

Homology of a bovine allergen and the oligomycin sensitivity–conferring protein of the mitochondrial adenosine triphosphate synthase complex

Sinikka Parkkinen, PhD, Marja Rytönen, MSc, Jaana Pentikäinen, PhD, Tuomas Virtanen, MD, and Rauno Mäntylä, MD, Kuopio, Finland

We have characterized bovine allergens by constructing and analyzing a complementary DNA library from bovine skin. Clones producing proteins that reacted with IgE antibodies from persons with allergy were purified and sequenced. One set of the allergen-coding clones showed an almost complete homology with the bovine oligomycin sensitivity–conferring protein of the mitochondrial adenosine triphosphate synthase complex. The IgE antibodies adsorbed with the recombinant allergen reacted with an 11 kd protein in the cow dander extract. Binding of the IgE from patients allergic to the recombinant allergen expressed in Escherichia coli confirmed the allergen-coding ability of the complementary DNA sequence. The prevalence of the IgE-positive sera among patients with cow allergy and control subjects suggests that the recombinant allergen represents one of the minor allergens in cow dander. This is the first time a mammalian allergen has been identified as a protein with a known function. (J ALLERGY CLIN IMMUNOL 1995;95:1255-60.)

Key words: Bovine allergen, complementary DNA cloning, oligomycin sensitivity–conferring protein, IgE binding

Cow dander and hair are important sources of allergens in dairy farms.¹ Sensitization to these allergens leads to both respiratory and cutaneous manifestations in persons with atopic disease. Sera of patients with cow allergy react with a number of proteins found in the bovine skin. Western blot analyses have revealed that the main IgE response is directed against 20 and 22 kd proteins, the major allergens of cow dander.^{1,2} A smaller proportion of the patients also reacts to the 24 and 16 kd proteins. Although amino acid composition, isoelectric point, values and some biochemical properties of the major bovine allergens have been well

Abbreviations used

ATP:	Adenosine triphosphate
cDNA:	Complementary DNA
OSCP:	Oligomycin sensitivity–conferring protein
TBS:	TRIS buffered saline solution
TRIS:	Tris(hydroxymethyl)aminomethane

characterized by biochemical methods² and by use of an affinity-purified protein,³ the basic molecular structure of these proteins still remains unknown. The amino acid sequences of many allergens have been deduced from the nucleic acid sequence by the complementary DNA (cDNA) cloning method. However, the basic molecular structure of most mammalian allergens has not yet been defined.⁴ An exception is the major allergen of the domestic cat, *Fel d I*, which has been examined in detail at the genome level by cDNA cloning and polymerase chain reaction amplification.⁵

The aim of this study was the molecular characterization of the bovine allergens by construction of a cDNA library from bovine skin. cDNA clones

From the Department of Clinical Microbiology, University of Kuopio. Dr. Parkkinen is now with the Department of Chemical Technology, Lappeenranta University of Technology, Lappeenranta, Finland.

Supported by the Finnish Foundation for Allergy Research and by the Emil Aaltonen Foundation.

Received for publication May 2, 1994; revised Sept. 23, 1994; accepted for publication Nov. 14, 1994.

Reprint requests: Rauno Mäntylä, MD, Department of Clinical Microbiology, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland.

Copyright © 1995 by Mosby–Year Book, Inc.

0091-6749/95 \$3.00 + 0 1/162017

producing recombinant proteins reacting with human IgE antibodies were isolated and sequenced. One set of clones representing a minor bovine allergen was found to be homologous with the oligomycin sensitivity-conferring protein (OSCP), a subunit of the mitochondrial adenosine triphosphatase complex.

METHODS

Isolation of messenger RNA and cDNA cloning

Cow epithelium was homogenized mechanically in guanidinium thiocyanate buffer and used for total RNA extraction by cesium chloride centrifugation.⁶ Poly(A)RNA was purified from the total RNA with oligo-dT cellulose (Promega, Madison, Wis.) and used for cDNA synthesis. cDNA was cloned into the Uni ZAP XR vector (ZAP-cDNA synthesis kit; Stratagene, La Jolla, Calif.).

