

The molecular basis of antigenic cross-reactivity between the group 2 mite allergens

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Background: Mite group 2 allergens Der p 2, Der f 2, and Eur m 2 are 14-kDa proteins of unknown function that share 83% to 85% amino acid sequence identity. Isoforms of the allergens within each genus have been identified which differ by 3 or 4 amino acids, but little is known of the influence of group 2 polymorphisms on human IgE antibody binding.

Objective: The purpose of this study was to investigate the importance of interspecies and isoform substitutions on murine mAb and IgE antibody binding and on the molecular structure of the group 2 allergens.

Methods: Site-directed mutagenesis was used to incorporate the isoform amino acid substitutions onto the Der p 2.0101 sequence. Recombinant allergens were expressed and purified from *Escherichia coli* and used to evaluate antibody binding by enzyme-linked immunosorbent assay (ELISA). Molecular modeling of the tertiary structure was used to analyze structural differences between the various group 2 allergens.

Results: The substitution of asparagine for aspartic acid at position 114 restored mAb binding of rDer p 2.0101; the other Der p 2 isoforms and the 3 rDer f 2 isoforms also reacted in the 2-site ELISA. The correlation of IgE binding to the Der p 2 isoforms was excellent and tended to be higher in the isoforms with the asparagine 114 substitution ($r^2 = 0.87$ vs $r^2 = 0.95$). rEur m 2.0101 bound to all mAb except 7A1; when compared with rDer p 2 for IgE binding, rEur m 2.0101 gave a correlation coefficient of $r^2 = 0.68$. Molecular modeling revealed that

Eur m 2 and the storage mite homologs Lep d 2 and Tyr p 2 retain the tertiary fold of Der p 2. Eur m 2 has a conserved surface, whereas Lep d 2 and Tyr p 2 present most of the amino acid substitutions on this surface. Lep d 2 and Tyr p 2 did not react with mAb or with sera from patients with IgE to *Dermatophagoides* species.

Conclusion: The isoform substitutions of rDer p 2 can be distinguished by mAb. The allergenic cross-reactivity between Der p 2, Der f 2, and Eur m 2 is a direct result of the conserved antigenic surface, whereas the lack of cross-reactivity with Lep d 2 and Tyr p 2 is a result of the multiple substitutions across this surface. (J Allergy Clin Immunol 2001;107:977-84.)

Key words: Mite, allergen, isoform, epitope

Dermatophagoides species mites are the most important source of indoor allergens associated with asthma.¹ Although more than 10 allergens have been identified by cDNA cloning, the prevalence of IgE antibody binding is highest for the 24-kDa group 1 proteins, Der p 1 and Der f 1, and the 14-kDa group 2 proteins, Der p 2 and Der f 2; 80% to 90% of mite-sensitive subjects have IgE antibody to these allergens.^{1,2} The group 1 allergens are cysteine proteases; however, the function of the group 2 allergens remains unknown. Previously, we determined the 3-dimensional structure of Der p 2 and showed that this allergen had structural homology to a moth molting protein and to a human epididymal protein.³⁻⁵

Homologs of Der p 2 and Der f 2 have been found in the closely related genus *Euroglyphus maynei*; in the genera *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Glycyphagus domesticus*, all of which are storage mites found in farming communities; and most recently, in *Blomia tropicalis* (Smith and Chapman, unpublished observation). Der p 2, Der f 2, and Eur m 2 share approximately 83% amino acid identity, in keeping with the close phylogenetic relationship of these species. Indeed, these 3 species often inhabit the same environment. However, the prevalence of sensitization to *E maynei* has been difficult to assess because of the lack of commercially available diagnostic reagents and because of allergenic cross-reactivity with the *Dermatophagoides* species.⁶⁻⁸ Microscopic examination of house dust has identified *E maynei* in numbers sufficient to cause sensitization.^{9,10} The group 2 allergens from the storage mites Lep d 2, Tyr p 2, and Gly d 2 share approximately 40% identity with

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This work was supported by grants from the American Lung Association, Virginia's Center for Innovative Technology (B10-01-008), and NIH AI34607, AI20565, and AI32557.

Presented in part at the 56th Annual Meeting of the American Academy of Allergy, Asthma and Immunology, San Diego, Calif.

Received for publication January 30, 2001; revised March 9, 2001; accepted for publication March 9, 2001.

