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Key Words

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Mite allergen
Dermatophagoides farinae

Structure of IgE Epitopes on a New 39-kD Allergen Molecule from the House Dust Mite, *Dermatophagoides farinae*

Abstract

Two IgE epitope sequences comprising Ser⁵⁶-Pro-Val-Thr-Lys-Arg-Ala-Ser-Leu-Lys-Ile-Asp-Ser-Lys-Lys⁷⁰ and Asp¹⁰⁴-Val-Glu-Leu-Ser-Leu-Arg-Ser-Ser-Asp-Ile-Ala¹¹⁵ were identified by deletion analysis of the cDNA encoding a new 39-kD protein of mite allergen. A synthetic dodecapeptide corresponding to the latter epitope sequence functioned as a monovalent and mite-specific hapten. Replacement of each of the 12 amino acid residues with Gly, using site-directed mutagenesis, indicated that Arg¹¹⁰ may play a central role in IgE binding. However, the 8 allergic sera tested exhibited a wide variation in their amino acid residues required for reactivity to IgE in allergic sera.

Introduction

Two predominant mite species, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, are the main sources of allergens [1] associated with immediate hypersensitivity reaction such as asthma, perennial rhinitis, and atopic dermatitis [2-4]. Although a large number of antigens and allergens was detected in a whole mite culture extract by immunoelectrophoretic analyses using mite-allergic sera [5-10], only the major allergens of *Der* I, II, and III groups have been extensively studied [11-19].

The structure and function of the major allergens has been studied using molecular cloning techniques [20-23] with a view to identifying and manipulating the important IgE-binding determinants on the proteins responsible for

provoking allergic diseases. However, attempts to identify amino acid sequences in the epitope region of the allergen molecule using random fragment libraries of cDNA were inconclusive and indicated that IgG- or IgE-binding regions were comprised of more than 30 amino acid residues distributed over the whole amino acid sequence of *Der p* I [24], and that the IgE-binding activity of the *Der p* II molecule was dependent on the integrity of the conformational structure [25]. On the other hand, the reactivities of synthetic overlapping peptides, spanning the entire sequence of *Der p* II, to IgE in mite-allergic sera suggested that a peptide comprising residues 65-78 was a sequential IgE-binding epitope [26]. Furthermore, among candidate peptides for the IgE epitope of *Der p* I, predicted by conventional algorithms (surface accessibil-

ity, mobility, and flexibility) and a three-dimensional model, two peptide regions (52–71 and 117–133) were more reactive to IgE from mite-allergic patients than the others [27]. However, apparent discrepancies in these IgE recognition sites has been left unsolved.

Much information on these and other important allergens remains to be accumulated [7, 8, 28–30]. Recently, we cloned and sequenced a cDNA encoding a new and potent mite allergen (*mag*, 1,144 bp). The gene product of the *EcoRI*-digested cDNA (*mag*1, 0.94 kbp) subcloned into plasmid pUEX2 was expressed as a fusion protein with β -galactosidase. This recombinant fusion protein reacted with about 30% frequency to IgE in sera from patients with mite allergy (unpublished data).

In this report, we described the identification of two important IgE-binding determinants on the allergen, and the immunochemical properties of a synthetic dodecapeptide which mimics one of the epitopes on the allergenic molecule species.

Materials and Methods

Restriction endonucleases, DNA-modifying enzymes, Deletion kit for kilo-sequence, and Mutan-G were purchased from Takara Shuzo Co. Ltd. (Kyoto, Japan).

Plasmid Vectors, Bacterial Strains, and Culture Media

The expression vector pAEX102 was constructed by insertion of *mag*1 cDNA (0.94 kb) that encodes a new mite allergen into the downstream region of the *lacZ* gene on a plasmid pUEX2 [31] [unpublished data]. Plasmid pUC18 for subcloning was obtained from Takara Shuzo. Phage vectors M13mp18 and tv18 (Takara Shuzo) were used for DNA sequencing [32]. The following *Escherichia coli* strains were used; pop2136, JM105 (Amersham International plc, Amersham, UK), XL1-Blue (Stratagene, La Jolla, Calif., USA) and BMH71-18 (Takara Shuzo). These strains were routinely grown at 28 or 37°C in LB, Super broth, 2xYT, or M9 glucose media.

Human Sera

Human allergic sera were supplied by the Hiroshima Prefectural Hospital (Hiroshima, Japan). Among 43 cases with positive skin reactions to house dust and mite of 97 patients with bronchial asthma, 13 sera were selected on the basis of the significant binding of IgE in the patient's serum to the translated product (Mag1) of *mag*1 on blot. Sera from a cedar pollen- or a *Candida*-allergic patient with a negative skin reaction to house dust and mite allergens were used as specificity controls together with sera from healthy donors with negative skin reaction to both allergens.

Deletion of *mag*1 DNA

Deletion of *mag*1 DNA from 3' Terminal. The plasmid pAEX102 DNA was linearized by digestions of *Sma*I and *Pst*I sites located at the downstream region of *mag*1. During digestion of the linearized DNA with Exonuclease III, ten aliquots were withdrawn at proper in-

tervals, followed by treatments with mung bean nuclease and Klenow fragment. The truncated plasmids were self-ligated with T4 DNA ligase.

