



IgE-binding epitopes of the American cockroach Per a 1 allergen

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Abstract

Cockroach is one of the major indoor allergens for IgE-mediated allergic respiratory illnesses throughout the world. The American cockroach (*Periplaneta americana*) Per a 1 allergen is antigenically cross-reactive with the German cockroach (*Blattella germanica*) Bla g 1 allergen. The aim of this study was to identify linear B cell epitopes of Per a 1 that are recognized by human IgE. Per a 1 deletion mutants were generated from the recombinant Per a 1.0104 allergen (274 amino acid residues), and antigenicities were assessed by immunoblotting, enzyme-linked immunosorbent assay (ELISA), and binding inhibition. Human atopic sera were not able to recognize deletion mutants consisting of amino acids 1–77, 86–205, and 200–266. However, human sera did recognize the N-terminal mutant containing amino acids 1–87 and the C-terminal mutant containing amino acids 200–274, demonstrating positive IgE binding that was heterogeneously distributed among the different sera tested. Amino acid residues 78–85 and 267–274, containing internal repeats, were shown to be required for IgE binding to the Per a 1.0104 protein. Two peptides corresponding to these IgE-binding amino acid sequences were synthesized. Peptide 78–85 showed a positive IgE interaction with 80% of the sera, while peptide 267–274 was capable of IgE binding to all of the sera tested. Moreover, preincubation of atopic sera with IgE-positive recombinants and peptides resulted in marked inhibition of the IgE binding to purified Per a 1.0104 allergen. Amino acid sequences ⁷⁸LIRALFGL⁸⁵ and ²⁶⁷IRSWFGLP²⁷⁴ of the American cockroach Per a 1.0104 allergen were involved in IgE binding. These findings will advance the understanding of the specific reactivity of the epitopes of cockroach allergens, thereby contributing to the development of specific immunotherapies for clinical use.

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1. Introduction

Airborne cockroach allergens play a central role in allergic disease, especially asthma (Helm, 1999). Protein allergens with masses ranging from 6 to 120 kDa from both American and German cockroaches, the two most common domiciliary species, have been identified by various techniques (Helm, 1999; Chapman et al., 1996; Wu and Lan, 1988; Wu et al., 1996a,b). Over the past few years, three *Periplaneta americana* allergens, Per a 3 (Wu et al., 1995, 1996a,b, 1997), Per a 1 (Wu et al., 1998; Melen et al., 1999; Wang et al., 1999; Yang et al., 2000) and Per a 7 (Astrurias et al., 1999; Santos et al., 1999), and six *Blattella germanica* allergens, Bla g 1 (Pomes et al., 1998), Bla g 2 (Arruda et al., 1995a,b), Bla g 4 (Arruda et al., 1995a,b), Bla g 5 (Arruda et al., 1997), Bla g Bd90K (Helm et al., 1996) and Bla g 6 (Chapman et al., 1996), have been cloned. Bla g 1 showed

94% nucleotide sequence homology to Bla g Bd90K and is now designated as Bla g 1.0102. Most cockroach allergens appear to be species specific. The only cross-reactive allergens that have so far been sequenced from both *P. americana* and *B. germanica* are Bla g 1 and Per a 1. Several cockroach allergens have already been cloned and expressed, but information concerning their allergenic determinants has been unavailable thus far.

The Per a 1 molecule containing isoallergens shares high sequence homology (52–71%) with Bla g 1, and both molecules contain internal repeat sequences, phosphorylation sites, and mitochondrial energy transfer protein signatures (Wu et al., 1998; Melen et al., 1999; Yang et al., 2000). In addition, Per a 1 and Bla g 1 contain no cysteine residues and no potential N-glycosylation sites. Per a 1 isoallergens show 54–77% skin reactivity in atopic patients, and are also found to have 27–32% sequence identity to the precursor protein of the female African mosquito (*Anopheles gambiae*) that is induced following a blood meal. Allergen-specific IgE plays an important role in hypersensitivity reactions (Sutton and Gould, 1993). The identification

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of IgE binding of cockroach allergens is essential in order to understand the antigenic structures responsible for allergenicity to the American cockroach. In the present study, linear B cell epitopes of Per a 1.0104 (GenBankTM accession number U69261 (Wu et al., 1998; Yang et al., 2000) that are recognized by atopic sera are characterized.

