The structure of the dust mite allergen Der p 7 reveals similarities to innate immune proteins

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Background: Sensitization to house dust mite allergens is strongly correlated with asthma. Der p 7 elicits strong IgE antibody and T-cell responses in patients with mite allergy. However, the structure and biological function of this important allergen are unknown. Allergen function might contribute to allergenicity, as shown for the protease activity of group 1 mite allergens and the interaction with the innate immune system by group 2 mite allergens.

Objective: We sought to determine the crystal structure of Der p 7 and to investigate its biological function.

Methods: X-ray crystallography was used to determine the Der p 7 structure. Nuclear magnetic resonance analysis and biochemical assays were used to examine the binding of Der p 7 to predicted ligands.

Results: Der p 7 has an elongated structure, with two 4-stranded antiparallel β-sheets that wrap around a long C-terminal helix. The fold of Der p 7 is similar to that of LPS-binding protein (LBP), which interacts with Toll-like receptors after binding LPS and other bacterially derived lipid ligands. Nuclear magnetic resonance and biochemical assays indicate that Der p 7 does not bind LPS but binds with weak affinity to the bacterial lipopeptide polymyxin B in the predicted binding site of Der p 7.

Conclusions: Der p 7 binds a bacterially derived lipid product, a common feature of some allergens. The finding that the group 7, as well as the group 2, mite allergens are structurally similar to different proteins in the Toll-like receptor pathway further strengthens the connections between dust mites, innate immunity, and allergy. (J Allergy Clin Immunol 2010;125:909-17.)

Key words: *Asthma, allergens, dust mites, Der p 7, LPS-binding protein, Toll-like receptor 4, lipopeptide, innate immunity*

The development of asthma associated with allergens is known as extrinsic asthma, which differentiates it from intrinsic asthma of unknown cause. Primarily, extrinsic asthma is associated with exposure to indoor allergens and occasionally with exposure to outdoor allergens.[1] Among indoor allergens, the most commonly associated with asthma are those of the house dust mite.[2] Indeed, greater than 80% of asthmatic patients show immediate hypersensitivity to allergens of the house dust mite.[3] A significant question remains as to why dust mite allergens are so strongly associated with the cause of asthma. Early studies to address this question focused on the discovery that many dust mite allergens are proteolytic enzymes.[4] Experiments showed that these proteases can damage lung epithelia, which might help explain the chronic inflammation of the lung that characterizes asthma symptoms.[5,6] Other studies have shown that digestion of specific proteins could encourage IgE synthesis, skew the immune response toward a T_H2 or symptomatic response instead of tolerance, or both.[7,8]

An important dust mite allergen for which there is not a known function from sequence analysis is Der p 7. The mature protein is 198 amino acids (molecular weight, 22.2 kDa) and is estimated to be found in dust at concentrations of 2.5 μg per gram of dust.[9] Natural Der p 7 has been described as a mixture of 3 molecules with molecular weights of between 24 and 31 kDa. These different molecular forms could represent different levels of glycosylation or isoforms.[10,11] More than 50% of patients with dust mite allergy react specifically to Der p 7, and some patients respond with IgE titers as strong as those to the major allergen Der p 2.[12] Previous studies suggested that Der p 7 promotes an abnormally high reactivity in T-cell proliferation assays among allergic and nonallergic patients.[9,11,13-15] In this study the 3-dimensional structure of the protein was determined to gain insight into the abnormal patient response and the natural function of the protein.

Allergens can be enzymes, structural proteins, or ligand-binding proteins that often show specific binding affinity for lipids.[16] The intrinsic adjuvant activity of lipid-binding proteins and their lipid cargo might be a general mechanism underlying allergenicity.[17] Supporting this hypothesis, coexposure to allergens and LPS from bacteria can drive the immune response away from benign tolerance to symptomatic response.[18,19] Interestingly, the dust mite allergen Der p 2 can bind to LPS and initiate the signaling of the Toll-like receptor (TLR) 4 complex in MD-2–deficient mice, thus functionally substituting for the mammalian innate immune protein MD-2. This raises many interesting questions about how other allergens, especially the lipid-binding proteins, might interfere with normal host defense signaling.

Herein, we report that structural analysis reveals Der p 7 to be distantly related to another family of innate immune proteins that

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Abbreviations used
BPI: Bactericidal/permeability-increasing protein
DSPC: Distearoyl phosphatidyl choline
HSQC: Heteronuclear single quantum coherence
JHBP: Juvenile hormone-binding protein
LAL: Limulus amoebocyte lysate
LBP: LPS-binding protein
MBP: Maltose-binding protein
NMR: Nuclear magnetic resonance
PB: Polymyxin B
TLR: Toll-like receptor
TO: Takeout

bind to various hydrophobic ligands. One such protein, LPS-binding protein (LBP), is in the TLR4 pathway. To explore the possibility of immune protein mimicry analogous to the recently reported behavior of Der p 2 and MD-2, we examined the binding of LPS and select other hydrophobic compounds to Der p 7.

METHODS

Chimeric ELISA for IgE antibodies to Der p 7

IgE binding to Der p 7 was analyzed by using a chimeric ELISA, as previously described.[20] The plates were coated overnight at 4°C with the anti–Der p 7 mAb WH9. Plates were blocked with PBS–Tween–1% BSA, followed by an incubation with 400 ng/mL recombinant (r) Der p 7 or natural (n) Der p 7 at room temperature. The source of nDer p 7 was a dust mite extract from *Dermatophagoides pteronyssinus* (HollisterStier, Spokane, Wash), which contained 126 μg/mL Der p 7 (30,000 AU/mL). Sera from allergic patients was purchased from PlasmaLab International (Everett, Wash). Thirty-three sera were added at 1:2 and 1:10 dilutions, and bound IgE was detected by using biotinylated goat anti-human IgE. The anti–Der p 2 mAb αDpX, purified nDer p 2 at 500 ng/mL, and the chimeric antibody 2B12-IgE were used for the standard curve to quantify the assay. Natural allergen (nDer p 7) and rDer p 7 expressed in *Pichia pastoris*[21] were compared with constructs of rDer p 7 expressed in bacteria (see below).