Deletion of *mag*1 DNA from 5' Terminal. A *Hinc*II- and *Bam*HI-digested fragment (nucleotide range 108–945) of *mag*1 DNA on pAEX102 was subcloned into the same sites on a plasmid pUC18. The resultant plasmid was redigested on *Pst*I and *Hinc*II sites located at the upstream region of *mag*1. The truncated and linear plasmids were prepared as described above and digested with *Bam*HI. After agarose gel electrophoresis, the truncated fragments were extracted by using a GeneClean II kit (Toyobo, Osaka, Japan), and then ligated to a plasmid pUEX2 DNA digested with *Sma*I and *Bam*HI.

Deletion of *mag*1 DNA Utilizing Restriction Enzyme Sites. Four internal fragments of *mag*1 were prepared by digestions of pAEX102 DNA with *Eco*RI and *Eco*RV (nucleotide position, 162) or *Eco*RI and *Pvu*I (nucleotide positions, 245 and 328). Each fragment was blunted at the terminals with Klenow fragment and then subcloned into the *Sma*I site on a plasmid pUEX2.

Nucleotide sequences of the deleted *mag*1 inserts were determined by the dideoxynucleotide chain termination method [33] using an auto-read sequencing kit and an ALF DNA sequencer (Pharmacia LKB Biotechnology, Uppsala, Sweden).

Site-Directed Mutagenesis

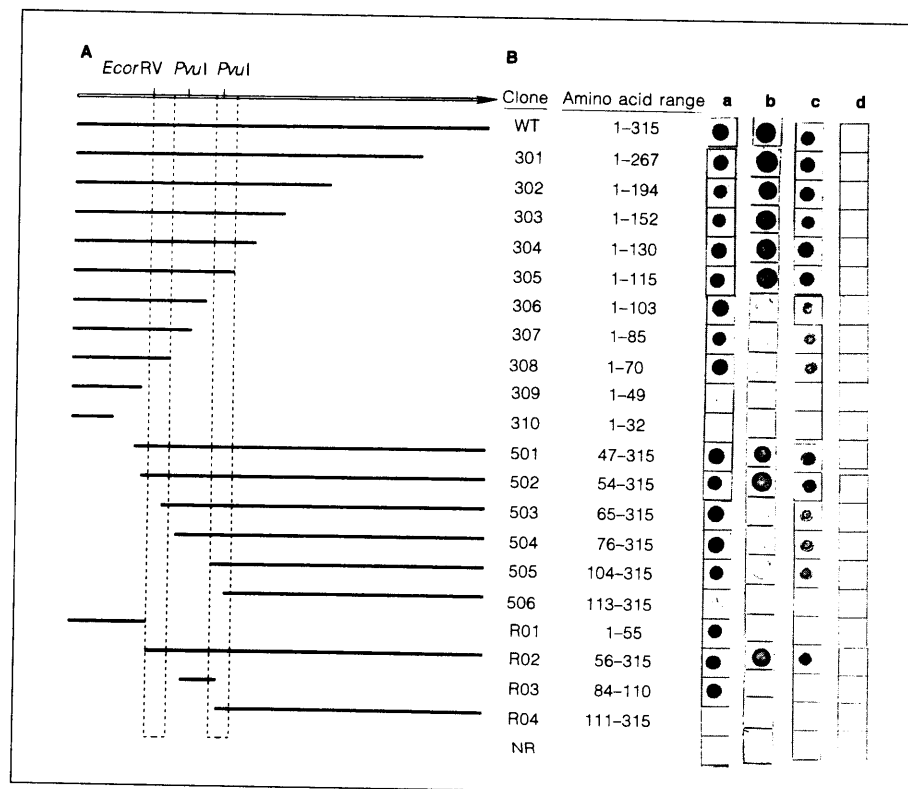
Oligonucleotides were synthesized on a DNA synthesizer (Model 381A, Applied Biosystems Japan Inc., Tokyo, Japan). Site-directed mutagenesis [34] was done using a Mutan-G kit (Takara Shuzo) in accordance with the manufacturer's protocol. In brief, an *Eco*RI-*Hind*III-digested fragment (nucleotide range 1–346) of a pAEX102-derived plasmid (clone 305) containing a truncated *mag*1 DNA from 3' terminal was subcloned into a vector M13-derived vector tv18. To replace each target amino acid residues by Gly, 19- or 20-meric oligonucleotides were designed in which the first and/or second base in the triplet corresponding to the target residue was replaced by G and was flanked on either side by nine nucleotides. The point-mutated *mag*1-derivative fragments were reinserted into the corresponding site on a plasmid pUEX2 DNA.

Nucleotide sequences of the point-mutated *mag*1 inserts were confirmed by DNA sequence analysis.