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0091-6749/2001 \$35.00 + 0 1/81/115629

doi:10.1067/mai.2001.115629

Abbreviations used

ABTS: 2,2'-azino bis(3-ethylbenzthiazoline sulfonic acid)
 ELISA: Enzyme-linked immunosorbent assay
 mAb: Monoclonal antibody
 NMR: Nuclear magnetic resonance

Der p 2.¹¹⁻¹⁴ Sensitization to these mites is often found in agricultural communities, and limited cross-reactivity has been reported between allergen extracts from the *Dermatophagoides* species and the storage mites.¹⁵

Natural preparations of Der p 2 are composed of multiple isoforms. Six isoforms of Der p 2 and 4 isoforms of Der f 2 have been cloned to date.^{16,17} These isoforms show 3 or 4 amino acid substitutions, and little is known of the influence of these substitutions on IgE binding. The Der p 2 isoform that was first described (rDer p 2.0101) had immunoreactivity comparable to that of the natural allergen.¹⁸⁻²⁰ Antibody binding to Der p 2 is dependent on the intact tertiary structure of the allergen and can be altered by breaking the 3 disulfide bonds that stabilize the allergen.^{20,21}

The present study was designed to determine the influence of the isoform and interspecies substitutions of the group 2 allergens on antibody binding. In combination with immunoassays using murine monoclonal antibodies (mAbs) and sera from dust mite-allergic subjects, molecular modeling was used to study the tertiary structure and the antigenic surface of the allergens.

METHODS**Systematic nomenclature for the group 2 isoforms**

Through use of the allergen nomenclature recommended by the World Health Organization and the International Union of Immunological Societies (WHO/IUIS),²² the group 2 allergen sequences were given the designations shown in Fig 1. The first Der p 2 isoform identified was designated *rDer p 2.0101*. Subsequent isoforms identified were assigned the designations *rDer p 2.0102* and *rDer p 2.0103*. In a similar fashion, the Der f 2 isoforms were designated *rDer f 2.0101*, *rDer f 2.0102*, and *rDer f 2.0103*. The sequences for the homologous allergens from *E maynei*,²³ *Lepidoglyphus*,¹² and *Tyrophagus*¹³ were previously assigned designations according to the WHO/IUIS recommended nomenclature.

Allergens

Recombinant Der p 2.0101 was produced from *E coli* expression cultures, as previously described.¹⁹ The asparagine substitution at position 114 was introduced into the rDer p 2.0101 sequence through use of oligonucleotide-directed mutagenesis (QuikChange, Stratagene, La Jolla, Calif). The substitution was confirmed by DNA sequence analysis, and variants were designated through use of the residue number, preceded by the amino acid of the Der p 2.0101 sequence and followed by the variant amino acid substitution: rDer p 2.0101 (D114N). Additional substitutions were introduced sequentially onto the Der p 2.0101 sequence, also by oligonucleotide mutagenesis, and designated *rDer p 2.0101* (D114N/T47S), *rDer p 2.0101* (D114N/V40L), *rDer p 2.0101* (D114N/I127L), *rDer p 2.0102*, and *rDer p 2.0103*. All of the rDer p 2 proteins carry the D1S substitution¹⁹ that allows for the efficient cleavage of the N-terminal methionine during expression in *E coli*.

The Eur m 2.0101 cDNA was amplified from the pUC18 plasmid²³ and subcloned into pET22b. rEur m 2 was produced with the natural amino terminus and retained the methionine initiator codon, showing the sequence MDQVD for positions -1 through 4. rEur m 2.0101 was expressed and purified as described above. Similar expression plasmids were used to produce rLep d 2.01 and rTyr p 2.01.^{13,24} rDer f 2 isoforms²⁵ were kindly provided by Dr Yasushi Okumura (Asahi Breweries, Tokyo).

Monoclonal antibodies

The mAbs used in this study have been described previously and include clones 1D8, 6D6, 2B12, 4G7, and 7A1.²⁶ Also included in this study were the mAb α DpX, produced by Dr R. Aalberse,²⁷ and the mAbs 15E11 and 13A4, kindly supplied by Dr H. Okudaira.²⁸ Through use of inhibition radioimmunoassay and enzyme-linked immunosorbent assay (ELISA), these mAbs were shown to define 3 antigenic regions on Der p 2,²⁶ and experiments in which hydrogen protection nuclear magnetic resonance (NMR) was used further defined these regions.²⁹ From these studies, the amino acids of Der p 2 that were protected by mAb 7A1 binding were found to be 94, 97, and 101; for mAb 6D6, an mAb that inhibits binding of mAb 1D8 and is therefore assumed to have the same specificity, protected residues were 111 and 116. Alanine scanning mutational analysis of the mAb 7A1 epitope identified H30, R31, K33, S57, K96, I97, and E102 as residues making contributions to the binding energy of the complex.²⁹

Patient sera

Sera from mite-allergic subjects were kindly provided by Drs Kunio Ichikawa (Tsukuba, Japan), Mark Larché and A. Barry Kay (London, United Kingdom), and Adnan Custovic (Manchester, United Kingdom). These subjects had positive CAP scores (class 1-5, Pharmacia CAP System) or positive radioallergen sorbent test results for *Dermatophagoides pteronyssinus*.