Crystallographic studies

The best results for crystallography were obtained with a customized maltose-binding protein (MBP) fusion with Der p 7. The MBP fusion was designed with surface entropy-reducing mutations in MBP to enhance crystallization.[22] DNA encoding residues 18 to 215 of Der p 7, representing the native expressed mite protein,[10] was cloned into a modified fixed-arm PMAL vector between the *Not*I and *Bam*HI restriction sites with MBP mutations D82A/K83A/E172A/N173A/K239A.[23,24] The MBP–Der p 7 fusion protein was expressed in *Escherichia coli* Rosetta2(DE3) pLacI cells (Novagen, Madison, Wis) and purified with the amylose affinity resin in batch followed by gel filtration with a 16/60 Superdex 200 column and an ionic exchange chromatography step with Q sepharose. The purified protein was dialyzed against 25 mmol/L HEPES (pH 7.4), 75 mmol/L NaCl, 5 and mmol/L maltose and concentrated to 27 mg/mL.

Crystals of the MBP–Der p 7 fusion protein were obtained at 4°C by mixing 1 μL of the protein solution with 1 μL of the reservoir consisting of 42.5 mmol/L Tris (pH 8.5), 12.75% PEG4000, 85 mmol/L lithium sulfate, and 7.5% glycerol. The crystal used in this experiment reached a maximum size of 0.2 × 0.2 × 0.2 mm after 1 week. Before data collection, the crystal was transferred to 63.8 mmol/L Tris (pH 8.5), 19.1% PEG4000, 128 mmol/L lithium sulfate, and 11.25% glycerol and then quickly transferred to 85 mmol/L Tris (pH 8.5), 25.5% PEG4000, 0.17 mol/L lithium sulfate, and 15% glycerol. The crystal was flash frozen in liquid nitrogen and placed in a nitrogen gas stream cooled to −180°C. Data were collected to a resolution of 2.35 Å and were scaled and processed with HKL2000.[25] Molecular replacement with the MBP from the MBP-RACK1 fusion structure was used to solve the position of the 3 molecules of MBP in the asymmetric unit.[24,26] The structures of the 3 Der p 7 molecules were built manually by means of iterative cycles of model building in O,[27] as well as density modification and refinement in CNS.[28] We were unable to identify a structure with sufficient homology to Der p 7 to allow solution by means of molecular replacement, but the coordinates from 1EWF.pdb (bactericidal/permeability-increasing protein) and 3E8T.pdb (Takeout 1) were consulted for help in chain tracing and secondary structure based on structural similarity suggested by GenTHREADER.[29-31] Table I lists important statistics for assessing the quality of the crystallographic data and the resulting structures.

Nuclear magnetic resonance constructs and spectroscopy

Recombinant Der p 7, residues 18 to 215, was subcloned and expressed in the Gateway system (Invitrogen, Carlsbad, Calif) with a cleavable 6-His tag to reduce the size of the protein for nuclear magnetic resonance (NMR) spectroscopic analysis. Optimal bacterial expression of this construct was obtained in the Rosetta2(DE3) pLacI cell line by growing the cells at 37°C to an OD_{600} of 0.6 and inducing overnight at 18°C. Stable isotopic labeling for NMR was done, as described previously.[32] The cells were lysed by means of sonication in 50 mmol/L HEPES (pH 7.4) and 500 mmol/L NaCl, and protein was found in the soluble fraction after centrifugation at greater than 40,000*g* for 30 minutes. The rDer p 7 was purified with Ni^{2+} affinity chromatography, followed by a Q-sepharose ion-exchange column. The affinity tag was cleaved with tobacco etch virus protease. Subsequently, cleaved protein was passed through another Ni^{2+} column and further purified with size exclusion chromatography.

NMR backbone assignments were obtained on a U-^{2}H, ^{13}C, ^{15}N–labeled sample of 0.5 mmol/L protein by using a Varian 600 MHz spectrometer. Standard backbone assignment experiments were used to establish the connectivity of resonances through the C′, CA, and CB.[32] The solution conditions for all NMR studies used PBS, with 5% to 10% $^{2}H_2O$ and 1 μmol/L 5,5-dimethylsilapentanesulfonate for referencing. Data were acquired at 25°C. The NMRViewJ RunAbout module was used for making the assignments.[33] Aiding the analysis were assignments from the automated algorithms MARS and PINE.[34,35] The assignments of manual inspection, MARS, and PINE agree very well.

RESULTS

IgE antibody responses to Der p 7

The prevalence of IgE antibody binding to natural allergen, rDer p 7 expressed in *P pastoris*, and bacterially expressed rDer p 7 constructs used for crystallography or NMR were very similar (range, 43% to 58%). There was an excellent correlation of IgE antibody binding between rDer p 7 expressed in *E coli* with or without MBP ($r = 0.94$, n = 18). IgE antibody binding to the rDer p 7 with a cleaved affinity tag showed an excellent correlation with IgE antibody binding to nDer p 7 from mite extracts ($r = 0.99$, n = 21; data not shown). Similarly, IgE antibody bound to the MBP–Der p 7 fusion protein showed a high correlation with IgE antibody to *P pastoris*–expressed Der p 7 ($r = 0.94$, n = 16) and to IgE bound to the natural allergen ($r = 0.96$, n = 21). There was an excellent correlation between IgE antibody binding to all the rDer p 7 allergens and to the natural allergen, indicating that the recombinant proteins were suitable for structural studies. Total IgE and specific IgE antibody levels to 4 allergens (Der f 1, Der p 1, Der p 2, and Der p 7) are reported in Table E1 (available in this article's Online Repository at www.jacionline.org).

Structural analysis

rDer p 7 without an affinity tag crystallizes readily at room temperature, although no crystals diffracted at high resolution.

TABLE I. Crystallographic data statistics

Data set	MBP–Der p 7 (18-215)
Unit cell	a = 193.35 Å, b = 117.90 Å, c = 92.44 Å; α = γ = 90°, β = 114.06°
Space group	C2
Resolution (Å)	50.0-2.35
No. of observations	407,861
Unique reflections	76,800
Rsym (%) (last shell)*	6.3 (35.5)
I/σI (last shell)	28.4 (2.5)
Mosaicity range	1.1-1.4
Completeness (%) (last shell)	97.8 (97.2)
Refinement statistics	
Rcryst (%)†	24.9
Rfree (%)‡	29.3
No. of waters	322
Overall mean B value (Å^2)	50.4
Average for molecule A	43.99
Average for molecule B	47.49
Average for molecule C	61.46
r.m.s. deviation from ideal values	
Bond length (Å)	0.004
Bond angle (°)	1.0
Dihedral angle (°)	22.5
Improper angle (°)	0.68
Ramachandran statistics§	
Residues in:	
Favored (98%) regions (%)	92.5
Allowed (>99.8%) regions (%)	99.5
PDB ID code	3H4Z

r.m.s, Root mean squared.
*Rsym = $\sum$ (| Ii − < I>|)/ $\sum$(Ii), where *Ii* is the intensity of the ith observation, and <*I*> is the mean intensity of the reflection.
†Rcryst = $\sum$|| Fo| − | Fc ||/ $\sum$| Fo| calculated from the working data set.
‡Rfree was calculated from 5% of data randomly chosen not to be included in refinement.
§Ramachandran results were determined by using MolProbity.