Screening of the cDNA library

The cDNA library was immunoscreened with sera of patients with cow allergy. Phage plaques, 1.4×10^5 plaque forming units per 500 cm² plate, were lifted onto the isopropylthio- β -D-galactoside-treated nitrocellulose filters. Filters were washed, blocked by 0.1% casein in tris (hydroxymethyl)aminomethane (TRIS)-buffered saline solution (TBS), consisting of 20 mmol/L TRIS HCl, and 150 mmol/L NaCl (pH 7.5) for 1 hour and left to incubate with diluted (1:5) patient serum for 6 hours. Iodine 125-labeled, diluted (1:10) anti-human IgE (Phadebas RAST, Kabi Pharmacia Diagnostics, Uppsala, Sweden) was used as the secondary antibody for overnight blotting. Filter washings and serum and conjugate dilutions were made in TBS with 0.05% polysorbate 20. After autoradiography, positive plaques were picked and purified with repeated phage dilutions and immunoblottings.

Affinity purification of IgE

Serum was adsorbed to the recombinant protein as described by Donovan and Baldo.⁷ Phage plaques were plated at a high density on a plate 100 mm in diameter. Synthesis of the fusion protein was induced by an isopropylthio- β -D-galactoside-treated nitrocellulose filter. The filter was lifted and washed briefly with TBS with 0.05% polysorbate 20. Proteins were fixed onto the filter by 0.5% glutaraldehyde in phosphate-buffered saline solution and blotted with a serum of a patient with cow allergy, diluted 1:4 in TBS with 0.1% casein. Antibodies were eluted with 0.1 mol/L glycine HCl (pH 3.0), neutralized by 1 mol/L TRIS (pH 9.5), concentrated by centrifugation through a Centricon (Amicon Inc., Beverly, Mass.) column and used for Western blotting.

Western blotting

Bovine dander extract was prepared from commercial raw material (Allergon AB),⁸ separated by sodium do-

decyl sulfate-polyacrylamide gel electrophoresis in a 12.5% gel and transferred to a nitrocellulose membrane.¹ IgE immunoblotting was carried out under conditions described earlier.

Sequencing of the cDNA clones

Inserts containing Bluescript SK(-) phagemids were excised from the bacteriophage Uni-ZAP XR λ and transformed to *Escherichia coli*, XL1-Blue cells (Stratagene). DNA for sequencing was purified by Wizard Minipreps DNA Purification System (Promega). Nucleotide sequencing was done with the automatic DNA sequenator A.L.F. with an Auto Read kit (Pharmacia Biotech, Uppsala, Sweden).⁹

RESULTS

We constructed a cDNA library from cow epithelium in an expression phage vector and screened it by immunoblotting with sera from patients with cow allergy. Plaques producing recombinant allergens as shown by binding of human IgE antibodies were selected and purified. Insert-containing phagemids were excised from the bacteriophage and sequenced. The sequencing revealed several cDNAs of different sizes, and it was apparent that some of the sequenced clones represented incomplete cDNAs (data not shown).

One phage clone, AS1, was selected for further studies. The frequency of the phage in the cDNA library was only 0.002%. We tested sera of nine patients with cow allergy for reactivity to the AS1 phage clone. The sera had been previously tested by Western blot, and they all exhibited IgE reactivity to various components in cow dander extracts (data not shown). Four of the patients had IgE antibodies to the recombinant allergen, although in the serum of one of them the level was low (Fig. 1). Five sera were completely negative even after 30 days' exposure to autoradiography.

To determine the prevalence of the IgE antibodies to the AS1 recombinant protein, the reactivity of 16 patients with cow allergy and of 10 control subjects was tested. The patients were confirmed as having cow allergy by clinical criteria and laboratory tests.¹ Control subjects were patients without allergy, from the clinic of respiratory diseases. Five of 16 patients with cow allergy (31%) had IgE antibodies to AS1 protein, all control subjects were negative for the protein. This experiment suggested that AS1 represents a real allergen but not one of the major allergens in cow dander.