Direct binding and 2-site ELISA

The ability of the anti-group 2 mAb to bind to the various group 2 allergens was determined through use of microtiter plates coated with allergen at 10 μ g/mL. mAb was added starting at 1 μ g/mL with serial 2-fold dilutions to 0.05 μ g/mL. Binding was detected through use of horseradish peroxidase-labeled goat antimouse IgG and the colorimetric substrate ABTS. The 2-site ELISA uses mAb 1D8 as the capture mAb and biotinylated 7A1 as the detecting mAb. Allergens were assessed in this assay over the concentration range of 0.25-250 ng/mL.

Chimeric ELISA for IgE to Der p 2

IgE antibody to the group 2 allergens was measured through use of the chimeric ELISA described by Schuurman et al.³⁰ The assay is quantified from a standard curve through use of the mAb α DpX to present nDer p 2 to the mouse-human chimeric IgE mAb, which carries the mAb 2B12 variable regions engineered onto a human IgE Fc domain. Allergen-specific IgE antibody was determined through use of a 1/10 serum dilution and allergen-coated microtiter plates.³¹

Molecular modeling

The crystal structure of rDer p 2.0101⁴ was used for molecular modeling of rEur m 2.0101, rLep d 2.01, and rTyr p 2.02; standard methods were used. SYBYL (Tripos, Inc, St Louis, Mo) was used for insertions or deletions, and CONGEN (ConGenomics, Inc, Princeton, NJ) was used for amino acid replacements, side chain reconstruction, energy minimization, and molecular dynamics.³²⁻³⁴ In all cases, the lowest energy conformer was selected for further consideration. Briefly, the sequences were aligned within SYBYL; only 2 or 3 single amino gaps were found. For Eur m 2, the sequence was 85% identical to Der p 2; thus the alignment and

	-1	10	20	30	40	50	60
Der p 2.0101	RDQVDVKDCANHEIKKVLVPGCHGSEPCIIHRGKPFQLEAVFEANQNTKTAKIEIKASIDGLEVDV						
0102	-----S-----						
0103	A-----L-----S-----						
Der f 2.0101	-----N-----M-D-----D-----T-----L-D-----L-----I-----						
0102	-----N-----M-D-----D-----T-----L-D-----L-----I-----						
0103	-----N-----M-D-----D-----T-----L-D-----L-----I-----						
Eur m 2.0101	-----I-----M-----K-----V-----TA-----D-----SNA-----T-----V-----I-----						
Lep d 2.01	GKMTF---GHG-VTELDIT--S-*DT-V---EKMT---K-A---D-AKVT-KVL-KVA-TT-Q-						
Tyr p 2.01	G--KFT--GKK--AS-A-D--E-*DL-V---KS--VHVI-E-T---D-SKIEVKVTGQLN---PI						

	70	80	90	100	110	120	129
Der p 2.0101	PGIDPNACHYMKCPLVKGQQYDIKYTNVVPKIAPKSENVVTVKVMGDDGVLACAIATHAKIRD						
0102	-----N-----L-----						
0103	-----N-----						
Der f 2.0101	---T---FV-----LI--N-----G---						
0102	---T---F-----LI--N-----G---						
0103	---T---F-----A-----LV--N-----						
Eur m 2.0101	---N-L--F-----E-----R-----LL--N-----						
Lep d 2.01	--LETDG-KFI---VK--EAL-FI-SGTI-A-T--V*KAD--AELI--H--M--G*TV-GQVE						
Tyr p 2.01	---ETDG-KVL---K--TK-TMN-SV---SVV-NI*KT--KLLAT-EH---G*VNTDVKP						

FIG 1. Amino acid sequences of the group 2 allergens. The single-letter amino acid code is used to show the sequences of the group 2 mite allergens used in this study. A *dash* indicates identity to rDer p 2.0101 at that position; an *asterisk* indicates a gap introduced for the purposes of alignment. The numeric isoform designation is given according to the WHO/IUIS nomenclature.²²

modeling was unequivocal. For Lep d 2 and Tyr p 2, the sequences were divided into core and surface residues on the basis of the Der p 2 structure. Of the 54 Der p 2 core residues, 83% either were identical or represented conservative changes in Lep d 2 and Tyr p 2. The core residues of Der p 2 were changed to those of the homologous allergen through use of the SPLICE module of CONGEN and then submitted to minimization with no constraints to stabilize the new core structure for each model. The surface residues, which showed 33% homology to Der p 2 surface residues, were then changed and subjected to minimization.