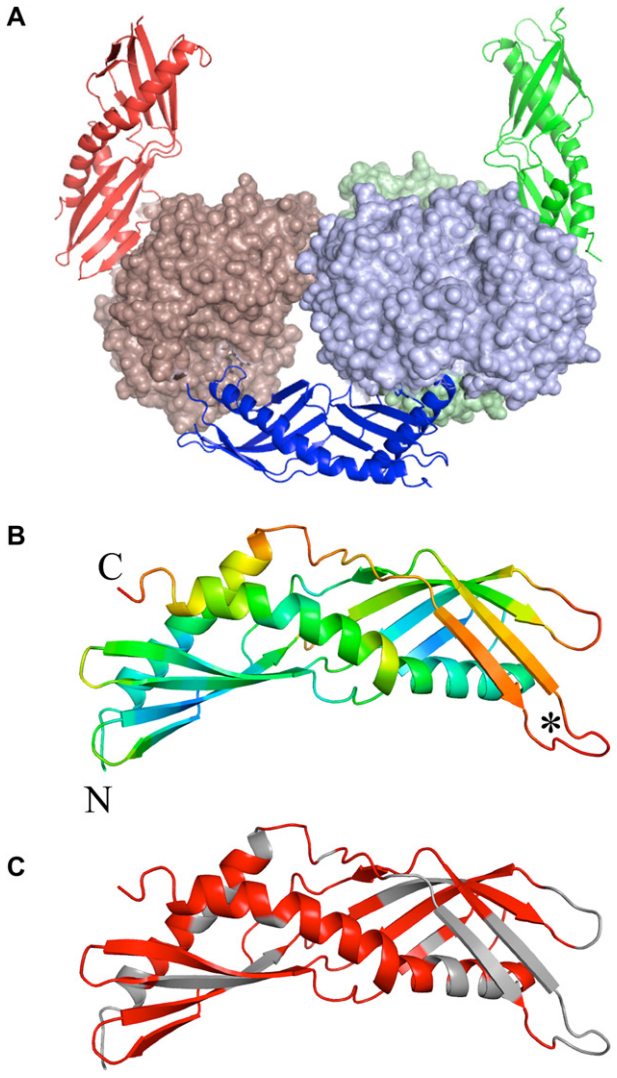

FIG 1. Der p 7 structural analysis. **A** shows the 3 molecules in the crystallographic asymmetric unit colored *green* (chain A), *blue* (chain B), and *red* (chain C). The Der p 7 sequence is rendered in ribbon style, whereas the MBP sequence is represented with a solid surface. **B** shows the Der p 7 molecule from chain A colored by relative B factor, where *red* is high (>80) and *blue* is low (<30). In **C** residues that could be assigned by NMR are colored *red*, and unassigned residues are *grey*. The N and C termini are annotated in Fig 1, *B*. The *asterisk* indicates a loop with variable density in chains A, B, and C of the asymmetric unit.

Therefore we used an alternate approach in which the Der p 7 was fused to an MBP mutant protein designed for optimal crystallization characteristics. The best Der p 7–MBP fusion protein crystals diffracted at 2.35 Å. The final model of the asymmetric unit consisted of 3 MBP–Der p 7 fusion proteins, as shown in Fig 1, *A* (PDB code 3H4Z). The most extensive interactions between symmetric units are among MBP molecules, although all permutations of interactions exist (ie, MBP-MBP, MBP–Der p 7, and Der p 7–Der p 7). The Der p 7 structure is elongated, with two 4-stranded β-sheets that wrap around a long C–terminal helix (Fig 1). These 2 β-sheets are situated in a "head-to-toe" orientation, appearing to form a single continuous β-sheet with a break in the middle. The N-terminal helix forms a cleft between the adjacent β-sheet and the end of the C-terminal helix, possibly for binding an unknown substrate.

Fig 1, *B*, shows the Der p 7 molecule from chain A colored by relative B factor. Of note, the Der p 7 molecules generally showed higher B factors than the attached MBP molecule, with the exception of chain C, which had high B factors for both the MBP and Der p 7 molecule (Table I). The regions with very high B factors were difficult to trace, and in some cases density could not be found in all 3 chains. For example, all Der p 7 residues are traced in molecule A, whereas molecules B and C are missing residues 63 to 67 and 59 to 67, respectively (see the asterisk in Fig 1, *B*). Additionally, the model of molecule C is missing residues 122 to 126 and the C-terminal residue Q215. All 3 of the Der p 7 molecules superimpose well, with a root mean squared deviation (RMSD) of less than 0.51 Å over 197 Cα atoms.

The NMR data acquired in solution are consistent with the structure determined by means of crystallography. The ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectrum of Der p 7 is characteristic of a generally well-folded protein with well-dispersed resonance intensities. The assignment of the backbone resonances was extremely challenging because of the high number of missing resonances in the HSQC and other 3-dimensional NMR experiments: 159 amide resonances appear of a possible 192 backbone amides. Of those that appear, 134 could be confidently assigned with manual inspection of the spectra. The assigned residues are colored red on the structure in Fig 1, *C*. In general, the assigned residues correlate with regions of lower B

TABLE II. Selected DALI matches to Der p 7

No.	PDB and chain	*z* Score	RMSD	Length align	No. of residues	Identity (%)	Name
1	2RCK-B	12.8	3.7	175	208	13	JHBP
2	1BP1-A	12.8	3.5	177	456	14	BPI
3	2RCK-A	12.7	3.6	174	221	13	JHBP
4	1BP1	12.6	3.6	174	456	14	BPI
5	1EWF	11.0	3.3	174	456	15	BPI
6	3E8W	10.7	4.4	171	219	11	TO-like protein 1
7	3E8T	10.6	4.5	172	296	10	TO-like protein 1
8	2OBD-A	10.4	3.5	169	427	12	Cholesteryl ester transfer protein
37	1VJH-B	4.5	3.7	99	120	11	Bet v 1 allergen family
290	2VJG-D	3.4	4.1	109	152	9	Major allergen Dau c 1
453	1H2O-A	2.5	4.0	92	159	10	Major allergen Pru av 1
531	2BK0-A	2.0	3.7	89	153	12	Major allergen Api g 1

factors in the crystal structure. The correlation of missing assignments with high B factors supports the contention that most of the assignment problem results from broadening because of conformational exchange. The secondary structure prediction from the assigned shifts[34] agrees very well with the secondary structure observed in the crystal, providing additional confidence in the assignments and showing that the solution conformation of the protein is similar to the crystal structure.