To characterize further the component of cow dander extract represented by the AS1 clone, we affinity purified the IgE fraction of a patient's serum reacting with the AS1 gene product. The

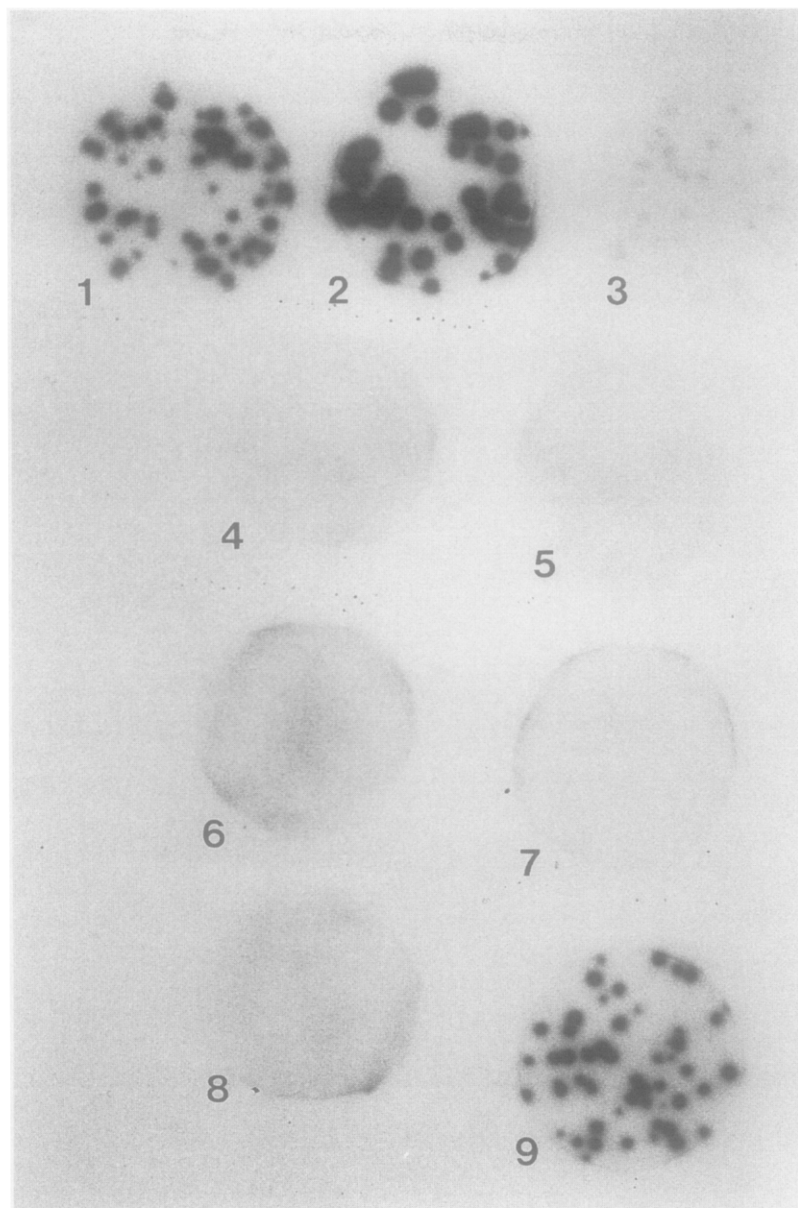


FIG. 1. IgE antibodies of patients with cow allergy to recombinant bovine AS1 allergen. Phages producing AS1 recombinant protein were transferred onto nitrocellulose filters, which were immunoblotted for IgE detection with nine sera (Nos. 1 to 9) of patients with cow allergy.

recombinant AS1 protein was immobilized on a nitrocellulose membrane, and it was allowed to adsorb IgE antibody from the serum.⁷ The antibody was eluted and used in immunoblotting with the bovine dander extract. The eluted IgE antibody attached to an 11 kd protein (Fig. 2). Western blots with the sera from patients with allergy revealed that the same sera (Nos. 1, 2, and 9; Fig. 1) that reacted strongly with the recombinant protein AS1 were also IgE reactive with an 11 kd protein in cow

dander extract. Sera that did not have IgE antibodies to the recombinant AS1 protein (Nos. 4 to 8) did not react with the 11 kd protein either. The negative reaction of the serum of one of the patients with allergy (No. 3) with the 11 kd protein in Western blot assay was probably due to a low level of specific IgE antibodies as shown by the very weak reaction to the recombinant protein expressed in plaques.