2. Materials and methods

2.1. Antibodies to cockroach allergens

Human serum samples were obtained from patients sensitive to American cockroach and assayed for Per 1.0104-specific IgE antibodies by enzyme-linked immunosorbent assay (ELISA). Equal volumes of sera of 10 patients with Per a 1.0104-specific IgE were used or pooled to constitute an atopic serum pool.

2.2. Synthetic peptides

The biotinylated and non-biotinylated were purchased from United Biochemical Research Inc. (Seattle, WA). They were purified by high-performance liquid chromatography (greater than 85% purity) and characterized using matrix-assisted laser desorption mass spectrometry by the manufacturer.

2.3. Deletion mutant generated by internal restriction enzyme sites

The cDNA of Per a 1.0104 was isolated from plasmid (pET 30) and purified by QIAprep[®] Miniprep (QIAGEN, Valencia, CA). The fragment encoding residue 1–87 were generated by digestion of purified Per a 1.0104 with restriction enzymes *Bam*HI/*Xho*I (Promega, Madison, WI) using standard methods (Sambrook et al., 1989).

2.4. Deletion mutants generated by PCR amplification

The gene segments of interest were generated by PCR to create restriction for cloning purposes. The cDNA of Per a 1.0104 was isolated from plasmid (pET 30) and purified by QIAprep[®] Miniprep (QIAGEN, Valencia, CA). Purified plasmid was combined with oligonucleotide primers specific for the target areas with appropriate restriction enzyme cleavage sites in a solution containing 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM MgCl₂ and 200 μ M of each dNTP (Pharmacia, Piscataway, NJ). The PCR was performed with an initial denaturation at 94 °C for 3 min, and subsequent 35 cycles of amplification was done under the following conditions: annealing at 55 °C for 2 min, extension at 72 °C for 3 min, and denaturation at 94 °C for 1 min. The PCR product was separated by agarose gel electrophoresis and purified.

2.5. Construction and expression of Per a 1.0104 deletion mutants

Restriction enzymes generated mutant residue1–87 was ligated into cloning vectors pET30a. The PCR-generated deletion mutant, residues 1–77, 85–205, 200–266 and 200–274, were ligated into pGEM-T vector plasmid (Promega, Madison, WI), double digested with *Bam*HI/*Sal*I, and cDNA were then ligated into cloning vectors pET30b. Sequences of deleted cDNA inserts were determined using a 310 DNA sequencer (Applied Biosystems, Foster City, CA). The resulting plasmids were then transformed into *E. coli* BL21 (DE3) for expression (Wu et al., 1998).

2.6. Purification of recombinant allergens

Recombinant proteins were over-expressed in *E. coli* BL21 (DE3). The culture was harvested and the cells were washed with sonication buffer (50 mM Tris–HCl, pH 8, 200 mM NaCl, 0.1 mM EDTA, and 0.1% Nonidet P-40). The pellets were resuspended in sonication buffer containing 2 mM phenylmethylsulfonyl fluoride and the cells were sonicated. The lysate was purified using the rapid affinity column chromatography with pET His-Tag System as described by Novagen (Madison, WI) (Wu et al., 1996a,b). Purified recombinant proteins were used for immunoblotting, ELISA and inhibition ELISA.

2.7. SDS-PAGE and immunoblotting

Recombinant proteins were suspended in SDS sample buffer containing 2-mercaptoethanol and loaded on a 5% polyacrylamide stacking gel above a 12% separating gel, and SDS-PAGE was run with discontinuous buffer according to Laemmli's method (Laemmli, 1970). After electrophoresis, gels were fixed and stained with 0.2% Coomassie Brilliant Blue R-250. For immunoblotting, gels were transferred electrophoretically (Towbin et al., 1979) to polyvinylidene difluoride (PVDF) membranes (Millipore Corp., Marlborough, MA). Immunodetection was performed using atopic human sera as previously described (Wu et al., 1998).