Structural homology

The software DALI[36] was used to identify related protein folds in the database to investigate whether the structure of Der p 7 provided clues to the function of the protein in mites or to its allergenic effect in human subjects. The search returned more than 500 structures with marginal match scores (z score $\sim$ 5) and 8 with very good matches (z score > 10). Table II shows the top 8 results, as well as a few select entries that are notable because they are also allergens. For example, the 37th best score is the birch pollen allergen Bet v 1. The fold superfamily of these allergens is classified as Bet v 1 like, also termed START.[37] The common feature of START domains is that they are known to bind hydrophobic ligands.

The best structural matches with Der p 7 include insect proteins that have very similar folds, such as juvenile hormone-binding protein (JHBP) and the takeout (TO) proteins, which is consistent with GenTHREADER predictions.[29,31,38] The RMSD of the superposition for Der p 7 with JHBP is 3.6 Å over 176 Cα atoms (Fig 2, *A*). Like the TO proteins, it superimposes well over the whole molecule and shows very similar secondary structure organization. JHBP and TO proteins bind to hydrophobic ligands, which is similar to Bet v 1 and the other START domain–like allergens.

Another significant match to Der p 7 based on the DALI evaluation was the N-terminal domain of bactericidal/permeability-increasing protein (BPI; PDB code 1BP130). A structural overlay is shown in Fig 2, *B*, and has an RMSD of 3.5 Å over 174 Cα atoms. BPI contains 2 domains, which have a very similar fold but are distantly related sequentially. The full structure of BPI is shown in Fig 2, *C*, with the domains shaded differently and bound ligands indicated with yellow spheres. The protein family database Pfam[39] designates this fold as "BPI/LBP/CETP N terminal," and the database CATH[40] classifies the architecture as a "super-roll" based on the way the long β-strands roll around the central long helix. The fold of Der p 7 is more similar to the super-roll than to the START domains.

Lipid-binding assays

Based on the structural similarities to other lipid-binding proteins, the lipid-binding potential of Der p 7 was examined. Because BPI and the related LBP bind to LPS, we first tested LPS binding to Der p 7. Biochemical assays indicated that Der p 7 does not bind tightly to LPS (see Fig E1 in this article's Online Repository at www.jacionline.org). Other potential Der p 7 ligands for evaluation were identified by comparison with BPI and LBP. One type of potential Der p 7 substrate is distearoyl phosphatidyl choline (DSPC), which is found complexed to BPI in the crystal structure (Fig 2, *C*).[30] Lyso-palmitoyl PC was tested for interactions with Der p 7 because of its similar structure to DSPC and much higher solubility. A sample containing 50 μmol/L U-[^{15}N]–labeled Der p 7 was titrated with increasing amounts of lyso-palmitoyl PC, and 2-dimensional ^{1}H-^{15}N HSQC spectra showed very few changes. Similar negative results were obtained on addition of SDS and N-octyl-glucoside. Fig 3, *A*, shows an example of an NMR spectrum showing minor perturbations that were obtained on addition of LPS. Polymyxin B (PB), which is known to sequester LPS, was added to determine whether the small changes caused by LPS could be reversed.[41] Surprisingly, the addition of PB produced much more significant spectral changes (data not shown). Figure 3, *B*, shows the changes in the NMR HSQC spectra of Der p 7 on addition of PB alone to ensure that the changes were due to PB. This result was followed up with a saturation transfer difference experiment[42] that demonstrated that Der p 7 and PB interact. Fig 4 shows the ^{1}H spectra of PB alone (Fig 4, *A*) and the results of the saturation transfer difference experiment with PB and Der p 7 (Fig 4, *B*). The same resonances appearing in Fig 4 and *B*, *A*, provide conclusive evidence of a binding interaction between PB and Der p 7.

The assignments of the resonances in the HSQC spectra were determined to map the site of the interaction to show that the binding of PB was a specific interaction (see Fig E2 in this article's Online Repository at www.jacionline.org). Fig 5, *A*, shows the residues involved in the interaction colored red. Many of the significant shift changes cluster near a cleft between the N and C termini of Der p 7. However, because of missing assignments, we are being cautious in our interpretation of the shift changes because it is possible that additional residues are