Sequencing of the AS1 clone revealed in the

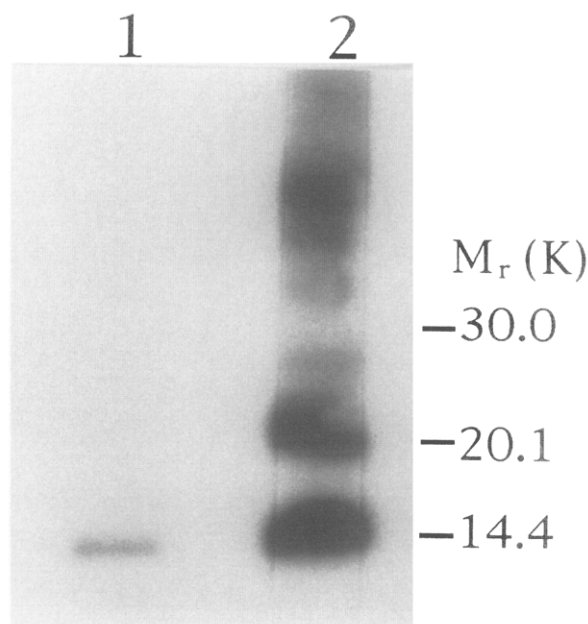


FIG. 2. Characterization of recombinant allergen coded by bovine cDNA clone AS1. IgE immunoblot of cow dander extract with serum from patient with cow allergy that had been affinity purified with recombinant AS1 phage protein (*lane 1*) and with same serum untreated as control (*lane 2*).

vector an insert of 330 nucleotides with an open reading frame of 238 nucleotides (Fig. 3). The AS1 sequence was compared with the sequences in the European Molecular Biology Laboratory Data Library (GCG program package; Center for Scientific Computing, Espoo, Finland), and an almost complete homology was found with the carboxy-terminal end of the bovine OSCP.¹⁰ To determine whether the whole OSCP messenger was present in the cDNA library, a probe was prepared from the cloning site of AS1 to the *Eco*RI site in the AS1 (nucleotide 468 → 736 of AS8; Fig. 3).

With this probe three longer clones containing AS1 sequences were picked from the cDNA library. Nucleic acid sequencing revealed that these clones were identical, 776 nucleotides long with an open reading frame of 705 nucleotides. The sequence of one of the clones, AS8, is shown in Fig. 3. The carboxy-terminal end was identical with AS1, and the homology with OSCP comprised the whole mature protein coding sequence of OSCP and the 3' part of the putative signal peptide.¹⁰ At the 5' end of the AS8, 111 nucleotides did not show any homology to the OSCP, but this sequence was found in the human and mouse genes of 28S ribosomal RNA. The most likely

explanation is an artefact caused by ribosomal RNA contamination of the messenger RNA used for cDNA synthesis.

DISCUSSION

An almost complete homology between a bovine allergen and the OSCP of the bovine mitochondrial adenosine triphosphate (ATP) synthase complex is demonstrated in this study. OSCP is one of the 14 subunits associated with the ATP synthase of bovine mitochondria.¹¹ This membrane-bound multisubunit enzyme catalyzes ATP production from adenosine diphosphate and inorganic phosphate by a transmembrane proton gradient. The size of the mature OSCP is 190 amino acids (21 kd),¹⁰ and the bovine cDNA clone AS8 has an open reading frame with a theoretical coding capacity of 220 amino acids (24 kd). In the Western blot, however, IgE antibodies adsorbed with the recombinant AS1 protein reacted only with an 11 kd protein of the cow dander extract. OSCP has been described to be sensitive to enzymatic degradation.^{12, 13} It is likely that during the preparation of the cow dander extract OSCP is degraded and only a carboxy-terminal fragment containing intact allergenic epitopes is present in the extract.

No data suggest that subunits of ATP synthase or other pump proteins of the cellular membranes have allergenic properties. Currently it is not clear why an evolutionarily conserved protein with a role in the energy metabolism of the mammalian cell has allergenic properties. Rat mitochondrial OSCP has been shown to have a high degree of homology with the bovine OSCP.¹⁴ Subunit d of *E. coli* ATP synthase and chloroplasts also bear partial homology with the bovine OSCP.¹¹ Immunologic cross-reactivity was demonstrated between bovine, human, and rat OSCP subunits.¹² However, no data are available of the degree of the nucleic acid sequence homology between human and bovine OSCP, but we assume that the nonhomologous part of the genes codes for the allergenic epitopes of the allergens.

