

MOLECULAR BIOLOGY OF ASPERGILLUS ALLERGENS

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Aspergilli are saprophytic fungi widely distributed in nature, and because of their universal presence, all individuals are exposed to these organisms. Among the various members of this genus, *Aspergillus fumigatus* is associated with over 80% of all human syndromes caused by aspergilli. Saprophytic presence to extensive invasion with this organism has been reported with various intermediary conditions depending on susceptibility and immunocompetence of the host.⁵⁵ Other species that have been associated with aspergillosis include *A. flavus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. oryzae*, and *A. ochraceus*.^{55, 57, 72, 88} The predominance of *A. fumigatus* over other aspergilli in human diseases may be due to its ability to grow at human body temperature, its small spore size, and its comparative resistance to oxidative killing.^{7, 8}

Three main processes are involved in the interaction between aspergilli and the lung: colonization, hypersensitivity, and invasion. Colonization is usually a passive process but may lead to immunologic tolerance and local production of potentially toxic fungal metabolites. Allergy or hypersensitivity to *Aspergillus* antigens depends on the mode and frequency of exposures to the organism as well as on the host immune responses. Aspergillomas (fungus ball) develop in pulmonary cavities secondary to other lung diseases, such as histoplasmosis, tuberculosis, and so forth, whereas invasive aspergillosis is usually associated with underlying defects in host-defense mechanism.^{7, 8}

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In this article, we review the recent advances in the field of molecular biology and immunology of *Aspergillus* allergens and how this new information will lead to enhance the knowledge for better diagnosis and management of allergic aspergillosis.

CHARACTERISTICS OF ALLERGENIC PROTEINS

The induction of allergic response in genetically predisposed individuals involves the cooperation of a number of functional and effector cells in recognizing allergen-induced stimulation leading to synthesis of IgE antibodies to *Aspergillus* allergens. A prerequisite for any allergen is the ability to activate T helper 2 (Th2) response in affected individuals. It is not known why certain proteins possess allergenicity, although allergens triggering immune responses must be polyvalent and should possess several epitopes to cross-link IgE antibodies on mast-cells, leading to mediator release. Thus, allergens are more or less constrained in their molecular conformations.

ASPERGILLUS-INDUCED ALLERGIC DISEASES

Aspergillus-mediated allergic disorders are allergic asthma, allergic rhinitis, allergic sinusitis, allergic bronchopulmonary aspergillosis (ABPA), and hypersensitivity pneumonitis. Patients with cystic fibrosis are often reported to have complication of ABPA due to *A. fumigatus* colonization in the lung.³¹

ABPA is the result of hypersensitivity to *Aspergillus* allergens in patients with atopic asthma. The immunologic responses in patients involving type I and III hypersensitivity range from asthma to fatal destructive lung disease with defined clinical, serologic, and pathologic features.⁵⁵ The immune response of the patients against fungal colonization is mainly mediated by elevated total serum IgE, *A. fumigatus*-specific IgE and IgG antibodies, and eosinophilia.⁸¹

Immunodiagnosis of aspergillosis is greatly effected by nonavailability of reliable antigen preparations. The complex nature of fungal morphology and metabolism could be the cause for the differences in antigenic profile of conidial, hyphal, and cytoplasmic preparations. The strain variability and diverse culture and extraction conditions may be responsible for batch-to-batch variation in the allergens. Moreover, sensitivity of serodiagnostic methods are greatly affected by the cross-reactivities among different species of *Aspergillus* as well as between aspergilli and other fungi and bacteria.^{36, 51, 54, 84} The toxins and proteases present in antigen preparations may degrade the allergens and may affect the potency of antigenic preparations.

Identification and Characterization of *A. fumigatus* Allergens

A number of investigators have attempted to characterize and purify relevant antigens of *A. fumigatus* using conventional purification methods such as gel filtration, ion exchange chromatography, preparative isoelectric focusing, hydrophobic interaction, and affinity chromatography.^{34, 35, 43, 53, 56, 77, 87} However, none of these methods provided sufficient quantities of purified relevant antigens.^{55, 60} Subsequently, hybridoma technology was introduced to purify relevant *Aspergillus* allergens. Although, still not available commercially for general use, two major allergens of *A. fumigatus*, Asp f 1 and Asp f 2, have been purified and characterized using this method.^{3, 4, 5, 29, 47, 48, 50, 83}

To circumvent the problems associated with purification and characterization of major allergens, interest has been focused in recent years on molecular biology technology as an effective alternative method for obtaining pure and invariant allergens. The advent of recombinant allergens not only improved diagnosis but also our knowledge of the amino acid sequences of the proteins and our understanding of the structure-function relationship of allergens.