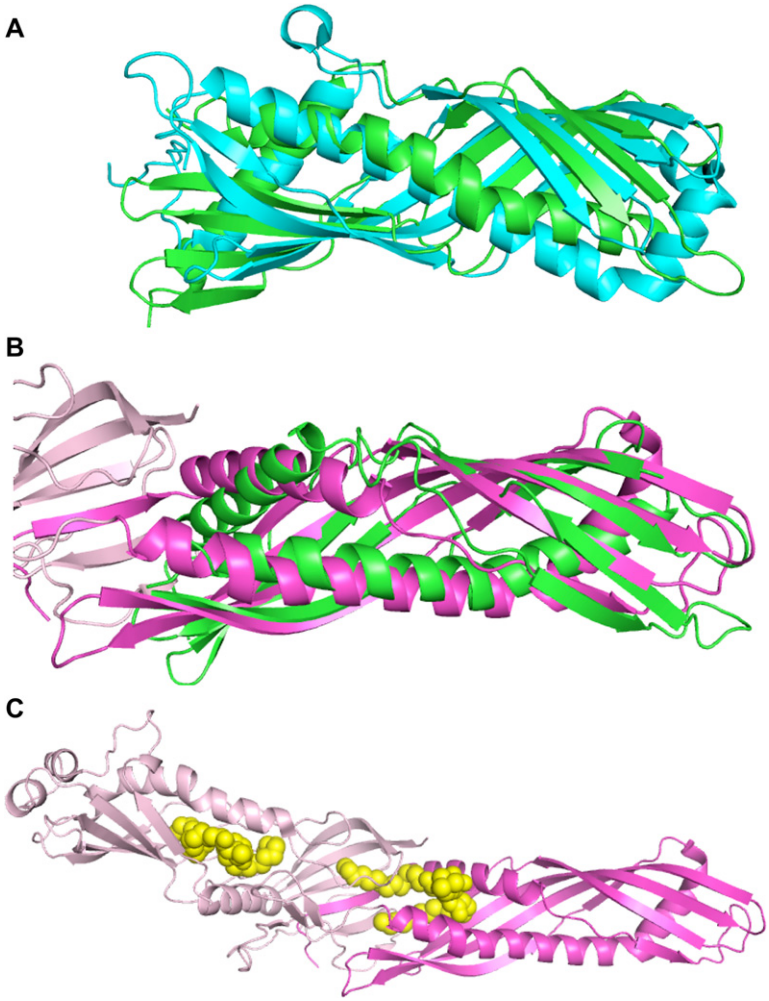

FIG 2. Examples of structurally similar proteins to Der p 7. Der p 7 is shown in *green* in all panels. **A** is the best alignment with JHBP (*cyan*). **B** shows the best alignment of Der p 7 with the N-terminal domain of BPI shaded *magenta*, whereas the C-terminal domain of BPI is shaded *pale pink*. **C** shows BPI with 2 bound DSPC molecules (*yellow spheres*).

involved. Fig 5, *B*, shows the N-terminal domain of the structure of BPI with the DSPC bound. BPI is aligned in a similar orientation to Der p 7. This figure shows the DSPC molecule bound in a cleft similar in location to the cleft in the Der p 7 molecule. Hence PB interactions with Der p 7 occur near the expected binding site.

DISCUSSION

Der p 7 appears closely related to several proteins known to bind hydrophobic compounds, including the TO proteins and BPI and LBP. The TO proteins are involved in signaling in a wide variety of biological functions of insects.[43] Because the class Insecta and the class Arachnida (including mites) are both in the phylum Arthropoda, this might provide some clues as to the natural function of Der p 7 in mites, but there is not a good enough match in the literature with a known natural ligand. Der p 7 does not appear to bind to LPS, like BPI or LBP, but exhibits affinity for PB, which is a 9-residue cyclic peptide with a lipid tail from the gram-positive bacteria *Bacillus polymyxa*. The weak binding of PB to Der p 7 does not imply that PB is the natural substrate. Future studies to identify the natural ligand or ligands will require screening of a broader spectrum of potential ligands, as well as a determination of the corresponding dissociation constants. However, the mapping of the interaction site to a similar position in homologous proteins validates the conclusion that Der p 7 has affinity for a lipid substrate, in this case a bacterially derived lipopeptide product. The importance of this is that lipid binding is a common feature of some allergens,[44] and natural lipid adjuvants are suspected to be important in sensitizing patients to allergens.[17]

LBP has been shown to interact with diacylated and triacylated lipopeptides, as well as LPS.[45,46] Bacterial and mycobacterial lipopeptides can induce the release of cytokines through interactions with TLR2 in cooperation with TLR1 or TLR6. Several TLRs might be involved in stimulating the allergic response through promotion of T_H2 immunity.[44] Indeed, stimulation of TLR2 in the presence of allergen enhances T_H2 responses,[47,48] as does TLR5 stimulation.[49] We speculate that if Der p 7 can bind bacterial lipopeptides other than PB as its natural ligand, it might promote T_H2 immunity (to itself) through costimulation of TLR2 pathways.