RESULTS

The group 2 family of mite allergens

The amino acid sequences of the group 2 isoforms were classified according to the WHO/IUIS nomenclature,²¹ and alignments were compared with the rDer p 2.0101 sequence (Fig 1). The sequences of Der p 2, Der f 2, and Eur m 2 are 85% identical. The storage mite allergens rLep d 2.01 and rTyr p 2.01 are respectively 40% and 41% identical to rDer p 2.0101. The structure of Der p 2 has been determined through use of NMR spectroscopy³ and x-ray crystallography⁴ and is composed of 2 antiparallel β -sheets (Fig 2, *top panel*). The positions that vary among the rDer p 2 isoforms are amino acids 40, 47, 114, and 127. The side chains at positions 47 and 114 are exposed to solvent, and substitutions at these positions would be expected to influence antibody binding. In contrast, the α carbon of residues 40 and 127 is on the surface and the side chains extend into the core of the molecule, making them unavailable to interact with antibody. Interestingly, the isoform substitutions are in regions of the allergen known to be important for mAb binding. Fig 2, *bottom panel* depicts the Der p 2 structure rotated 90°, showing the amino acid side chains of the

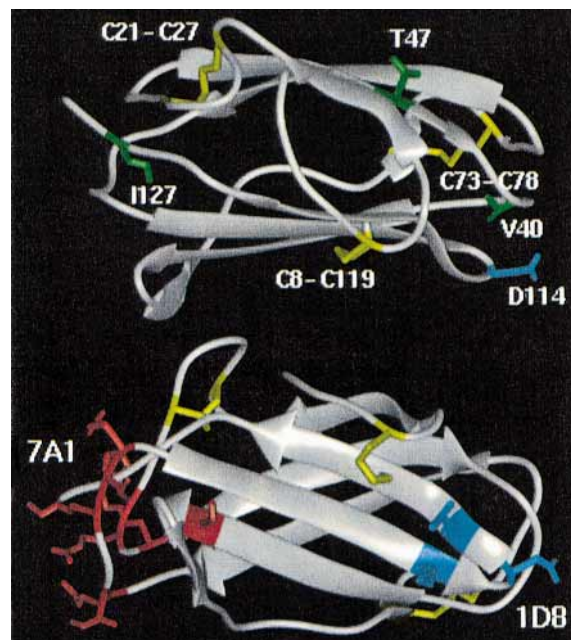


FIG 2. Location of isoform substitutions and mAb binding regions on the ribbon structure of rDer p 2.0101. The backbone structure and disulfide bonds of rDer p 2.0101 are shown (the latter in yellow); positions that vary among the rDer p 2 isoforms are shown in green (V40, T47, I127) and blue (D114) in the top panel. In the bottom panel, the molecule has been rotated 90° to show the β strand structure and the residues involved in binding mAb 7A1 (in red) and mAb 1D8 (in blue).

residues involved in mAb binding.^{29,35} The mAb 7A1 binds residues 30, 31, 33, 57, 96, 97, 100, and 102, and positions 111 and 116 are protected by mAb 1D8.