After sequencing several cDNA clones from the bovine dander, we found only one set of related clones that had a homology with sequences in the European Molecular Biology Laboratory Data Library gene bank. Only a few of the mammalian allergens are well characterized,⁴ and none of them has nucleotide sequences corresponding to an identified functional protein. Cat allergen *Fel d* I chain 1 has sequence similarities with the rabbit uteroglobin and with a protein of human Clara cells.¹⁵ On the other hand, house dust mite aller-

```

1  TGACTGGGGC GGTACACCTG TCAAACNGTA ACGCAGGTGT CCTAAGGCGA

51  GCTCAGGGAG GACAGAAACC TCCCGTGGAG CAGAAGGGCA AAAGCTCGCT

      28S rRNA ← | → OSCP leader                | → mature OSCP
101 TGATCTTGAT TTTCAGTACA TCTGTGGTCA GGCCATTTC CAAGCTTGTG

151 AGGCCACCTG TTCAGATATA TGGCATCGAA GGCCGCTACG CCACAGCTCT

201 GTATTCCGCT GCATCTAAAC AGAATAAACT GGAGCAAGTA GAGAAGGAGT

251 TGTTCGAGT AGGACAAATC TTGAAGGAAC CCAAATGGC TGCTTCTCTT

301 TTGAATCCCT ATGTAAAGCG TTCAGTTAAA GTGAAAAGCC TAAGTGACAT

351 GACGGCAAAG GAGAAGTTCT CTCCTCTCAC GTCCAACCTG ATCAATTTGC

401 TTGCTGAAA TGGTCGCTTG ACTAATACCC CTGCGGTGAT TTCCGCATTT

      | → AS1
451 TCTACCATGA TGAGTGTTCA CCGTGGAGAA GTACCATGCA CAGTTACCAC

      A
501 TGCATCTGCT TTAGATGAAG CCACTCTGAC TGAATTGAAA ACGGTCCTGA

      A G
551 AGAGCTTCCT AAGTAAAGGC CAGGTTTTGA AGTTAGAAGT TAAGATTGAT

601 CCGTCGATCA TGGGTGGAAT GATTGTCCGT ATTGGAGAGA AATACGTTGA

651 TATGTCTGCA AAAACCAAGA TTCAGAAGCT GAGCAGAGCA ATGCGGGAGA

      Eco RI
701 TTCTGTAAAA GTGTTGATTT TCTAAAAGTG AGAATTCTTA AAGTTGGAGC
      ***

751 AACAATAAAC ACTTCCTGAA CAGGGCAAAAAA

```

FIG. 3. cDNA sequence of bovine allergen AS8. Region homologous with OSCP begins from nucleotide 112 and continues until poly(A) site. Three nonidentical nucleotides are indicated. Coding region for mature OSCP is from nucleotide 136 until stop codon (***). Predicted polyadenylation signal is underlined. Nucleotide 112 → 136 is homologous with putative signal peptide of OSCP. Sequence nucleotide 1 → nucleotide 112 is homologous with sequence in gene for human or mouse 28S ribosomal RNA. Shorter cDNA clone, AS1, starts at nucleotide 468. *Eco*RI site in AS1 clone is double underlined.

gens are well characterized. Many of them possess proteolytic enzyme activity, which could be involved in the pathogenesis of allergy.¹⁶⁻²⁰ The major heat-stable shrimp allergen, *Sa* II, has been recently reported to represent tropomyosin, a protein of thin muscle filaments.²¹ However, no cross-reactivity could be demonstrated with tropomyosins from other vertebrate species.

Some plant allergens have homologies with known plant proteins. Birch, alder, and hazel pollens have related major allergens (*Bet v* I, *Aln g* I, and *Cor a* I, respectively) that have a homology

with a group of plant pathogenesis-related proteins.²² Another birch pollen allergen, *Bet v* II, has been shown to be a member of the family of plant profilins, provoking IgE reactivity in about 20% of patients with allergy.²³

The low frequency of the cDNA encoding the AS8 phage protein allowed us to assume that the proportion of the OSCP protein of cow dander proteins is also low. Because 31% of patients with cow allergy have IgE antibodies to the AS1 recombinant protein, we conclude that OSCP is a real but a minor allergen of the bovine dander. Epitope

mapping and site-directed mutagenesis will further clarify the allergenic character of this protein.

The technical assistance of Mrs. Riitta Korhonen is gratefully acknowledged.