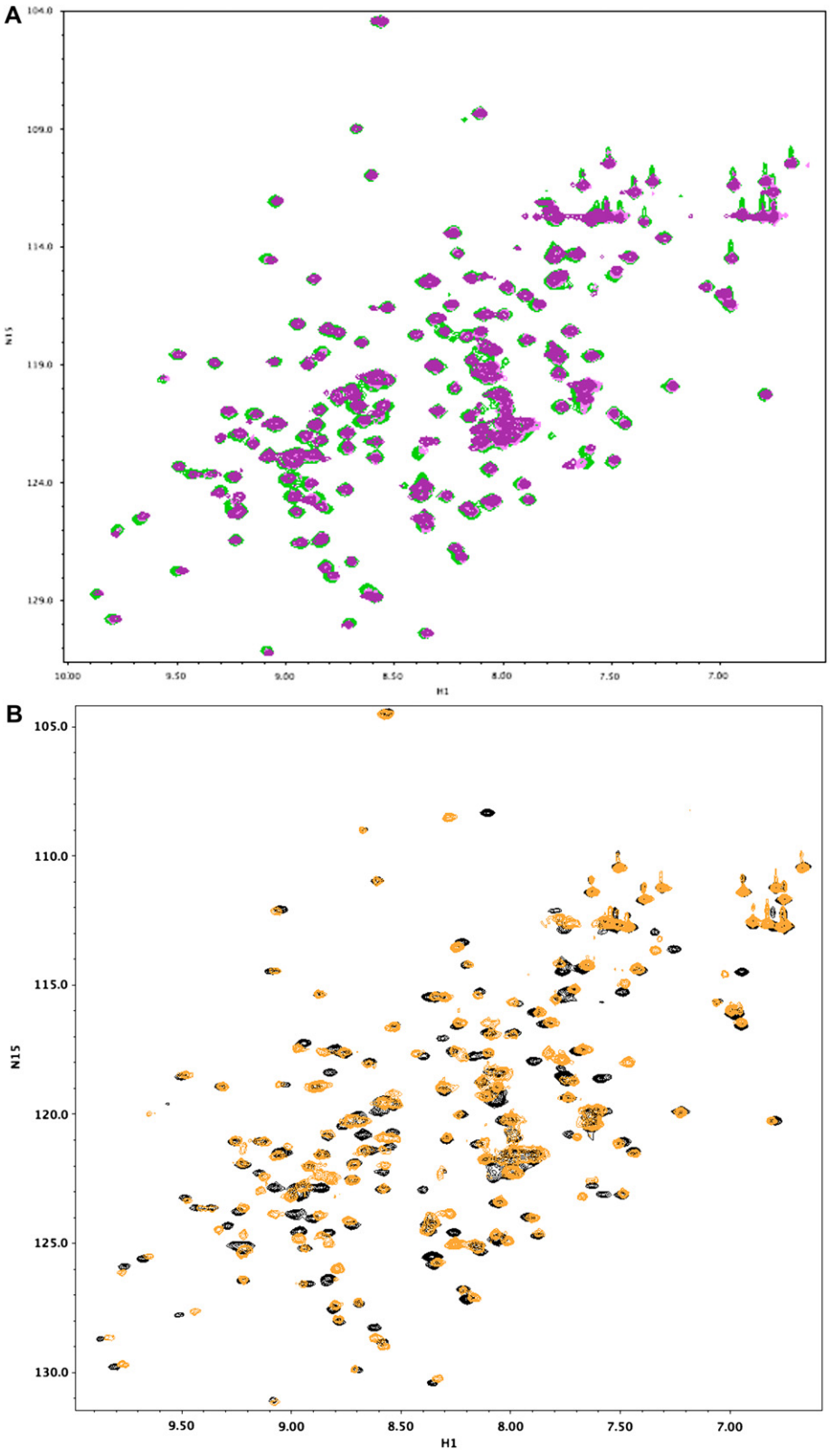

FIG 3. Changes in the ^{15}N-^{1}H HSQC spectra of Der p 7 on addition of compounds. **A** shows very little change in the HSQC spectra of 50 μmol/L Der p 7 going from apo (*green*) to 100 μmol/L (*pink*) to 200 μmol/L (*purple*) LPS. In contrast, addition of PB caused dramatic changes, as shown in **B**. Der p 7 apo spectra are *black*, and on addition of PB, the spectra are colored *orange*.

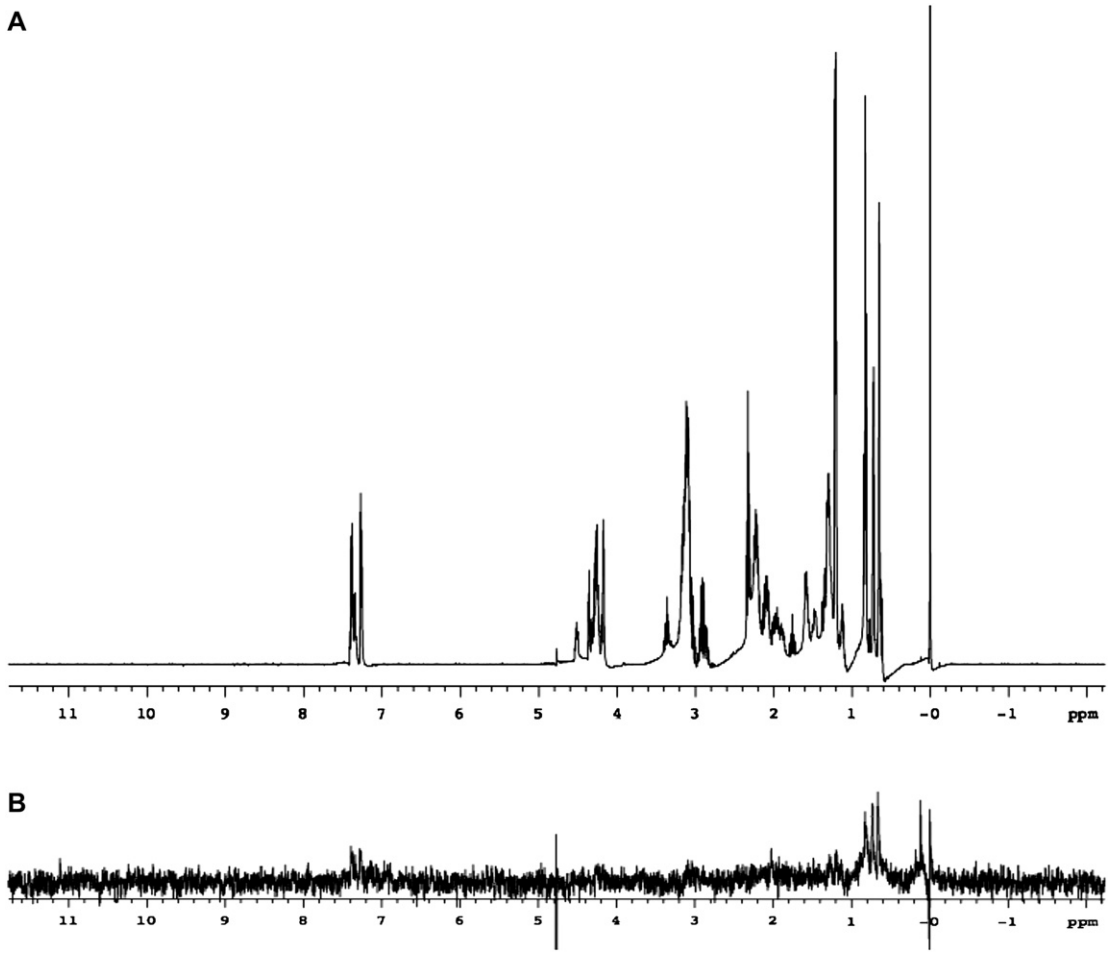

FIG 4. ^{1}H NMR of PB. **A** shows the ^{1}H NMR spectrum of 1 mmol/L PB in $^{2}H_2O$ with 10 μmol/L 5,5-dimethylsilapentanesulfonate (at 0 ppm) for chemical shift referencing. **B** shows the result of the saturation transfer difference experiment[42] with 1 mmol/L PB and 50 μmol/L Der p 7; the saturation frequency was 0.2 ppm.

In addition to understanding allergen function, structural characterization of allergens can provide detailed knowledge of potential B-cell epitopes and aid in mapping antibody interactions. We previously used hydrogen exchange NMR to map mAb binding sites on Der p 2, and NMR techniques have recently been used to define a mAb epitope on the *Blomia tropicalis* allergen Blo t 5.[50,51] Several x-ray crystal structures of allergen/antibody complexes have also been reported, including Bet v 1, β-lactoglobulin, and Bla g 2.[52-54] Modification of structure has been an effective way to design recombinant hypoallergenic variants of allergens, which have potential clinical application for immunotherapy in patients with mite allergy.[55,56] Our IgE binding data confirm that Der p 7 is an important allergen and shows that the prevalence of IgE antibody to rDer p 7 is comparable with that of the natural allergen.[12] The purified rDer p 7 was of sufficiently high quality for crystallographic studies. These observations suggest that rDer p 7 could be used in a cocktail of recombinant mite allergens for use in subcutaneous immunotherapy and for rational design of hypoallergenic variants. A cocktail of 5 recombinant timothy pollen allergens was successfully used in a placebo-controlled trial and resulted in a 39% reduction in symptom scores and medication use.[57] Clinical improvement was associated with a strong increase in allergen-specific IgG4 antibody production, reduced levels of IgE antibody, and induction of IL-10–producing regulatory T cells. Current data suggest that rDer p 1, rDer p 2, and rDer p 7 are important immunologic targets and should be included in similar vaccine trials for mite allergen immunotherapy. The structure presented here will be useful in the rational design of hypoallergenic mutant proteins and in understanding future studies of the cross-reactivity between the group 7 mite allergens.