2.8. Immunodetection by direct binding ELISA

The reactivities of purified intact Per a 1.0104 and IgE-positive deletion mutant proteins, and synthetic peptides with IgE antibodies were evaluated in a direct binding ELISA. ELISA was performed as described previously (Wu et al., 1996a,b). Optimal concentration of antigen and conjugate were determined by checkerboard titration. Plate was prewashed with washing buffer, PBS, pH 7.4 containing 0.05% Tween 20 (PBST). Purified recombinants (3.0 μ g per well) or biotin-labeled synthesized peptides (10 ng per well) in coating buffer (0.1 M sodium carbonate, pH 9.6) were coated onto the microtiter plate (Immunolon II; Dynatech, Alexandria, VA) or streptavidin immobilizer

plate (Exiqon, Bygstubbe, Denmark) in triplicate and incubated at 37 °C for 2 h. Plate was washed with PBST and incubated overnight at 4 °C with human IgE (1:10 dilution). After washing, antibody binding was detected using peroxidase-labeled goat anti-human IgE along with 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) containing 0.03% H₂O₂ as a substrate (Wang et al., 1999). The optical density was determined at 415 nm on a SLT SPECTRA Reader (SLT Labinstruments, Salzburg, Austria).

2.9. Inhibition ELISA

Optimal concentration of antigen and conjugate were determined by checkerboard titration. Purified Per a 1.0104 recombinant allergen (3.0 µg per well) in coating buffer was coated onto each well in triplicate and incubated at 37 °C for 2 h. After incubation, wells were washed five times with PBST and then filled with 1.0% normal goat serum and incubated for 2 h at room temperature. Wells were washed and then incubated with patient serum pool (1:10) which have been preincubated 2 h at room temperature with the solution of the different concentrations of non-biotin labeled peptide or purified recombinants. The wells were incubated at 4 °C overnight, and then processed as standard ELISA (described above) using peroxidase-conjugated goat anti-human IgE as the secondary antibody. Per a 1.0104 was used as inhibitor for positive control, and preincubation of inhibitor with non-allergic sera was used as negative control. Results were expressed as the percent inhibition of the reaction of antibody without any inhibitor (Wang et al., 1999).

3. Results and discussion

3.1. Identification of IgE-binding epitopes of Per a 1.0104

Per a 1.0104 serial deletion mutant cDNAs were expressed as fusion proteins and assayed for IgE reactivity. Affinity-purified recombinant proteins were subjected to SDS-PAGE and the results of immunoblotting using Per

1.0104-sensitive atopic sera are shown in Fig. 1. The polypeptides 1–87 lacking the C-terminus (Fig. 1B, lane c), deletion mutant 200–274 without the N-terminus (Fig. 1B, lane g) and Per a 1.0104 (Fig. 1B, lane b) recognized atopic IgE. On the other hand, no detectable reactivity was found for deletion mutants 1–77, 86–205 and 200–266 (Fig. 1B, lanes d–f). These preliminary results indicate that the human linear IgE-binding epitopes are located within the internal repeat amino acid sequences 78–85 and 267–274 of Per a 1.0104 (Fig. 2). Two biotinylated and non-biotinylated peptides corresponding to these two IgE-binding amino acid sequences, peptide 78–85 and peptide 267–274 were synthesized.

3.2. IgE binding of recombinant and synthesized peptides

Interactions of specific atopic IgE antibodies with intact Per a 1.0104, deletion mutant proteins, and synthesized peptides were demonstrated by ELISA with 10 sera obtained from Per a 1.0104 skin test-positive patients and two sera from healthy non-allergic adult subjects. All of the atopic sera recognized intact Per a 1.0104, and no IgE binding was detected in deletion mutants 1–77, 86–205, 200–266, or in the negative control sera (Figs. 3 and 4). Deletion mutants 1–87, 200–274, peptide 78–85 and peptide 267–274 showed positive IgE binding that was heterogeneously distributed among the different sera tested (Figs. 3 and 4). The IgE from patients 2 and 3 recognized deletion mutant 200–274 and peptide 267–274, but were unable to bind 1–87 or peptide 78–85, while IgE from the remainder of patients recognized deletion mutant 1–87, peptide 78–85, and peptide 267–274. Truncated protein 1–87 and peptide 78–85 were capable of IgE binding to 80% (8/10) of atopic sera tested at a dilution of 1:10. Truncated protein 200–274 and peptide 267–274 showed a positive IgE interaction with 100% (10/10) of the sera analyzed. Both our IgE-E binding results and our immunoblotting results indicate that amino acid sequences 78–85 and 267–274 of Per a 1.0104 are directly involved in the interaction to human IgE. Results are summarized, and the schematic map of the location of