Allergen Nomenclature

As the number of identified allergens is increasing continuously, specific designation needs to be assigned to well characterized allergens according to the accepted taxonomic nomenclature. The International Union of Immunologic Societies through the Subcommittee for Allergen Nomenclature presented a standard designation system for purified allergens and individual components detected within complex allergen extracts.^{39, 44, 63} The first three letters of the genus and the first letter of the species are used to indicate the organism from which the allergens are derived. An Arabic numeral is assigned according to the order of their identification, and the allergens are further labeled by the strain number. The Roman numerals are now used for gene designation and Arabic numerals for protein designation. The biologic names of some of the well-characterized *A. fumigatus* allergens are listed in Table 1.

Molecular Cloning of *A. fumigatus* Allergens

Almost a decade ago, researchers began to isolate and characterize complementary DNAs (cDNA) encoding allergens from various sources in order to determine which proteins induce IgE response in humans.⁹⁷ Indeed, striking sequence similarities between allergens and known proteins were found; the major allergen Asp f 1 from *A. fumigatus* showed more than 99% sequence homology to restrictocin, a ribotoxin from *A. restrictus*. A sequence homology of more than 77% was present between heat shock protein (Hsp) allergen from *A. fumigatus* and Hsp

Table 1. RECOMBINANT *A. FUMIGATUS* ALLERGENS

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*From the official list of allergens/IUIS Allergen Nomenclature Subcommittee.

90 family of proteins present in other organisms.^{2, 49, 64} Another *Aspergillus* allergen identified as manganese superoxide dismutase (MnSOD) exhibited 67.2% sequence homology to the corresponding human enzyme.²¹ Among other allergic proteins from different sources, the white face hornet venom allergen Dol m 5 and major pollen allergen Bet v 1 showed sequence homology to the pathogenesis-related proteins,^{13, 27} the major house dust mite allergen Der p 1 contained sequence motifs of a protease, and Bet v 2, the birch pollen allergen, was identified as the actin-binding protein profilin.^{17, 96} On the other hand, no significant homology was detected with the major allergens of ragweed Amb a 1, cat allergen Fel d 1, and group I, group II, and group V allergens from grass pollen. Therefore, a common structural or functional feature is yet to be found among the characterized allergens, which would have allowed the prediction of whether a certain protein possesses an intrinsic property to behave as an allergen.^{26, 32, 67, 76, 78, 89, 90, 98}

Molecular cloning of allergens consists of the isolation of messenger RNA (mRNA) from the *Aspergillus* strains and the reverse transcription of the mRNA into cDNA. The allergen-encoding genes were identified by Southern hybridization using radiolabeled oligonucleotides or by antibody screening of cDNA libraries with sera from allergic patients. Various methods involved in cloning and sequencing of allergens are shown in Figure 1. The amino acid sequences of the cDNA-encoded proteins can be deduced from the nucleotide sequences of the cDNA. To date, the majority of *A. fumigatus* allergens have been cloned in the phage expression vectors lambda gt11 and lambda ZAP II. Recently, cloning and sequencing based on polymerase chain reaction has been reported, although this method requires knowledge of the partial se-

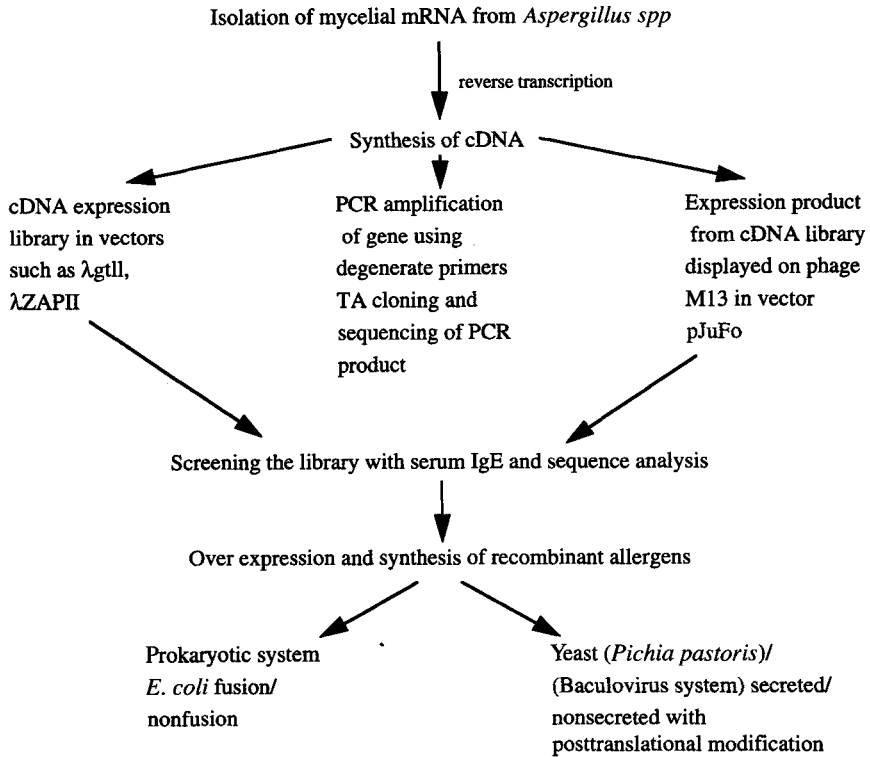


Figure 1. Molecular cloning methods used in expression cloning of *Aspergillus* allergens. PCR = polymerase chain reaction.