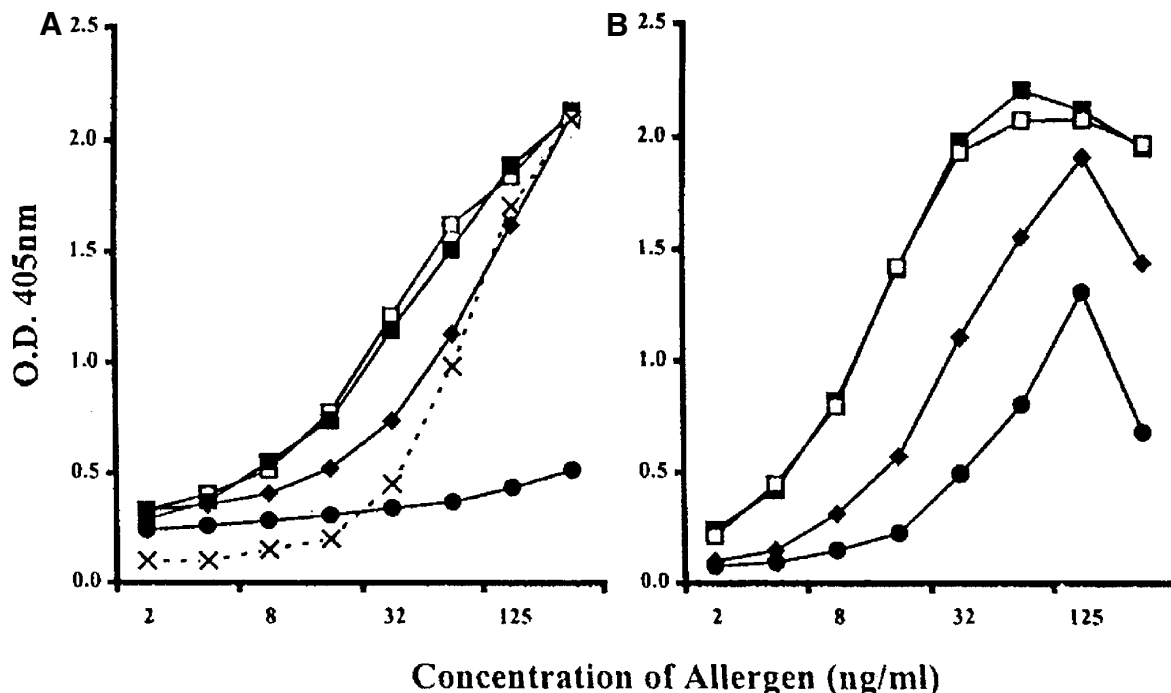


FIG 3. Binding of rDer p 2 and rDer f 2 isoforms in 2-site ELISA. In this assay, mAb 1D8 was used as the capture antibody and biotinylated mAb 7A1 was used as the detecting mAb. **A**, Reactivity of increasing concentrations of rDer p 2.0101 (●), rDer p 2.0102 (◆), rDer p 2.0103 (■), and rDer p 2.0101 (D114N) (X) compared with the nDer p 2 standard (□). **B**, Reactivity of increasing concentrations of rDer f 2.0101 (◆), rDer f 2.0102 (●), and rDer f 2.0103 (■), compared with the nDer f 2 standard (□). Allergens that did not bind in this assay include rEur m 2.0101, rLep d 2.01, and rTyr p 2.01 (not shown).

Reactivity of mAb with isoforms and homologs

By direct binding ELISA, all mAbs bound rDer p 2.0102 and rDer p 2.0103, whereas rDer p 2.0101 was recognized by all mAbs except 1D8 and 4G7, which gave only a weak signal at 250 ng/mL of allergen. This was confirmed through use of inhibition ELISA (not shown). The mAbs 1D8, α DpX, and 7A1 bound to the rDer f 2 isoforms, and rEur m 2.0101 was recognized by all except mAbs 7A1 and 13A4. None of the mAbs bound to rLep d 2.01 or rTyr p 2.01. In the 2-site ELISA, rDer p 2.0101, rEur m 2.0101, rLep d 2.01, and rTyr p 2.01 did not react, in keeping with the direct binding ELISA results (Fig 3).

To investigate the inability of mAb 1D8 to bind to rDer p 2.0101, we made the D114N substitution. We focused initially on this residue because the side chain was exposed on the surface of the allergen (Fig 2, A) and because it was near amino acids 111 and 116, those residues predicted to be within the 1D8 epitope. Fig 3, A shows the 2-site ELISA binding curves for the rDer p 2 isoforms and the rDer p 2.0101 (D114N) variant. The substitution of asparagine for aspartic acid at position 114 restored binding of rDer p 2.0101. The variants rDer p 2.0101 (D114N, T40S) and (D114N, T47S) and (D114N, I127L) gave binding curves similar to that of the D114N variant (not shown). Asparagine is found at position 114 in rDer p 2.0102 and

rDer p 2.0103, and these isoforms reacted in the assay. The binding curve of rDer p 2.0103 was nearly overlapping with the nDer p 2 standard, and the curve of rDer p 2.0102 was shifted to the right. This isoform required approximately 2-fold more allergen to give an optical density at 405 nm equivalent to the nDer p 2 standard.

All 3 rDer f 2 isoforms reacted in the 2-site ELISA (Fig 3, B). Recombinant Der f 2.0103 gave a parallel and nearly overlapping binding curve with the natural Der f 2 standard, and binding curves for rDer f 2.0101 and rDer f 2.0102 were parallel and shifted to the right, requiring 5- and 13-fold more antigen, respectively, to give equivalent binding in the assay. All 3 Der f 2 isoforms have asparagine at position 114. The shift in the binding curves might be due to substitutions elsewhere within the mAb 7A1 (position 125) or the mAb 1D8 (positions 76 and 111) epitopes.