Colony Blot Test

Antigenicity of the recombinant mite proteins was measured by essentially the same method as Stanley [35]. Overnight cultures of *E. coli* pop2136 transformants carrying the intact, deleted, or point-mutated plasmids were adjusted to an OD₆₀₀ unit of 1.2. One microliter of each culture was spotted onto a Hybond-C membrane (Amersham International plc) overlaid on an LB medium plate containing 100 μ g/ml ampicillin. The plate was incubated at 28°C for about 6 h and for an additional 2 h after raising the temperature to 42°C. The membrane was placed on Whatman 3 MM papers (Whatman International Ltd., Maidstone, UK) soaked with 5% SDS solution and incubated at 100°C for 20 min to lyse the host cells and simultaneously to adsorb proteins exuded by cell lysis on the membrane. Furthermore, the proteins were firmly fixed on the membrane under ordinary electroblotting conditions. The membrane was washed twice with 10 mM Tris-buffered saline (TBS, pH 7.5) for 5 min and incubated at room temperature for 10 min with TBS containing 10 μ g/ml DNaseI. After a wash, the membrane was blocked with 5% skim milk in TBS for 1 h, incubated with pooled or individual allergic patients' sera ($\times 5$) preadsorbed with *E. coli* lysates for 1 h and washed 3 times with the

Fig. 1. Epitope mapping of the recombinant allergen molecule. **A** Restriction map of *mag1* insert depicted by the horizontal arrow is shown at top of the figure. The horizontal lines below the *mag1* insert illustrate the extents of the insert DNA fragments from *mag1*. The vertical bars surrounded with a broken line indicate the extent of each IgE epitope as defined by the minimum overlap among clones that produce a positive signal with the pooled allergic sera. **B** Plasmid pAEX102 and intact plasmid pUEX2 were used as positive (clone WT) and negative controls (clone NR). Transformants of *E. coli* pop 2136 carrying pAEX102 or deleted pAEX102-derived plasmids, or pUEX2 were expressed on a Hybond-C membrane, followed by the colony blot test. The precise insert endpoints (amino acid range) and the IgG-binding (**a**) and IgE-binding (**c-d**) data for each DNA clone are tabulated: **a** pooled IgG sera from 10 mite-allergic patients; **b** pooled IgE sera from the same patients; **c** individual IgE serum from a patient allergic to mite, and **d** individual IgE serum (600 IU/ml) from a patient allergic to cedar pollen (specificity control).



same buffer containing 0.05% Tween 20. Peroxidase-conjugated goat anti-human IgE or IgG antibody ($\times 50$, Tago Inc., Burlingame, Calif., USA) and 3,3'-diaminobenzidine tetrahydrochloride were used for detection.

Peptide Synthesis

Two peptides comprising SPVTKRASLKIDSKK and DVELSLRSSDIA were synthesized on a peptide synthesizer (9050, Millipore Corp., Bedford, Mass., USA) by the Fmoc method. The peptide was purified to homogeneity on the reversed-phase chromatography using a Cosmosil 5C18-P-300 column (4.8 $\times$ 250 mm, Nakarai Tesque, Tokyo, Japan).

Competitive Inhibition of the Binding of IgE in Allergic Sera

An ELISA plate (Falcon 3912, Becton Dickinson and Co., Oxford, Calif., USA) was coated for 2 h at 37°C with 50 μ l of 0.1 M sodium bicarbonate buffer (pH 9.5) containing 20 μ g/ml of mite body extract (Dfb), cedar pollen extract, or *Candida* extract. The washed wells were blocked with 100 μ l of 10 mM Tris-buffered saline (pH 8.0) containing 1% bovine serum albumin, 0.02% Thimerosal, and 0.05% Tween 20 (blocking solution). Pooled sera of allergic subjects diluted 5-fold with the blocking solution were preincubated for 30 min at 37°C in the presence or absence of a series of properly diluted Dfb, cedar pollen extract, *Candida* extract, synthetic peptide (Syn-E2), or irrelevant peptide (bovine insulin chain-A) solutions and added to the antigen-coated wells. The washed wells were overlaid with biotin-conjugated goat anti-human IgE ($\times 1,200$, Tago), followed by incubation with peroxidase-conjugated streptavidin ($\times 3,000$, Tago) for 2 h at 37°C. Absorbance at 415 nm was measured after 10 min of the enzyme reaction in 10 mM citrate buffer (pH 4.2) containing 1 mg/

ml 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) and 0.03% H_2O_2 .

Histamine Release and Skin Reaction Tests

Assay of released histamine from washed peripheral blood cells of mite-allergic subjects was performed using a liquid chromatograph apparatus (Shimadzu LC-6A, Kyoto, Japan) equipped with a fluorescence HPLC monitor [manuscript in preparation]. Total histamine (complete) and spontaneous release (blank) were determined for perchloric acid lysis of cell aliquots and cells incubated in the buffer, respectively. Results are expressed as the mean percentage of histamine released for triplicated assays, where: percentage histamine release = (sample - blank)/(complete - blank) $\times$ 100.

In *in vivo* skin reaction tests, 20 μ l of 0.005% sample solution containing 0.9% NaCl and 0.5% phenol was injected into patients with mite allergy. Allergenic activity was evaluated from the mean diameters of erythema appearing after 15 min.