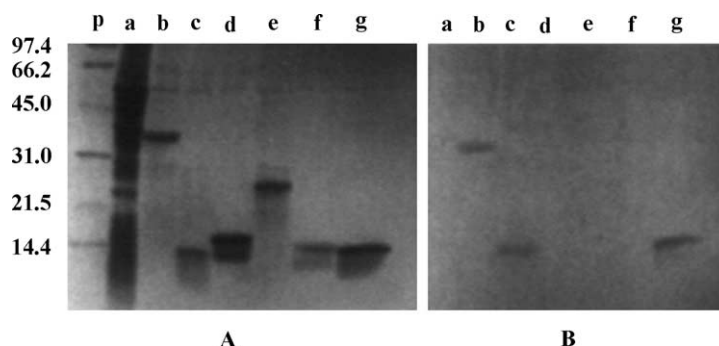


Fig. 1. SDS-PAGE (A) and immunoblotting (B) of proteins expressed from deleted Per a 1.0104 cDNA. Proteins were stained with Coomassie brilliant blue (A), or probed with atopic sera (B). *E. coli* BL21(DE3) (lane a), Per a 1.0104 (lane b), Per a 1.0104:1–87 (lane c), Per a 1.0104:1–77 (lane d), Per a 1.0104:86–205 (lane e), Per a 1.0104:200–266 (lane f) and Per a 1.0104:200–267 (lane g). Numbers at left indicate sizes (kDa) of protein marker (lane p).

1	VGVDGLIDDIIAILPIDDLKALFQEKLETSLDFKAFYDAVRSPEFQSI VQTLNAMPEYQD	60
61	LLQKLRDKGVDVDHYIEL IRALFGL TREARNLQDDLNDFLALIPTDQILAIAMDYLANDA	120
121	EVQELVAYLQSDDFHKIINTIEALPEFANFYNFLKGHGLDVANYINEIHSIIGLPPFVPP	180
181	SRRHARRGVGINGLIDDVIAILPVDELKTLFQEKLETSPDFKALYDAIRSP EFQSIISTL	240
241	NAMPEYQELLQNLRLDKGVDVDHFIEL IRSWFGLP	274

Fig. 2. The deduced primary structure of Per a 1.0104. Internal repeat sequences (amino acid residues 5–85 and 93–273) are underlined, and sequences required to bind IgE (sequences 78–85 and 267–274) are shown in bold.