To investigate the loss of binding of mAb 7A1 to rEur m 2.0101, we used molecular modeling of rEur m 2 and examined the region of the allergen known to be involved in 7A1 binding. Fig 4 is a space-filling model showing the residues involved in the mAb 7A1 epitope. The residues in red and blue on Der p 2 have recently been identified through use of hydrogen exchange antibody protection NMR and site-directed mutagenesis as contributing to the binding of mAb 7A1.²⁹ Three of these position differ in the Eur m 2 model: K33T, S57T,

and K96R. The failure of mAb 7A1 to bind to Eur m 2 is most likely a direct result of one or more of these substitutions. In addition, the model of Eur m 2 shows that the substitutions cause subtle shifts in the position of adjacent side chains, which might also influence antibody binding.

IgE binding to group 2 allergens

IgE antibody binding to the Der p 2 isoforms was comparable, as measured in an ELISA through use of sera from dust mite-allergic patients from Japan ($n = 72$), and from the United Kingdom ($n = 67$). When the 139 sera were analyzed, there was an excellent correlation between IgE antibody binding to nDer p 2 and rDer p 2.0101, rDer p 2.0102, and rDer p 2.0103 ($r^2 = 0.85$, 0.87 , and 0.88 , respectively; Fig 5, A-C). When the sera from subjects living in Japan were analyzed, the correlation coefficients for IgE antibody binding comparing nDer p 2 and the 3 rDer p 2 isoforms were 0.83 , 0.91 , and 0.91 , respectively. The values were slightly higher for subjects living in the United Kingdom: 0.87 , 0.93 , and 0.95 . The modest increase in the correlation coefficient suggests that the antigenic determinant influenced by asparagine at position 114 is recognized by some sera.

In vivo IgE reactivity confirmed the in vitro IgE binding data. Three subjects with positive results of skin prick testing for *D pteronyssinus* extract were tested for reactivity to nDer p 2 and the rDer p 2 isoforms. All 3 subjects had positive prick test results for each allergen when a $5\text{-}\mu\text{g/mL}$ preparation was used, and intradermal skin testing showed comparable endpoint dilutions of 10^{-6} to 10^{-7} $\mu\text{g/mL}$ (not shown).

Because *E maynei* mites have been found in environmental samples from the United Kingdom,⁷ we used the sera from subjects living in the United Kingdom to test IgE binding to rEur m 2.0101. IgE binding to this allergen also correlated with binding to nDer p 2 ($r^2 = 0.69$; Fig 5, D). Fifteen sera showed higher binding to nDer p 2; these sera contained 7 to 25 ng/mL of Der p 2-specific IgE and 1 ng/mL of Eur m 2.0101-specific IgE antibody. Two sera had higher IgE to rEur m 2.0101 with 8 and 18 ng/mL, respectively, and 1 ng/mL of nDer p 2-specific IgE antibody. When 24 sera randomly selected from the panel were used, no IgE binding was detected to rLep d 2 and rTyr p 2 (not shown).

Modeling of rEur m 2.0101, rLep d 2.01 and rTyr p 2.01 tertiary structures and analysis of the allergenic surfaces

The crystal structure of Der p 2 was used for molecular modeling of rEur m 2.0101, rLep d 2.01, and rTyr p 2.01 through use of standard homology modeling methods. The amino acid differences between Der p 2 and Eur m 2 are located on the surface, but because only 21 residues differ and because these are spaced uniformly across the surface, this surface is largely conserved (Fig 6). This is also the case for the Der f 2 isoforms, which have 15 amino acid substitutions spread over the surface

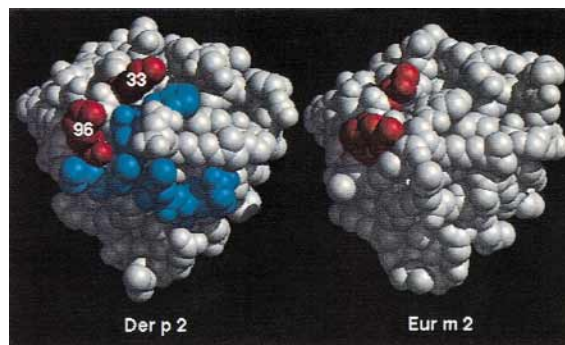


FIG 4. The mAb 7A1 epitope. The molecule shown in Fig 2, bottom panel has been rotated by 90° to show a space-filling view of the residues on rDer p 2.0101 (red and blue) involved in binding mAb 7A1. The amino acids shown in red differ between rDer p 2.0101 (K33, K96) and rEur m 2.0101 (T33 and R96); those shown in blue are common to the 2 allergens. Position 57 also differs (S $\rightarrow$ T) but is not seen in this view.