Results

Identification of IgE Epitope Sequence on the Recombinant Allergen Molecule

The expression vector pAEX102 inserting *mag1* cDNA into a plasmid pUEX2 was used as the source of wild-type antigen. Each truncated *mag1* DNA illustrated in figure 1A was expressed as β -galactosidase-fused protein in *E. coli* pop2136. Reactivities of these proteins against IgE

in mite-allergic sera were detected in the colony blot test (fig. 1B).

Significant decreases in color intensity of colony blots occurred at two stages of deletion of *mag1* DNA from either side. Deletion analysis from the downstream side of *mag1* DNA, using pooled sera from mite-allergic patients (column b), showed that two regions are essential for IgE binding. Comparison of the color intensities of the pAEX102-derived plasmid clones 305 and 308, respectively, indicates that these regions are in amino acid ranges 50?–70 and 104?–115. Essentially the same regions (amino acid ranges 54–64? and 104–112?) were shown to be required for IgE-binding by deletion analysis from the upstream side of *mag1* DNA. The color intensity for the transformant carrying the clone R02 (amino acid ranges 56–315) was not greatly different from that for the intact pAEX102 plasmid (WT). Similarly, IgE from a patient allergic to mite was much more reactive to these two peptide regions than to the others (column c) and the other 5 mite-allergic patients tested gave the same results (data not shown). However, IgE from a patient allergic to either cedar pollen (column d) or *Candida* (data not shown) did not bind to any peptide region. Thus, specific IgE-binding regions in the translated product of *mag1* localized within the amino acid ranges of 56–70 and 104–115 in the molecule correspond to the epitope regions on Mag1 molecule and are referred to as Mag1-E1 and Mag1-E2, respectively.

Except for clones 309, 310, 506, and R04, almost the same color intensities were observed for IgG binding when the pooled allergic sera were used. This suggests that the IgG-binding region is distributed over the range of amino acid residues 50–110 in the Mag1 molecule.

The antigenicity values of the IgE-epitope regions, Mag1-E1 and E2, were calculated from the deduced amino acid sequence of *mag1* on the basis of a predictive algorithm [36]. The amino acid sequence of Mag1-E1, Ser⁵⁶-Pro-Val-Thr-Lys-Arg-Ala-Ser-Leu-Lys-Ile-Asp-Ser-Lys-Lys⁷⁰, coincided with the predicted high antigenic region, while Mag1-E2, comprising Asp¹⁰⁴-Val-Glu-Leu-Ser-Leu-Arg-Ser-Ser-Asp-Ile-Ala¹¹⁵, was less coincident (data not shown). However, Mag1-E2 seemed to have a slightly predominant ability to bind IgE in the colony blot test and, therefore, the Mag1-E2 region was selected for the following study.

Immunochemical Properties of a Synthetic Peptide Corresponding to the IgE Epitope

To reconfirm that Mag1-E2 corresponds to IgE epitope, the dodecapeptide with the same sequence as Mag1-E2 was synthesized. The synthetic peptide, referred to as

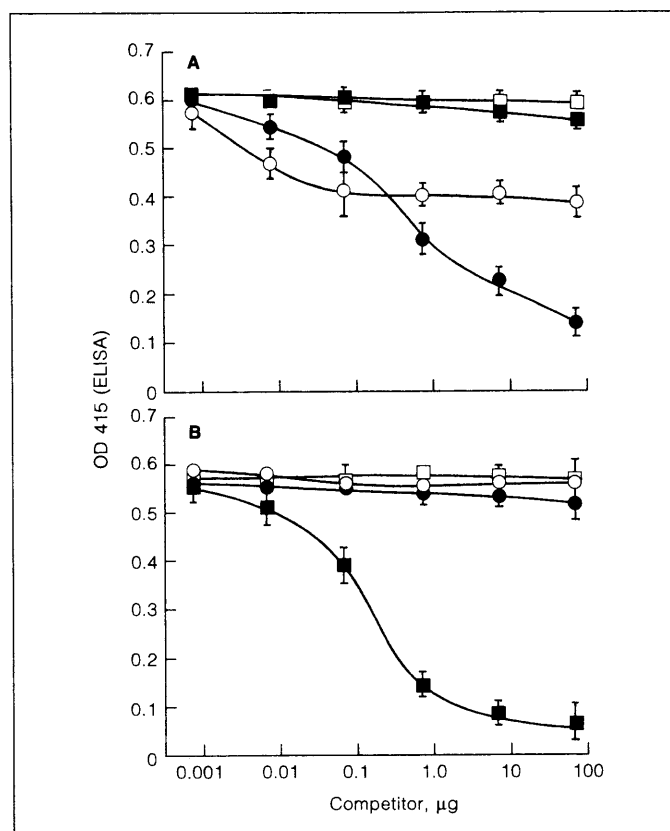


Fig. 2. Competitive inhibition of IgE-binding to Dfb with Syn-E2. The reaction of Dfb or cedar pollen extract to IgE in the pooled sera from mite-allergic patients (A) or in a serum from a cedar pollen-allergic subject (B), respectively, was inhibited on an ELISA plate by a series of properly diluted Syn-E2 solution (○). As negative controls an irrelevant peptide, bovine insulin chain-A (□), was used, whereas incubation of the pooled sera with Dfb (●) or cedar pollen extract (■) served as positive control. The bars represent the deviation between triplicate assays.