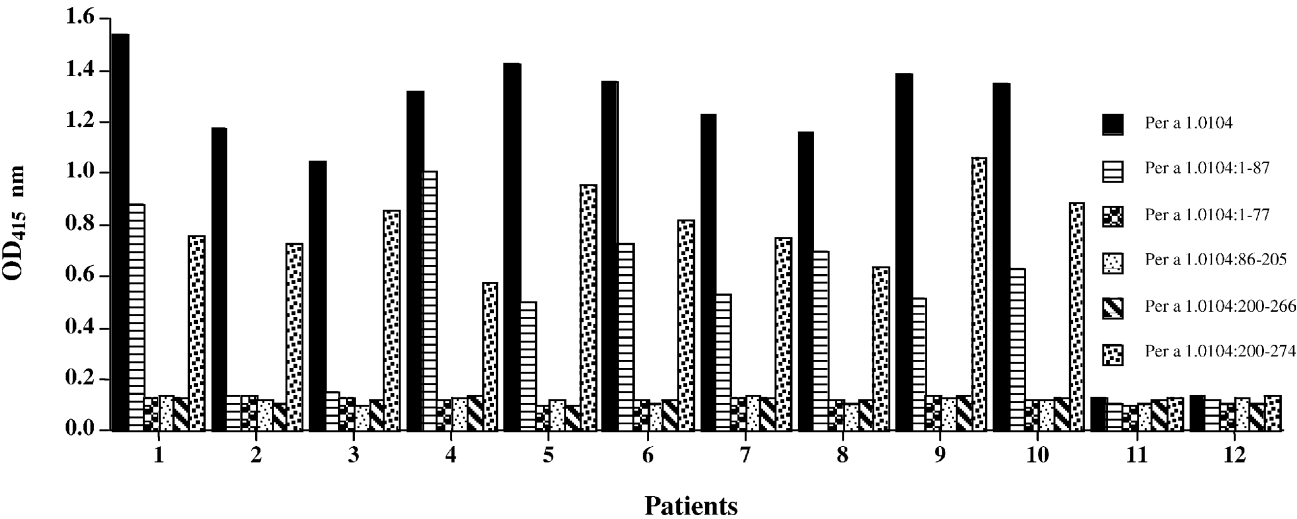


Fig. 3. Binding profiles of IgE antibodies to Per a 1.0104 and deletion mutant proteins by ELISA. Ten human atopic sera and two sera from health non-allergic adults (patients 11 and 12) were analyzed.

recombinant fragments on the complete Per a 1.0104 molecule is presented in Fig. 5.

3.3. IgE binding inhibition of recombinants and synthetic peptides

To confirm the binding specificity of IgE antibodies to deletion mutant proteins and synthesized peptides, compe-

titive inhibition assays were performed using purified intact Per a 1.0104, deletion mutant proteins, and non-biotinylated synthetic peptides as competitors. Pooled sera from the 10 Per a 1.0104-positive patients were used for the inhibition studies. Full-length Per a 1.0104, deletion mutant 1–87, deletion mutant 200–274, peptide 78–85, and peptide 267–274 inhibited 84, 69, 67, 54, and 50% of IgE binding to Per a 1.0104 at concentration of 1.0 µg/ml in solution,

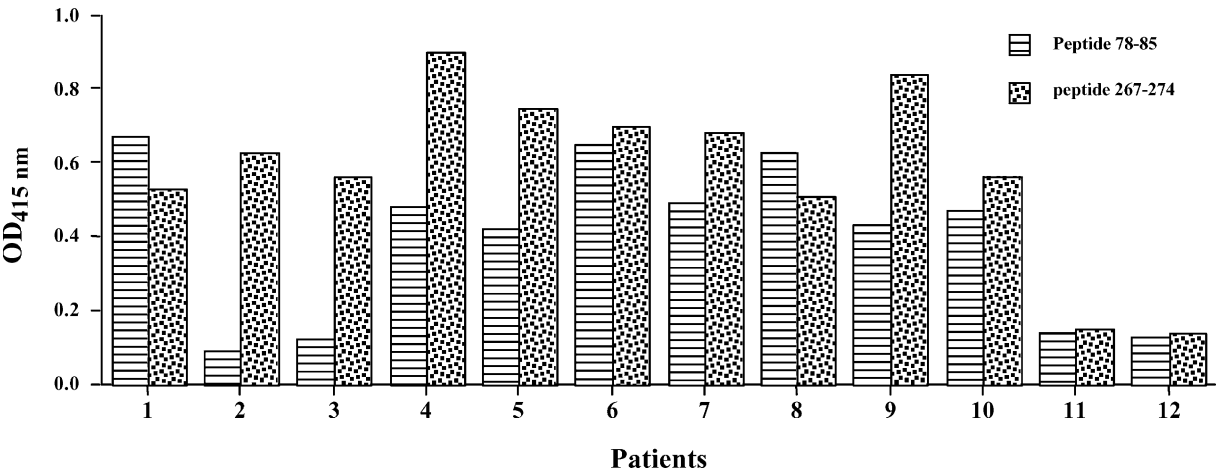


Fig. 4. Binding profiles of IgE antibodies to synthetic peptides by ELISA. Ten human atopic sera and two sera from health non-allergic adults (patients 11 and 12) were analyzed.

Endonuclease/PCR			Human IgE	
			Immunoblotting	ELISA
cDNA	1-274	————— 78 ↓ 85	+	+
<i>Bam</i> HI/ <i>xho</i> I	1-87	—————■	+	+
PCR	1-77	—————	—	—
PCR	86-205	—————	—	—
PCR	200-266	————— 267 ↓ 274	—	—
PCR	200-274	—————■	+	+

Fig. 5. B cell epitopes mapping on the Per a 1.0104. Schematic map location of deletion mutants on the complete Per a 1.0104. The symbol (■) represent the regions recognized by human IgE.

respectively (Fig. 6). No significant inhibition was observed when peptides were preincubated with non-allergic sera (Fig. 6), and no significant inhibition was detected on deletion mutants 1–77, 86–205 and 200–266 (data not shown). These results reconfirmed those amino acid sequences 78–85 and 267–274 of Per a 1.0104 are directly involved in binding of human IgE antibodies.