(not shown). This highly conserved antigenic surface accounts for the extensive allergenic cross-reactivity between these allergens. For the storage mite allergens Lep d 2 and Tyr p 2, some differences occur in the core residues, but these are conservative substitutions, and most of the substitutions are at surface residues.

DISCUSSION

In this study, we examined the amino acid polymorphisms of the group 2 house dust mite allergens. Preparations of natural Der p 2 have been shown to contain as many as 10 isoforms, or variants.¹⁷ Although the proportion of each isoform in natural Der p 2 preparations is not known, the Der p 2.0101 sequence appears in 4 of 9 Der p 2 cDNA sequences from environmental mite isolates.³⁶ The mAb and IgE binding studies presented here suggest that variation at position 114 influences antibody binding. Similar results were reported by Haakart et al.³⁷ We have been able to support our findings using structural studies that showed that the side chain of residue 114 is located on the solvent-accessible surface of Der p 2 and is within the antigenic determinant defined by mAb 1D8. Antigen-antibody interactions are stabilized by a variety of intermolecular interactions, including van der Waal's forces, hydrogen bonds, and salt bridges.³⁸ The substitution of asparagine for aspartic acid at position 114 would remove the side chain charge that might contribute to the binding energy of the allergen-antibody complex. The isoform substitutions at positions 40, 47, and 127 are more conservative, and their influence on antibody binding is more subtle. Substitutions at these positions might, however, be responsible for the shift in the binding curve of the rDer p 2.0102 isoform. Studies of the group 1 mite allergens have also used structural models to investigate antibody binding to homologous allergens.^{39,40}

The 2-site ELISA for the group 2 allergens is used worldwide for evaluating environmental exposure as well as for monitoring allergen avoidance measures.^{26,41} The results of the binding studies showed that the 2.0103 iso-

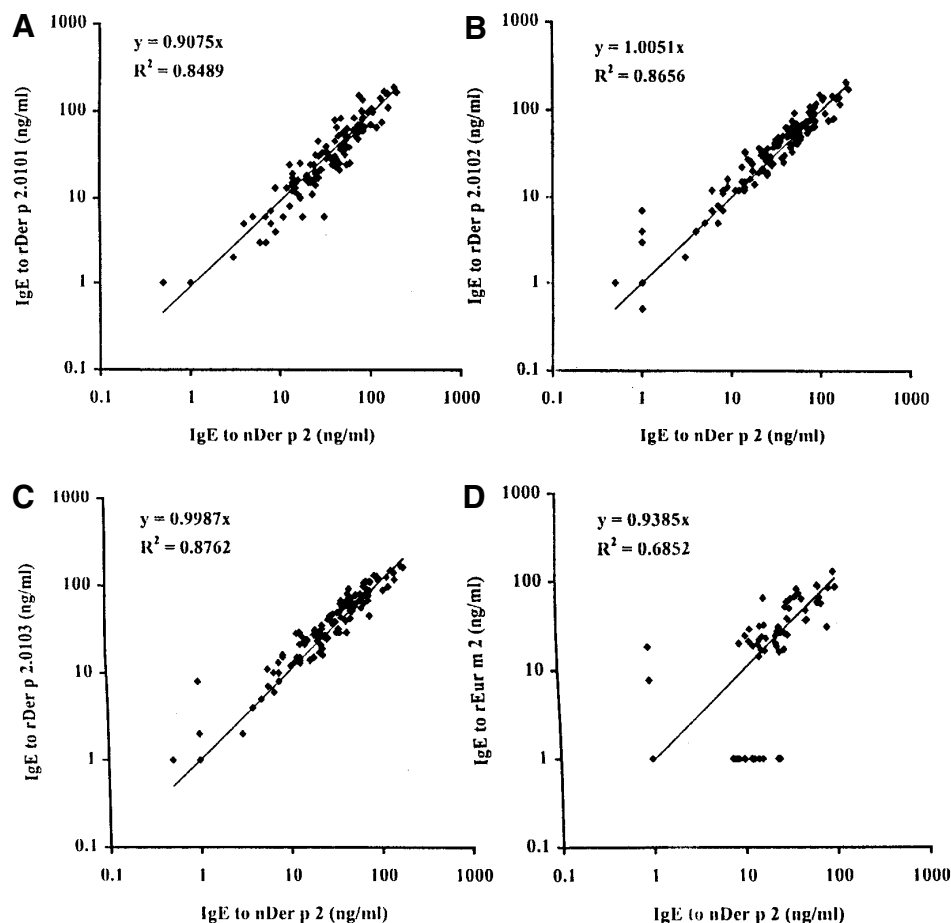


FIG 5. Correlation of IgE binding to natural Der p 2 with rDer p 2 isoforms and rEur m 2.0101. IgE antibody binding was quantified in an ELISA through use of the mouse-human chimeric IgE mAb assay and sera from 139 mite-allergic subjects. For each correlation shown, $P < .001$.