Syn-E2, inhibited the binding of IgE in mite-allergic sera to Dfb as examined by the ELISA inhibition test (fig. 2). The maximum extent of the inhibition was about 25–30% even when the concentration of Syn-E2 was increased to more than 0.1 µg/ml (fig. 2A) and the extent of the inhibition was dependent on the pooled sera tested. In contrast with the inhibition observed with Syn-E2, the irrelevant peptide (bovine insulin chain-A) hardly affected the IgE binding to Dfb. When IgE from patients sensitive to cedar pollen (fig. 2B) or *Candida* (data not shown) were incubated with Syn-E2, no significant inhibition of the IgE binding to the corresponding antigens were observed, although the IgE binding to the corresponding antigens on wells were inhibited with free antigens. These suggest that chemically synthesized Syn-E2 possesses mite-specific

IgE-binding activity, but that it occupies only one fourth to one third of the surface-located epitopes on the well-bound allergen molecules present in Dfb extract.

In vivo allergenic activity of Syn-E2 against 8 mite-allergic patients or healthy subjects was not detected by the intracutaneous skin test (data not shown). Moreover, no significant histamine release was observed by stimulation of washed blood cells of a mite-allergic subject (fig. 3), a cedar pollen- or a *Candida*-sensitive subject (data not shown), or a healthy subject (data not shown) with Syn-E2 or bovine insulin chain-A. However, Syn-E2 did inhibit histamine release in a dose-dependent manner, histamine release elicited with Dfb being depressed by about 20 or 40% by equal or 5-fold higher concentrations of Syn-E2, respectively, but the depression of histamine release was not detectable with the irrelevant peptide (data not shown). Moreover, any concentration of Syn-E2 used could not suppress histamine release induced by stimulation of washed blood cells of a cedar pollen- or a *Candida*-sensitive patient with cedar pollen or *Candida* extract, respectively (data not shown). These results suggest that the synthetic peptide functions as a monovalent and mite-specific epitope, and that only mite-sensitive patients could specifically react to Syn-E2.

Identification of the Residue that Participates in Binding to Specific IgE Antibody

To identify the amino acid residues in Mag1-E2 essential for IgE-binding activity, each amino acid residue was replaced by Gly in site-directed mutagenesis. Twelve transformants carrying these point-mutated plasmids expressed recombinant fusion proteins after heat induction. Reactivities of the translated products to IgG or IgE in allergic sera were evaluated from the color intensities observed in the colony blot test and immunoblotting (fig. 4).

Replacement of any amino acid in the 104–115 region of Mag1-E2 by Gly caused a small loss of reactivity to IgE, but large decreases in reactivity were observed only for replacements of the Asp, Glu, Ser, or Arg residues. The reactivity to IgG in pooled mite-allergic sera was not influenced by replacement of any of the amino acid residues by Gly.

The reaction patterns of the sera from 8 individuals varied greatly in the extent of their reduction in reactivity and in the specific amino acid residues required for binding to specific IgE. Generally, the essential amino acid residues for specific IgE-binding were identified as Asp¹⁰⁴, Glu¹⁰⁶, Ser¹⁰⁸, Arg¹¹⁰, Ser¹¹¹, Ser¹¹², and Asp¹¹³. Of these, Arg¹¹⁰ seemed to play the most important role in IgE-binding.

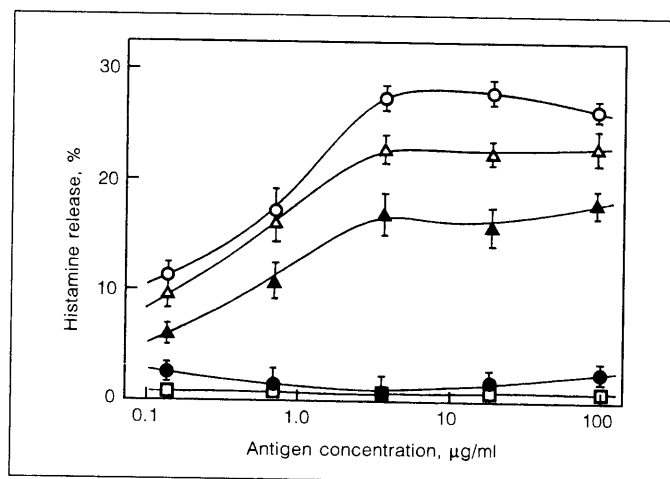
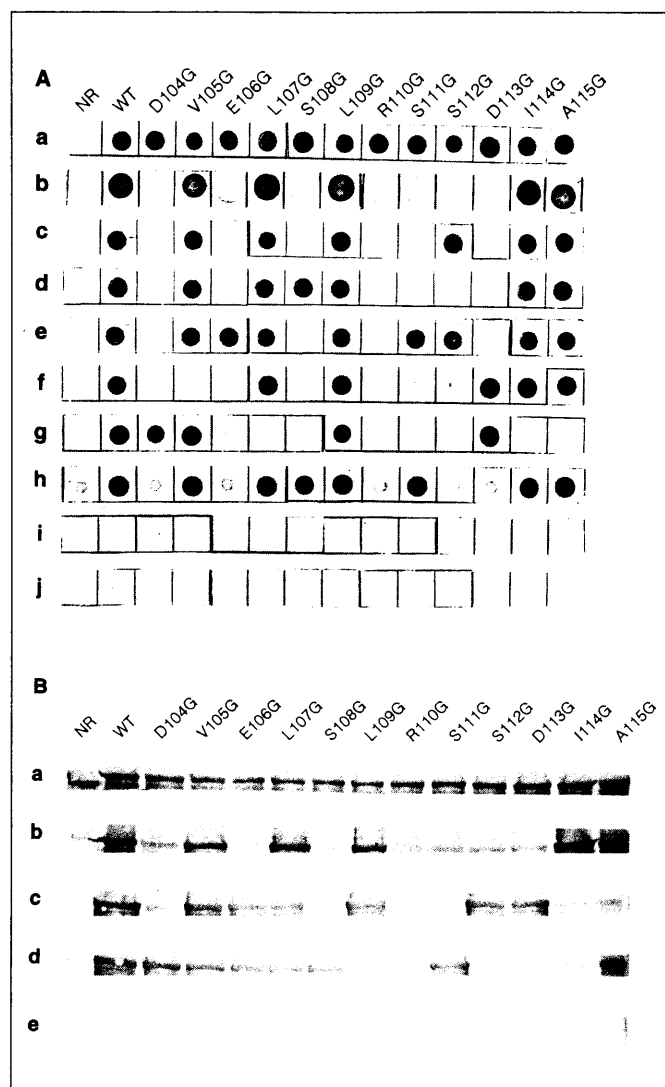