Knowledge of allergen function has contributed to an improved understanding of allergenicity. This was demonstrated for the protease activity of group 1 mite allergens on the adaptive immune system[7,8] and the interaction with the innate immune system by group 2 mite allergens.[17] We suggest that there might be similarities between the biological functions of the group 7 and group 2 mite allergens that involve binding of lipid substrates and that both allergens might co-opt innate immune responses into promoting allergenicity. Further studies are needed to investigate this hypothesis. The structure of Der p 7 presented here will contribute to an improved understanding of the complex interactions between the innate and adaptive immune systems in the development of allergy.

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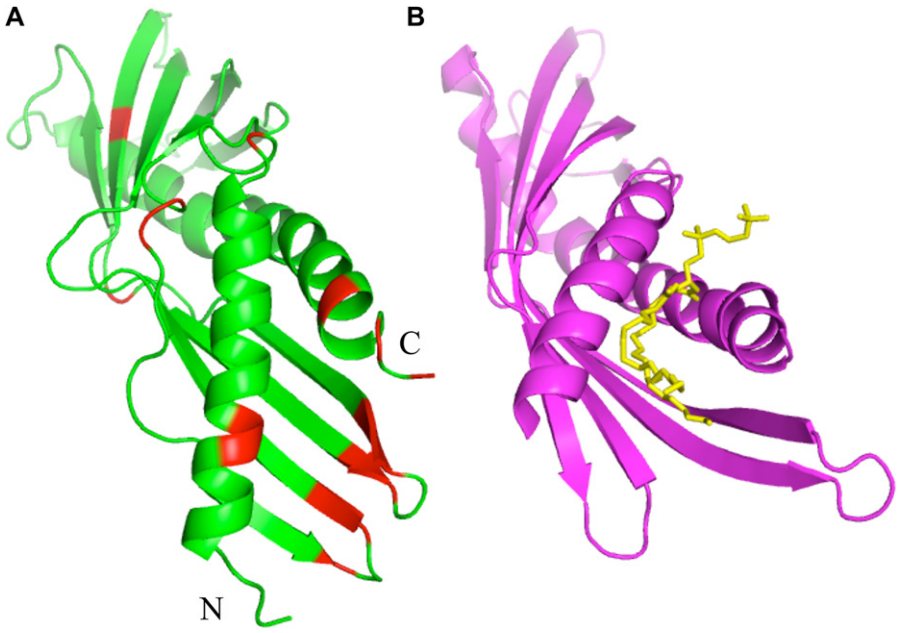

FIG 5. Proposed binding site of PB in Der p 7. The significant chemical shift changes in Fig 3, *B*, correspond to the residues colored *red* in **A**. The residues map to a cleft between the N and C termini, as labeled in Fig 5, *A*. This location is similar to a cleft in the N-terminal domain of BPI that contains DSPC in the crystal structure **(B)**.

Key messages

- **The structure of the dust mite allergen Der p 7 reveals a distant homology to a family of proteins involved in human innate immune recognition of bacterial lipid products.**
- **Der p 7 binds specifically to a bacterially derived lipopeptide.**
- **Der p 7 is the second dust mite allergen identified to be structurally related to a protein in the TLR pathway.**

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METHODS

Patient sera IgE

Sera from allergic patients were obtained from PlasmaLab International, which operates in full compliance of US Food and Drug Administration regulations. An informed donor's consent was obtained from each person before the first donation. Levels of IgE were measured by using Fluorescent Multiplex Array analysis (total IgE, Der f 1, Der p 1, and Der p 2) or by using a chimeric ELISA (rDer p 7 without MBP expressed in *E coli* and nDer p 7).

LPS-binding experiments

The primary role of BPI and LBP is to bind LPS, also known as endotoxin. The binding of LPS to Der p 7 was assessed with 2 biochemical assays. First, we compared the ability of Der p 7 to inhibit the Limulus amoebocyte lysate (LAL) assay like BPI.[E1,E2] BPI is known to inhibit the binding of LPS to the chromogenic substrate in the LAL. Second, we used ELISA techniques.[E3]

The LAL assay for quantification of endotoxin was used according to the manufacturer's instructions (Lonza, Walkersville, Md). Inhibition of the LAL assays was performed similarly to previously described methods.[E1,E2] Biotinylated LPS was purchased from Invivogen (San Diego, Calif) to test binding by using ELISA techniques.[E3]

To remove contaminating LPS from the bacterially expressed proteins, the Der p 7 samples were passed twice through an LPS-removal column (Proteospin; Norgen Biotek, Kyoto, Japan). After column passage, there remained a small, residual, less than 0.1 EU/mL of endotoxin that could not be removed, which was subtracted from the absorbance reading because it was a constant in all samples.

NMR analysis

The assignments of the resonances in the HSQC spectra were determined to map the site of the interaction to show that the binding of PB was not a nonspecific interaction. A weighted shift perturbation parameter was used as a measure of the binding-induced perturbation to evaluate the significance of the chemical shift changes observed in Fig 3, *B*, as follows:

$$\Delta\delta = \sqrt{(5\Delta\omega_{1_H})^2 + \Delta\omega_{15_N}{}^2}.$$

The factor of 5 in the above equation compensates for the more limited parts per million (ppm) range of the ^{1}H axis. The coordinate peaks in Fig 3, *B*, were assigned to residues in the protein. The differences in position with and without PB were evaluated.

RESULTS

Patient sera IgE

Table E1 shows the total and specific IgE levels in patient sera reactive to Der p 7. Specifically measured were IU (IgE/mL) of Der f 1, Der p 1, Der p 2, rDer p 7 (without affinity tag and expressed in *E coli*), and nDer p 7 from mite extract.