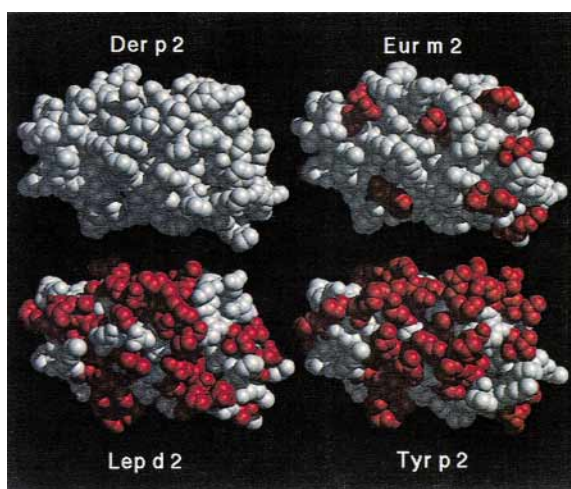


FIG 6. Surface topology of the group 2 allergens. Shown is a space-filling view of the crystal structure of rDer p 2.0101, in the same orientation shown in Fig 2, *bottom panel*, and models of rEur m 2.0101, rLep d 2.01, and rTyr p 2.01. The amino acids shown in red differ from the rDer p 2.0101 sequence.

forms of Der p 2 and Der f 2 gave parallel or nearly overlapping binding curves with the natural allergen standards in the ELISA. However, the Der p 2 isoform with the D114 substitution showed no binding, and binding of Der f 2.0101 and Der f 2.0102 isoforms was significantly reduced. These results suggest that the ELISA might underestimate group 2 levels in extracts in which these isoforms are present in large amounts relative to the 2.0103 isoforms. The relative proportions of different isoforms in either mite extracts or environmental dust samples have not yet been established but are now open to study through use of mAbs in isoform-specific ELISA.

Natural standards are prepared from spent mite culture media and are a limited resource. Our binding studies show that these standards could be replaced with recombinant allergen preparations, which can be consistently produced in large (g) quantities. The recombinant allergens also showed a strong correlation with natural Der p 2 for IgE binding through use of sera from mite allergic subjects. This suggests that the recombinant allergens could be used for in vitro diagnosis.

The complexity of the indoor environment with respect to the variety of mite species has been investigated by microscopic examination and visual identification. Although the *Dermatophagoides* mites are the predominant species in most temperate regions of the world, *Euroglyphus* and the non-proglyphid storage mites are also found in the indoor environment at levels sufficient to cause sensitization.^{9,10} Commercial extracts are not readily available for these species, so the prevalence of sensitization to species other than *Dermatophagoides* might be underestimated. Using rEur m 2, we investigated cross-reactivity with Der p 2 and found a strong correlation for IgE binding. The molecular basis for this cross-reactivity lies in the conserved antigenic surface of these proteins. Two *Dermatophagoides*-specific mAbs did not bind to Eur m 2, suggesting that a Eur m 2-specific mAb can be isolated that binds to the antigenic region containing the mAb 7A1 epitope. Development of a specific immunoassay will allow quantitative measurements of allergen levels and address the issue of the prevalence of this mite species in indoor environments.

Molecular modeling of rLep d 2.01 and rTyr p 2.01 showed that while the tertiary structure is conserved, the surfaces of the storage mite allergens display many amino acid substitutions and this most likely accounts for the lack of cross-reactivity. One report has demonstrated reactivity of the mAb 7A1 and 1D8 with a 16-kDa protein from a *Tyrophagus* extract.⁴² We have been unable to detect binding of these mAbs to rTyr p 2.01 by ELISA and immunoblot. It might be that additional isoforms of Tyr p 2 exist that retain the mAb 7A1 and 1D8 epitopes.

This study demonstrates that isoforms are recognized differently by allergen-specific mAbs despite the extensive sequence and structural homology. For selected sera, IgE antibody binding tended to correlate better with the isoforms with the asparagine substitution at position 114. Overall however, all 3 Der p 2 isoforms investigated showed excellent IgE reactivity. Indeed, this appears to be a common finding; similar results have been reported for isoforms of major pollen allergens.^{43,44} Our findings will be applied to the development of recombinant allergen standards for assays to assess environmental exposure and the effectiveness of avoidance measures, as well as the development of diagnostic reagents and allergen-specific therapies.

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