Fig. 3. Inhibitory activity of Syn-E2 toward in vitro histamine release eliciting with Dfb. Two hundred microliters of the washed cell suspension was added to that of Hanks' buffer containing a series of appropriately diluted Dfb (○), Syn-E2 (●), or bovine insulin chain-A (□). Simultaneously, the reaction mixture containing Dfb supplemented with equal (△) or 5-fold higher concentrations (▲) of Syn-E2 competitor was prepared. The concentration of histamine in the supernatant of the incubation mixture was measured by HPLC. The percent histamine release is plotted on the ordinate. The bars represent the deviation between triplicate assays.

Discussion

Most approaches to the analysis of epitopes on mite allergens have used monoclonal antibodies raised against the target molecules [37, 38]. This has the advantage that epitopes can be detected on the intact molecule of interest. However, it is difficult to identify the structures of epitopes on the molecule. Recently, the IgG and IgE reactivities have been tested on the translated products of DNA fragments encoding parts of the major allergen proteins [24, 25]. This technique allows identification of important IgE-binding determinants on proteins responsible for provoking allergic diseases. However, IgG- or IgE-binding regions on the *Der p* I molecule seem to be distributed over the whole amino acid sequence resulting in a complex antibody-binding profile [24], whilst the IgE epitopes of *Der p* II are highly conformational [25, 39]. The synthesis and screening of peptide regions, spanning the entire sequence of *Der p* II, or predicting as candidates for IgE epitope of *Der p* I by conventional algorithms and a three-dimensional model, are one of advantageous strategies for the identification of IgE recognition sites. The peptide regions comprising residues 65–78 of *Der p* II or residues 52–71 and 117–133 of *Der p* I have proved to be an IgE-binding epitope [26, 27]. Deletion analysis of *mag1*

Fig. 4. Identification of amino acid residues essential for IgE-binding activity in Syn-E2 molecule. The expression vector pAEX102-derived plasmid (clone 305) was used as the source of wild-type (WT) antigen. Intact plasmid pUEX2 was also used as negative control (NR). **A** Transformants of *E. coli* pop2136 carrying intact or point-mutated clone 305-derived plasmids were expressed on Hybond-C membrane after a temperature switch to 42°C. The detection was carried out by probing with peroxidase-conjugated anti-human IgG (**a**) and IgE (**b**) after the membrane was overlaid with the pooled allergic sera. Simultaneously, the membrane was probed with peroxidase-conjugated anti-human IgE after overlaying with 6 individual sera (**c–h**), a cedar pollen-allergic serum (**i**), or a healthy serum (**j**). **B** Whole cell lysates prepared from 10-μl cultures of *E. coli* pop2136 carrying these plasmids were separated on 12.5% polyacrylamide gel. After SDS-PAGE, target proteins were visualized with Coomassie brilliant blue R-250 (**a**). Semi-dried blotting was performed on Immobilon membranes. The membrane was overlaid with pooled mite-allergic sera (**b**), individual mite-allergic sera (**c**, **d**), a cedar pollen-allergic sera (**e**) and then visualized by immunostaining.



cDNA identified two different amino acid regions as candidates for IgE epitopes. In fact, one candidate peptide was chemically synthesized and found to possess a monovalent, haptenic activity capable of competing with the IgE epitope region. Thus, deletion analysis of allergen cDNA seemed to offer an additional method for elucidation of the primary sequence of the linear epitope.