quences of the protein for the construction of oligonucleotide primers. In another new approach, filamentous phage M13 has been used to express the *A. fumigatus* proteins from cDNA library on phage surface and screened for allergens binding to IgE antibody of allergic aspergillo-sis patients.^{20, 22} Molecular cloning and sequencing of relevant *Aspergillus* genes encoding antigens and allergens reported in recent years are listed in Table 1. The application of molecular biology technologies for allergen characterization involves the high expression cloning of biologically active recombinant allergen that has structural and functional properties comparable to its natural counterpart. The detailed characterization of the biochemical and immunochemical properties of recombinant allergens is necessary before they can be considered for diagnosis and therapy (see display box 1). A competitive antibody inhibition assay using recombinant and native Asp f 2 showed more than 90% similarity in antibody binding, indicating the structural and functional similarities between native and recombinant *A. fumigatus* allergens (Fig. 2).

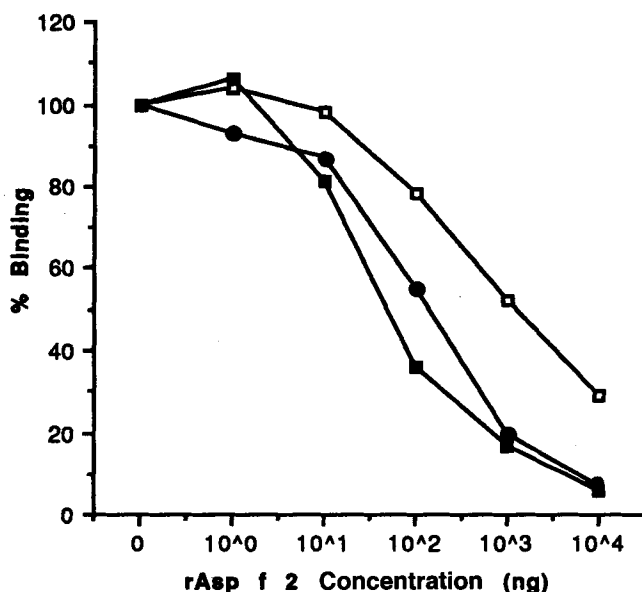


Figure 2. Competitive inhibition study using native and recombinant Asp f 2 allergens and anti-Asp f 2 antibodies raised in mice. Polyclonal anti-Asp f 2 sera were used at a dilution of 1:500 and inhibited with various concentrations of recombinant Asp f 2. The native Asp f 2 purified from *A. fumigatus* strain Af104 was the coating allergen. Three different anti-Asp f 2 antibodies were used in this study raised against native Asp f 2 isolated from *A. fumigatus* strains Af104 and Af102 and against recombinant Asp f 2: open squares = anti-Af 104 (37 kD); circles = anti-Af 102 (37 kD); solid squares = anti-r Asp f 2.

Display Box 1. Biochemical and Immunologic Characterization of Recombinant Allergens

1. Biochemical characterization
2. Purity (recommended > 95%)
3. Lack of toxicity in animals
4. Stability under laboratory storage condition
5. Lack of biological activity when suitably modified
6. Immunologic

Humoral immune responses

- Direct IgE binding by ELISA or PCA assays
- Competitive inhibition of IgE binding using native allergens
- In vivo IgE binding by skin test or bronchial challenge

Cell-mediated immune responses

- T-cell stimulation using PBMC proliferation
- Proliferation of specific T-cell lines
- Cytokine expression (IL-2, IL-4, IL-5, IFN- γ)

Among different expression vectors in use for recombinant allergens, pET has been the most favored prokaryotic expression system. It is a tightly controlled T7 polymerase-based gene expression in *E. coli*. The coding sequence of interest is cloned in pET vector under control of bacteriophage T7 transcriptional and translational signals. The expression of the target protein is induced by a T7 RNA polymerase in the host *E. coli*.^{80, 93} The purification of the protein expressed along with a histidine tag either at the N- or C-terminal end can be accomplished using a single step Ni^{2+} - agarose affinity chromatography. The majority of *A. fumigatus* allergens have been cloned in a high-expression prokaryotic system to obtain the recombinant purified proteins for serodiagnosis and skin-prick test.^{6, 21, 68, 69} The IgE antibody binding of rAsp f 1 and rAsp f 2 purified by pET system are comparable to the native proteins, suggesting that the IgE antibody binding is directed mainly to the protein part of the molecule, and posttranslational modification is not essential for obtaining the proper conformation for antibody-epitope interaction. pET expression of three major *A. fumigatus* allergens, Asp f 1, Asp f 2, and Asp f 12, is shown in Figure 3.