Certain evidence has indicated that allergen degradation products are natural targets of B cell recognition, reinforcing the importance of continuous epitopes (Becker, 1997). Group V timothy grass pollen allergen (Phl p va) (Bufe et al.,

1994), birch pollen allergen (Bet v 1) (Vik et al., 1993), *Chironomus thummi* allergen (Chi t I) (Liebers et al., 1993), shrimp allergen (*Penaeus aztecus*, Pen a 1) (Reese et al., 1995), and latex allergen (Hev b 5) (Beezhold et al., 1999) all contain continuous epitopes. Group I velvet grass pollen allergen (Hol I 1) (Schramm et al., 1997) and house dust mite allergen Der p 2 and Der f 2 (Smith and Chapman, 1996; Takai et al., 1997) contain conformational and continuous epitopes for IgE recognition. These findings suggest that both linear and conformational epitopes are important in allergic reactions. Immunotherapy by repeated subcutaneous injection has been shown to be an effective method in the treatment of allergies (Ohman, 1991). Immunotherapies containing specific recombinant allergens or epitopes, rather than crude allergen vaccine mixtures, may prove to be more efficacious. Encouraging results using recombinant allergens for immunotherapy have already been reported (Valenta and Kraft, 1995; Hoyne et al., 1995). Recently, small peptides carrying only one IgE-binding epitope have been proposed for immunotherapy in order to increase specificity of the immune response (Ball et al., 1994). Information on IgE-binding epitopes is required for further immunotherapy studies. The CD4⁺ allergen-specific T cell is crucial for induction of IgE. Nevertheless, allergens with no or very low IgE-binding capacities are also important in immunotherapy.

In this study, we have performed B cell linear epitope analysis of the major American cockroach allergen Per a 1.0104 using a number of deletion mutants. We have shown that the amino acid sequences ⁷⁸LIRALFGL⁸⁵ and ²⁶⁷IRSWFGLP²⁷⁴, located within internal repeats of Per a 1.0104, are the minimum sequences required for IgE binding. Our current findings are important for the understanding of the antigenic structures responsible for allergenicity to the American cockroach, thereby contributing to the strategic development of more specific and potentially more efficacious immunotherapy.

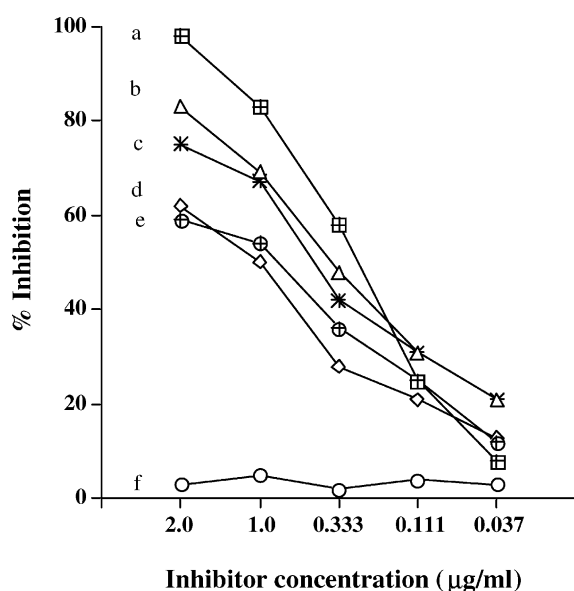


Fig. 6. Binding inhibition of IgE to Per a 1.0104 by Per a 1.0104 (a), Per a 1.0104:200–274 (b), Per a 1.0104:1–87 (c), peptide 78–85 (d), peptide 267–274 (e), and non-allergic sera (f). Microtiter plates were coated with purified Per a 1.0104, and results are average of experiment performed in triplicate as described.