The dimensions of the epitope regions are defined by the primary amino acid sequences in the Mag1 molecule required for interaction with IgE as predicted by the color intensity of clones in the colony blot assays. A drastic change in color intensities between clone 502 and clone 503 may be caused by a reduction in IgE-binding activity with scission of the Mag1-E1 epitope region. Loss of IgE-binding activities of clones 506, R03, and R04 may be due to cleavage of the Mag1-E2 epitope region.

Miller et al. [40] reported that content of Gly is highest among amino acids consisting of protein and is distributed evenly on the surface and in the interior of protein molecule. As Gly is the structural unit of variety of amino acids, we considered that the substitution to Gly would be influenced least of all on the neighboring environment. The failure of clones R03 and R04 to bind IgE in mite-allergic sera indicates that the deleted hydrophilic amino acids, such as Asp¹⁰⁴, Asp¹¹³, Glu¹⁰⁶, Ser¹⁰⁸, Ser¹¹¹, and Ser¹¹², are essential for activity although Arg¹¹⁰ certainly plays the most important role as shown by the replacement of the corresponding residues to Gly (fig. 4). Antigenic determinants are surface-located and often contain charged and polar residues [41]. Hydrophobic amino acid residues are also often present in antigenic determinants, as pointed out by Welling et al. [36], but the hydrophobic amino acid

residues of the Mag1-E2 region do not participate in IgE-binding activity. Site-directed mutagenesis in combination with the colony blot test would be effective to determine which amino acid residues in each epitope are important for the specificity of mite-allergic sera.

Syn-E2 inhibits the binding of specific IgE to Dfb by about 25–30%. A similar result was obtained in the histamine release test in that about 30% of maximum histamine release was observed by stimuli of blood cells of mite-allergic patients with β -Gal-Mag1 at a concentration of less than one hundredth that of Dfb (unpublished data). Contrary to our expectation, Syn-E2 inhibited the

Dfb-elicited histamine release in a dose-dependent manner. This is of great interest and our future studies will be directed towards elucidating whether this phenomenon is caused by a specific interaction.

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References

- Voorhorst R, Spieksma-Boezeman MIA, Spieksma FTM: Is a mite (*Dermatophagoides* sp.) the producer of the house-dust allergen? *Allergie Asthma* 1964;10:329–334.
- Voorhorst R, Spieksma FTM, Varekamp H, Leupen MJ, Lyklema AW: The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house-dust allergen. *J Allergy* 1967;39:325–339.
- Miyamoto T, Oshima S, Ishizaki T, Sato S: Allergenic identity between the common floor mite (*Dermatophagoides farinae* Hughes, 1961) and house dust as a causative antigen in bronchial asthma. *J Allergy* 1968;42:14–28.
- Tovey ER, Chapman MD, Platts-Mills TAE: Mite faeces are a major source of house dust allergens. *Nature* 1981;289:592–593.
- Lind P, Weeke B, Loewenstein H: A reference allergen preparation of the house dust mite *D. pteronyssinus*, produced from whole mite culture. A part of the DAS 76 study. Comparison with allergen preparation from other raw materials. *Allergy* 1984;39:259–274.
- Tovey ER, Baldo BA: Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987;79:93–102.
- Baldo BA, Ford SA, Tovey ER: Toward a definition of the 'complete' spectrum and rank order of importance of the allergens from the house dust mite: *Dermatophagoides pteronyssinus*. *Adv Biosci* 1989;74:13–31.
- Arlian LG, Bernstein IL, Geis DP, Vyszynski-Mohler DL, Gallagher JS: Investigations of culture medium-free house dust mites. IV. Cross antigenicity and allergenicity between the house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*. *J Allergy Clin Immunol* 1987;79:467–476.
- Nakanishi K, Shimokata K: Immunoblot analysis of *Dermatophagoides farinae* antigen. *Ann Allergy* 1990;64:219–223.
- Wahl R, Weber BF: Investigation of house dust mite (*Dermatophagoides pteronyssinus*) allergen extracts prepared from purified mite bodies and whole mite culture. *Int Arch Allergy Appl Immunol* 1991;94:373–375.
- Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980;125:587–592.
- Stewart GA: Isolation and characterization of allergen Dpt 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int Arch Allergy Appl Immunol* 1982;69:224–230.
- Stewart GA, Lake FR, Thompson PJ: Faecally derived hydrolytic enzymes from *Dermatophagoides pteronyssinus*: Physicochemical characterization of potential allergens. *Int Arch Allergy Appl Immunol* 1991;95:248–256.
- Lind P: Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp42, of the house dust mite, *D. pteronyssinus* and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm6). *Int Arch Allergy Appl Immunol* 1986;79:60–65.
- Holck A, Dale S, Sletten K: Purification and characterization of a major allergen from the house dust mite *Dermatophagoides farinae*. *Allergy* 1986;41:408–417.
- Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE: Antigenic and structural analysis of group II allergens (*Der f I* and *Der f II*) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83:1055–1067.
- Haida M, Okudaira H, Ogita T, Ito K, Miyamoto T, Nakajima T, Hongo O: Allergens of the house dust mite *Dermatophagoides farinae*. Immunological studies of four allergenic fractions. *J Allergy Clin Immunol* 1985;75:686–692.
- Yasueda H, Mita H, Yui Y, Shida T: Isolation and characterization of two allergens from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1986;81:214–223.
- Stewart GA, Ward LD, Simpson RJ, Thompson PJ: The group III allergens from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. *Immunology* 1992;75:29–35.
- Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, Turner KJ: Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p I*. Homology with cysteine proteases. *J Exp Med* 1988;167:175–182.
- Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR: Isolation of cDNA coding for the major mite allergen *Der p II* by IgE plaque immunoassay. *Int Arch Allergy Appl Immunol* 1990;91:118–123.
- Dilworth RJ, Chua KY, Thomas WR: Sequence analysis of cDNA coding for a major house dust mite allergen, *Der f I*. *Clin Exp Allergy* 1991;21:25–32.
- Yuuki T, Okamura Y, Ando T, Yamakawa H, Suko M, Haida M, Okudaira H: Cloning and expression of cDNA coding for the major house dust mite allergen *Der f II* in *Escherichia coli*. *Agric Biol Chem* 1991;55:1233–1238.
- Greene WK, Cyster JG, Chua KY, O'Brien RM, Thomas WR: IgE and IgG binding of peptides expressed from fragments of cDNA encoding the major house dust mite allergen *Der p I*. *J Immunol* 1991;147:3768–3773.
- Chua KY, Greene WK, Kehal P, Thomas WR: IgE binding studies with large peptides expressed from *Der p II* cDNA constructs. *Clin Exp Allergy* 1991;21:161–166.
- Van't Hof W, Driedijk PC, Van den Berg M, Beck-Sickinger AG, Jung G, Aalberse RC: Epitope mapping of the *Dermatophagoides pteronyssinus* house dust mite major allergen *Der p II* using overlapping synthetic peptides. *Mol Immunol* 1991;28:1225–1232.

