds to antigenicity of Lep d 2, the major allergen of the dust mite *Lepidoglyphus destructor*. *Mol Immunol* 1998;35:1017–1023.
- Johansson E, Eriksson TLJ, Olsson S, Kronqvist M, Whitley P, Johansson SGO, Gafvelin G, van Hage-Hamsten M: Evaluation of specific IgE to the recombinant group 2 mite allergens Lep d 2 and Tyr p 2 in the Pharmacia CAP system. *Int Arch Allergy Immunol* 1999;120: 43–49.
- Magnusson CGM, Nelson DF, Magnusson MAJ: Disproportional distribution of isotype specific and non-isotype specific IgG subclass anti-IgE autoantibodies in cord serum. *Int Arch Allergy Immunol* 1996;110:31–40.
- Magnusson CGM, Aalberse RC, Johansson SGO: Monoclonal antibodies against human IgE. Identification of a determinant restricted to IgE of the lambda light chain type. *Int Arch Allergy Appl Immunol* 1986;80:329–332.
- Gehlhar K, Schlaak M, Becker W-M, Bufer A: Monitoring allergen immunotherapy of pollen-allergic patients: The ratio of allergen-specific IgG4 and IgG1 correlates with clinical outcome. *Clin Exp Allergy* 1999;29:497–506.
- Smith AM, Yamaguchi H, Platts-Mills TAE, Fu SH: Prevalence of IgG anti-Der p 2 antibodies in children from high and low antigen exposure groups: Relationship of IgG and subclass antibody responses to exposure and allergic symptoms. *Clin Immunol Immunopathol* 1998;86:102–109.
- Oshika E, Kuroki Y, Sakiyama Y, Matsumoto S: Measurement of IgG subclass antibodies to the group II antigen of *Dermatophagoides pteronyssinus* (Der p II) in sera from children with bronchial asthma. *Ann Allergy* 1992;69: 427–432.
- Tame A, Sakiyama Y, Kobayashi I, Terai I, Kobayashi K: Differences in titres of IgE, IgG4 and other IgG subclass anti-Der p 2 antibodies in allergic and non-allergic patients measured with recombinant allergen. *Clin Exp Allergy* 1996;26:43–49.
- Kemeny DM, Urbanek R, Ewan P, McHugh S, Richards D, Patel S, Lessof MH: The subclass of IgG antibody in allergic disease. II. The IgG subclass of antibodies produced following natural exposure to dust mite and grass pollen in atopic and non-atopic individuals. *Clin Exp Allergy* 1989;19:545–549.
- Johansson E, Härfast B, Johansson SGO, van Hage-Hamsten M: IgG1 and IgG4 antibody responses to the dust mite *Lepidoglyphus destructor* in a naturally exposed farming population. *Allergy* 1995;50:473–477.
- Jeannin P, Delneste Y, Tillie-Leblond I, Wal-laert B, Carlier A, Pestel J, Tonnel AB: Abnormal IgG4 antibody response to aeroallergens in allergic patients. *Int Arch Allergy Immunol* 1994;104:191–198.

- 29 van Milligen FJ, Craig S, Rogers BL, van Swieten P, Aalberse RC: Differences between specificities of IgE and IgG4 antibodies: Studies using recombinant chain 1 and chain 2 of the major cat allergen *Felis domesticus* (Fel d) I. *Clin Exp Allergy* 1995;25:247–251.
- 30 Kobayashi I, Sakiyama Y, Tame A, Kobayashi K, Matsumoto S: IgE and IgG4 antibodies from patients with mite allergy recognize different epitopes of *Dermatophagoides pteronyssinus* group II antigen (Der p 2). *J Allergy Clin Immunol* 1996;97:638–645.
- 31 Snow RE, Chapman CJ, Holgate ST, Stevenson FK: Clonally related IgE and IgG4 transcripts in blood lymphocytes of patients with asthma reveal differing patterns of somatic mutation. *Eur J Immunol* 1998;28:3354–3361.
- 32 Jabara HH, Loh R, Ramesh N, Vercelli D, Geha RS: Sequential switching from μ to ϵ via $\gamma 4$ in human B cells stimulated with IL-4 and hydrocortisone. *J Immunol* 1993;151:4528–4533.
- 33 Akdis CA, Blesken T, Wymann D, Akdis M, Blaser K: Differential regulation of human T cell cytokine patterns and IgE and IgG4 responses by conformational antigen variants. *Eur J Immunol* 1998;28:914–925.
- 34 Elfman LHM, Whitley P, Schmidt M, van Hage-Hamsten M: IgE binding capacity of synthetic and recombinant peptides of the major storage mite (*Lepidoglyphus destructor*) allergen, Lep d 2. *Int Arch Allergy Immunol* 1998;117:167–173.
- 35 Duchateau J, Michils A, Michel O, Baras L: Mite allergy is associated with a specific profile of IgG epitopes recognized on antigen p1 of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1997;27:296–305.
- 36 Duchateau J, Michils A, Lambert J, Gossart B, Casimir G: Anti-betalactoglobulin IgG antibodies bind to a specific profile of epitopes when patients are allergic to cow's milk proteins. *Clin Exp Allergy* 1998;28:824–833.
- 37 Upham JW, Holt BJ, Baron-Hay MJ, Yabuhashi A, Hales BJ, Thomas WR, Loh RKS, O'Keefe PT, Palmer L, Le Soueuf PN, Sly PD, Burton PB, Robinson BWS, Holt PG: Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: Covert responses are unmasked by serum-free medium. *Clin Exp Allergy* 1995;25:634–642.

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