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References

- Arruda, L.K., Vailes, L.D., Mann, B.J., Fox, J.W., Vedvick, T.S., Hayden, M.L., Chapman, M.D., 1995a. Molecular cloning of a major cockroach (*Blattella germanica*) allergen Bla g 2. *J. Biol. Chem.* 270, 19563–19568.
- Arruda, L.K., Vailes, L.D., Hayden, M.L., Benjamin, D.C., Chapman, M.D., 1995b. Cloning of cockroach allergen, Bla g 4, identified ligand binding proteins (or calycins) as a cause of IgE antibody responses. *J. Biol. Chem.* 270, 31196–31201.
- Arruda, L.K., Vailes, L.D., Platts-Mills, T.A.E., Hayden, M.L., Chapman, M.D., 1997. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). *J. Biol. Chem.* 272, 20907–20912.
- Astrurias, J.A., Gomez-Bayon, N., Arilla, M.C., Martinez, A., Pala, R., Sanchez-Gascom, F., Martinez, J., 1999. Molecular characterization of American cockroach tropomyosin (*Periplaneta americana* allergen 7), a cross-reactive allergen. *J. Immunol.* 162, 4342–4348.
- Ball, T., Vrtala, S., Sperr, W.R., Valent, P., Susani, M., Kraft, D., Valenta, R., 1994. Isolation of an immunodominant IgE hapten from an epitope expression cDNA library. Dissection of the allergic effector reaction. *J. Biol. Chem.* 269, 28323–28328.
- Becker, W.M., 1997. Allergen epitopes. In: Ring, J., Behrendt, H., Vieluf, D. (Eds.), *New Trends in Allergy*, vol. IV. Springer, Heidelberg, pp. 339–44.
- Beezhold, D.H., Hickey, V.L., Slater, J.E., Sussman, G.L., 1999. Human IgE-binding epitopes of the latex allergen Hev b 5. *J. Allergy Clin. Immunol.* 103, 1166–1172.
- Bufe, A., Becker, M.M., Schramm, G., Peterson, A., Mamat, U., Schlaak, M., 1994. Major allergen Phl p Va (timothy grass) bears at least two different IgE-reactive epitopes. *J. Allergy Clin. Immunol.* 94, 173–181.
- Chapman, M.D., Vailes, L.D., Hayden, M.L., Platts-Mills, T.A.E., Arruda, L.K., 1996. Cockroach allergens and their role in asthma. In: Kay, A.B. (Ed.), *Allergy and Allergic Diseases*. Blackwell Scientific Publications, Oxford, UK, pp. 942–951.
- Helm, R., Cockrell, G., Stanley, J.S., Brenner, R.J., Burks, W., Bannon, G.A., 1996. Isolation and characterization of a clone encoding a major allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. *J. Allergy Clin. Immunol.* 98, 172–180.
- Helm, R.M., 1999. Cockroach and other inhalant insect allergens. In: Lockett, R.F., Bukantz, S.C. (Eds.), *Allergens and Allergen Immunotherapy*, 2nd ed. Marcel Dekker, New York, pp. 203–223.
- Hoyne, G.F., Kristensen, N.M., Yessl, H., Lamb, J.R., 1995. Peptide modulation of allergen-specific immune response. *Curr. Opin. Immunol.* 7, 757–761.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Liebers, V., Raulf, M., Mazur, G., Modrow, S., Baur, X., 1993. Epitope mapping with peptides of Chi t I component III and immunomodulation of the Chi t immune response. *J. Allergy Clin. Immunol.* 92, 334–339.
- Melen, E., Pomes, A., Vailes, L.D., Arruda, L.K., Chapman, M.D., 1999. Molecular cloning of Per a 1 and definition of the cross-reactive group I cockroach allergens. *J. Allergy Clin. Immunol.* 103, 859–864.
- Ohman Jr., J.L., 1991. *Clinical and Immunologic Responses to Immunotherapy*. Marcel Dekker, New York, pp. 209–232.
- Pomes, A., Melen, E., Vailes, L.D., Retief, J.D., Arruda, L.K., Chapman, M.D., 1998. Novel allergen structures with tandem amino acid repeats derived from German and American cockroaches. *J. Biol. Chem.* 273, 30801–30807.