- 27 Jeannin P, Didierlaurent A, Gras-Masse H, Elsass AA, Delneste Y, Cardot E, Joseph M, Tartar A, Vergoten G, Pestel J: Specific histamine release capacity of peptides selected from the modeled *Der p* I protein, a major allergen of *Dermatophagoides pteronyssinus*. *Mol Immunol* 1992;29:739-749.
- 28 Tovey ER, Cobon GS, Johnson MC, Baldo BA: The cloning of a mite (*Dermatophagoides pteronyssinus*) recombinant protein that shares IgE-binding determinants with a 14-15 kD component of mite extracts. *Adv Biosci* 1989;74:33-42.
- 29 Van der Zee JS, Van Swieten P, Jansen HM, Aalberse RC: Skin tests and histamine release with P1-depleted *Dermatophagoides pteronyssinus* body extracts and purified P1. *J Allergy Clin Immunol* 1988;81:884-896.
- 30 De la Hoz F, Carreira J: Identification of main allergens from *Dermatophagoides farinae* and their properties under native and dissociating conditions. *Int Arch Allergy Appl Immunol* 1986;79:238-245.
- 31 Bressan GM, Stanley K: pUEX, a bacterial expression vector related to pEX with universal host specificity. *Nucleic Acids Res* 1987;15:10056.
- 32 Yanisch-Perron C, Vieira J, Messing J: Improved M13 phage cloning vectors and host strains: Nucleotide sequence of the M13mp18 and pUC19 vectors. *Gene* 1985;33:103-119.
- 33 Sanger F, Nicklen S, Coulson AR: DNA sequencing with chainterminating inhibitors. *Proc Natl Acad Sci USA* 1977;74:5463-5467.
- 34 Kramer W, Frits HJ: Oligonucleotide-directed construction of mutations via gapped duplex DNA; in Wu R, Grossman L (eds): *Methods in Enzymology*. London, Academic Press, 1987, vol 154, pp 350-367.
- 35 Stanley KK: Solubilization and immune-detection of β -galactosidase hybrid proteins carrying foreign antigenic determinants. *Nucleic Acids Res* 1983;11:4077-4092.
- 36 Welling GW, Weijer WJ, Van den Zee R, Welling-Wester S: Prediction of sequential antigenic regions in proteins. *FEBS Lett* 1985;188:215-218.
- 37 Lind P, Hansen OC, Horn N: The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, *Der p* I, of *Dermatophagoides pteronyssinus*. Relative binding site location and species specificity studied by solid-phase inhibition assays with radiolabeled antigen. *J Immunol* 1988;140:4256-4262.
- 38 Chapman MD, Heymann PW, Platts-Mills TAE: Epitope mapping of two major inhalant allergens, *Der p* I and *Der f* I, from mites of the genus *Dermatophagoides*. *J Immunol* 1987;139:1479-1484.
- 39 Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD: Conformational stability of B cell epitopes on group I and group II *Dermatophagoides* spp. allergens. Effect of thermal and chemical denaturation on the binding of murine IgG and human IgE antibodies. *J Immunol* 1990;144:1353-1360.
- 40 Miller S, Janin J, Lesk AM, Chothia C: Interior and surface of monomeric proteins. *J Mol Biol* 1987;196:641-656.
- 41 Hopp TP, Woods KR: Prediction of protein antigenic determinants from amino acid sequences. *Proc Natl Acad Sci USA* 1981;78:3824-3828.