LPS-binding experiments

Fig E1 shows that after LPS removal, we could detect no inhibition of the LAL assay, even at concentration ranges 15 times greater than that at which BPI will inhibit the LAL assay.[E2] Second, in data not shown, we attempted to detect LPS binding to Der p 7 by using an ELISA in which Der p 7 was coated to the plate and used to capture biotinylated LPS in solution, which is similar to a reported assay used to show MD-2 binding to LPS.[E3] However, there was no detectable binding of LPS greater than the nonspecific binding of LPS to the plates. As a positive control, immobilized PB did capture the biotinylated LPS, as expected.[E4] Thus it appears that LPS does not bind to Der p 7 with high affinity.

NMR analysis

A plot of $\Delta\delta$ versus residue is shown in Fig E2, along with a solid line for the mean and a dashed line at the mean + 1 standard deviation. In Fig 4, *A*, those residues with $\Delta\delta$ values greater than or approximately equal to 1 σ from the mean are shown in red in the ribbon diagram of Der p 7. Additionally colored red are residues 213 and 215 because the resonance broadened below the noise threshold on addition of PB.

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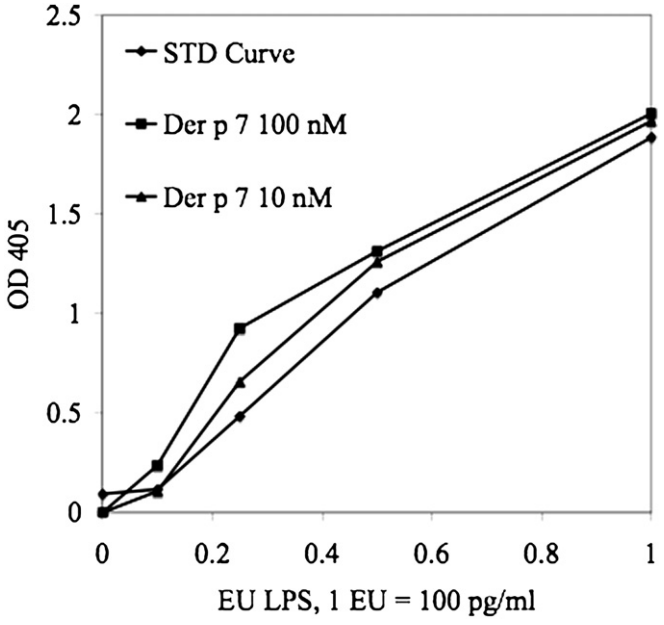

FIG E1. LAL assay. The standard curve for determining LPS concentration versus absorbance at 405 nm is plotted *(diamonds)*. On addition of 10 nmol/L *(triangles)* and 100 nmol/L *(squares)* Der p 7, no inhibition of absorbance is observed. *STD*, Saturation transfer difference.

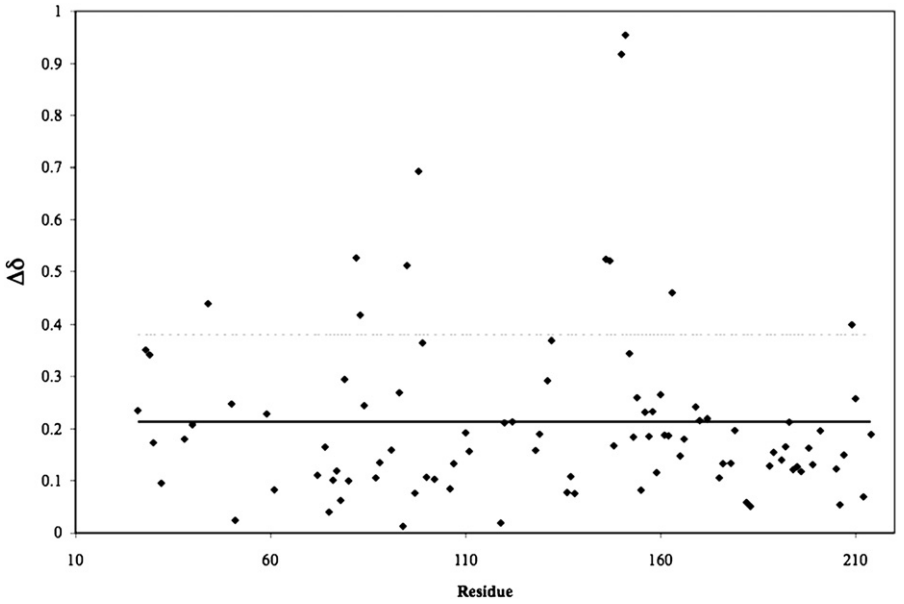

FIG E2. NMR analysis. Δδ versus residue is shown, along with a *solid line* for the mean and a *dashed line* at the mean + 1 standard deviation.

TABLE E1. Total and specific IgE in sera reactive to Der p 7

		Specific IgE antibody binding (IU/mL) to:				
Serum	**Total IgE (IU/mL)**	**Der f 1**	**Der p 1**	**Der p 2**	**rDer p 7**	**nDer p 7**
1	220	29	51	42	0.4	0.4
2	535	13	149	80	0.4	<0.3
3	1,001	7	92	233	1.5	<0.3
4	492	10	40	13	3.4	4.2
5	261	1	151	61	1.7	1.6
6	459	4	31	17	1.7	2.2
7	580	5	119	44	6.5	6.8
8	615	16	134	37	1.1	1.3
9	853	5	75	42	0.3	2.0
10	6,047	75	1,072	743	32.0	35.8
11	611	10	156	47	0.4	3.4
12	2,559	<0.3	<0.3	<0.3	<0.3	1.0
13	413	0.3-3	34	15	1.4	1.4
14	4,877	23	82	42	2.6	1.5
15	14,981	79	429	262	2.9	4.8
16	13,146	18	57	117	25.8	30.5
17	751	10	83	22	<0.3	0.4
18	594	37	147	67	3.5	5.8
19	456	0.3-3	87	28	1.1	1.1
20	693	21	17	44	4.9	5.0
21	3,162	17	93	93	<0.3	0.3

Results are reported as international units (IgE per milliliter), and values were measured by using Fluorescent Multiplex Array analysis (total IgE, Der f 1, Der p 1, and Der p 2) or by using a chimeric ELISA (rDer p 7 without MBP expressed in *E coli* and nDer p 7).