REFERENCES

1. Ylönen J, Mäntyjärvi R, Taivainen A, Virtanen T. Comparison of the antigenic and allergenic properties of three types of bovine epithelial material. *Int Arch Allergy Appl Immunol* 1992;99:112-7.
2. Prahl P, Bucher D, Plesner T, Weeke B, Løwenstein H. Isolation and partial characterisation of three major allergens in an extract from cow hair and dander. *Int Arch Allergy Appl Immunol* 1982;67:293-301.
3. Ylönen J, Virtanen T, Horsmanheimo L, Parkkinen S, Pelkonen J, Mäntyjärvi R. Affinity purification of the major bovine allergen by a novel monoclonal antibody. *J ALLERGY CLIN IMMUNOL* 1994;93:851-8.
4. Schou C. Defining allergens of mammalian origin. *Clin Exp Allergy* 1993;23:7-14.
5. Griffith IJ, Craig S, Pollock J, Yu X-B, Morgenstern JP, Rogers BL. Expression and genomic structure of the genes encoding FdI, the major allergen from the domestic cat. *Gene* 1992;113:263-8.
6. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press, 1989.
7. Donovan GR, Baldo BA. Recombinant DNA approaches to the study of allergens and allergenic determinants. In: Baldo BA, ed. Molecular approaches to the study of allergens. *Monogr Allergy* 1990;28:52-83.
8. Virtanen T, Vilhunen P, Husman K, Mäntyjärvi R. Sensitization of dairy farmers to bovine antigens and effects of exposure on specific IgG and IgE titers. *Int Arch Allergy Immunol* 1988;87:171-7.
9. Ansorge W, Sproat B, Stegemann J, Schwager C, Zenke M. Automated DNA sequencing: ultrasensitive detection of fluorescent bands during electrophoresis. *Nucleic Acids Res* 1987;15:4593-602.
10. Walker JE, Gay NJ, Powell SJ, Kostina M, Dryer MR. ATP synthase from bovine mitochondria: sequences of imported precursors of oligomycin sensitivity conferral protein, factor 6, and adenosinetriphosphatase inhibitor protein. *Biochemistry* 1987;26:8613-9.
11. Walker JE, Lutter R, Dupuis A, Runswick MJ. Identification of the subunits of F_1F_0 -ATPase from bovine heart mitochondria. *Biochemistry* 1991;30:5369-78.
12. Hekman C, Hatefi Y. The F_0 subunits of bovine mitochondrial ATP synthase complex: purification, antibody production, and interspecies cross-immunoreactivity. *Archs Biochem Biophys* 1991;284:90-7.
13. Joshi S, Burrows R. ATP synthase complex from bovine heart mitochondria. *J Biol Chem* 1990;265:14518-25.
14. Higuti T, Kuroiwa K, Kawamura Y, Morimoto K, Tsujita H. Molecular cloning and sequence of cDNAs for the import precursors of oligomycin sensitivity conferring protein, ATPase inhibitor protein, and subunit c of H^+ -ATP synthase in rat mitochondria. *Biochim Biophys Acta* 1993;1172:311-4.
15. Morgenstern JP, Griffith IJ, Brauer AW, et al. Amino acid sequence of Fel dI, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci U S A* 1991;88:9690-4.
16. Chua KY, Stewart GA, Thomas WR, et al. Sequence analysis of cDNA coding for a major house dust mite allergen *Der p I*: homology with cysteine proteases. *J Exp Med* 1988;167:175-82.
17. Ando T, Ino Y, Haida M, et al. Isolation of cysteine protease in the crude mite extract, *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1991;96:199-205.
18. Kent NA, Hill MR, Keen JN, Holland PWH, Hart BJ. Molecular characterisation of group I allergen *Eur m I* from house dust mite *Euroglyphus maynei*. *Int Arch Allergy Immunol* 1992;99:150-2.
19. Stewart GA, Ward LD, Simpson RJ, Thompson PJ. The group III allergen from the house dust mite *Dermatophagoides pteromyssinus* is a trypsin-like enzyme. *Immunology* 1992;75:29-35.
20. Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA. House dust mite-derived amylase: allergenicity and physicochemical characterization. *J ALLERGY CLIN IMMUNOL* 1991;87:1035-42.
21. Shanti KN, Martin BM, Nagpal S, Metcalfe DD, Subba Rao PV. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. *J Immunol* 1993;151:5354-63.
22. Scheiner O. Recombinant allergens: biological, immunological and practical aspects. *Int Arch Allergy Immunol* 1992;98:93-6.
23. Valenta R, Duchene M, Ebner C, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992;175:377-85.