- Reese, G., Dual, C.B., Lehrer, S.B., 1995. Antigenic analysis IgE and monoclonal antibodies of the major shrimp allergen Pen a 1 (Tropomyosin) from *Penaeus aztecus*. *Int. Arch. Allergy Immunol.* 107, 245–247.
- Sambrook, J., Fritsch, E.F., Maniatis T., 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed., Cold Spring Harbor Press, New York.
- Santos, A.B.R., Chapman, M.D., Arruda, L.K., Aalberse, R.C., Vailes, L.D., Ferriani, P.L., Oliver, C., Rizzo, M.C., Naspitz, C.K., Arruda, L.K., 1999. Cockroach allergens and asthma in Brazil: identification of tropomyosin as a major allergen with potential cross-reactivity with mite and shrimp allergens. *J. Allergy Clin. Immunol.* 104, 329–337.
- Schramm, G., Bufe, A., Peterson, A., Hass, H., Schlaak, M., Becker, W.M., 1997. Mapping of IgE-binding epitopes on the recombinant major group I allergen of velvet grass pollen rHol I 1. *J. Allergy Clin. Immunol.* 99, 781–787.
- Smith, A.M., Chapman, M.D., 1996. Reduction in IgE binding to allergen variants generated by site-directed mutagenesis: contribution of disulfide bonds to the antigenic structure of the major house dust mite allergen Der p 2. *Mol. Immunol.* 35, 399–405.
- Sutton, B.J., Gould, H.J., 1993. The human IgE network. *Nature* 366, 412–418.
- Takai, T., Yuuki, T., Okumura, Y., Mori, A., Okudaira, H., 1997. Determination of N- and C-terminal sequences required to bind human IgE of the major house dust mite allergen Der f 2 and epitope mapping for monoclonal antibodies. *J. Allergy Clin. Immunol.* 34, 255–261.
- Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoresis transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. U.S.A.* 76, 4350–4354.
- Valenta, R., Kraft, D., 1995. Recombinant allergens for diagnosis and therapy of allergic diseases. *Curr. Opin. Immunol.* 7, 751–756.
- Vik, H., Steinvag, S.K., Elsayed, S., 1993. Studies on allergenicity of the amino-terminal epitope (Bet v 1 23–28) from Birch pollen allergen. *Int. Arch. Allergy Immunol.* 101, 89–94.
- Wang, N.M., Lee, M.F., Wu, C.H., 1999. Immunological characterization of a recombinant American cockroach (*Periplaneta americana*) Per a 1 (Cr-PII) allergen. *Allergy* 54, 119–127.
- Wu, C.H., Lan, J.L., 1988. Cockroach hypersensitivity: isolation and partial characterization of major allergens. *J. Allergy Clin. Immunol.* 82, 727–735.
- Wu, C.H., Lee, M.F., Liao, S.C., 1995. Isolation and preliminary characterization of cDNA encoding American cockroach allergens. *J. Allergy Clin. Immunol.* 96, 352–359.
- Wu, C.H., Hsieh, M.J., Huang, J.H., Luo, S.F., 1996a. Identification of low molecular weight allergens of American cockroach and production of monoclonal antibodies. *Ann. Allergy Asthma Immunol.* 76, 195–203.
- Wu, C.H., Lee, M.F., Liao, S.C., 1996b. Sequencing analysis of cDNA clones encoding the American cockroach Cr-PI allergens. Homology with insect hemolymph proteins. *J. Biol. Chem.* 271, 17937–17943.
- Wu, C.H., Lee, M.F., Wang, N.M., Luo, S.F., 1997. Sequencing and immunochemical characterization of the American cockroach Per a 3 (Cr-PI) isoallergenic variants. *Mol. Immunol.* 34, 1–8.
- Wu, C.H., Wang, N.M., Lee, M.F., Kao, C.Y.Y., Luo, S.F., 1998. Cloning of the American cockroach Cr-PII allergens: evidences for the existences of cross-reactive allergens between species. *J. Allergy Clin. Immunol.* 101, 832–840.
- Yang, C.Y., Wu, J.D., Wu, C.H., 2000. Sequence analysis of the first complete cDNA clone encoding an American cockroach Per a 1 allergen. *Biochim. Biophys. Acta* 1517, 153–158.