Pichia expression is a eukaryotic system designed for high-level expression of recombinant protein in the yeast *Pichia pastoris*. The target gene under alcohol oxidase promoter expresses as high as 12 mg/L of recombinant protein. Recently, an antigenic protein of *A. fumigatus* with dipeptidyl peptidase activity was cloned and expressed in *Pichia pastoris*.⁹ The new method of displaying functional cDNAs or other gene

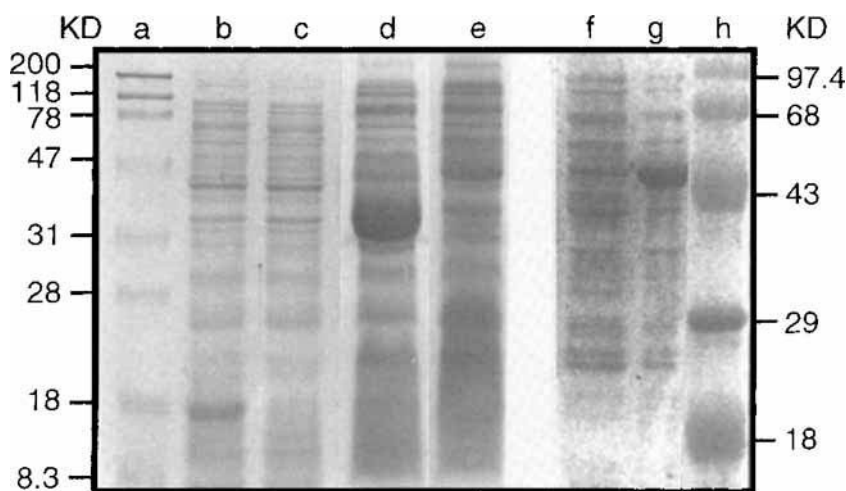


Figure 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of *E. coli* cell lysates expressing recombinant Asp f 1, Asp f 2, and polypeptide of Asp f 12 in pET expression vectors. Lane a = molecular weight markers; b = induced *E. coli* expressing Asp f 1; c = uninduced *E. coli*; d = induced *E. coli* expressing Asp f 2; e = uninduced *E. coli* with Asp f 2; f = uninduced *E. coli*; g = induced *E. coli* expressing Asp f 12 fragment; h = molecular weight markers.

products on the surface of filamentous phage M13 has been developed, exploiting the high-affinity interaction of the Jun and Fos leucine zippers.^{20, 25} The pJuFo cloning vector was designed to display cDNA library from *A. fumigatus* on the surface of filamentous phage, and selective isolation of surface-expressed allergens was carried out by screening IgE antibody binding with sera from *A. fumigatus*-sensitized patients. The phages carrying specific gene products on their surface via leucine zippers could be selectively enriched to 10^4 to 10^6 over nonspecific phages using antibody-based screening procedures.²⁰ The enzyme MnSOD, a major allergen from *A. fumigatus*, has been cloned and characterized using phage display method.^{21, 64}

Allergens from *A. fumigatus*

A large number of genes have been cloned and recombinant proteins obtained from *A. fumigatus* during recent years. However, the allergenicity of the majority of these recombinant proteins has not been determined. We describe here the major *A. fumigatus* allergens isolated by molecular cloning.

Asp f 1

Asp f 1 is a 18 kD major allergen from *A. fumigatus* belonging to the mitogillin family of cytotoxins.^{2, 3, 61, 82} The nucleotide sequence of Asp f 1 gene was determined by sequencing polymerase chain reaction (PCR) products amplified from *A. fumigatus* conidial DNA. The gene encoding 149 amino acid residues includes an 81 base pair (bp) leader sequence, a 52 bp intron, and a 444 bp open reading frame. The culture filtrate of *A. fumigatus* has shown thousand-fold more Asp f 1 than mycelial and spore extracts, suggesting that germination of spores and growth of the fungus are essential for this allergen production. An increased level of Asp f 1 specific IgE and/or IgG antibodies are found in patients with ABPA, aspergilloma, and cystic fibrosis patients with complication of ABPA.⁶⁸ The production of IgG antibodies may have a protective effect to restrict the growth of the fungus in immunocompetent patients. Asp f 1 specific DNA could be amplified by PCR using genomic DNA from *A. fumigatus* and *A. restrictus*, but not from other *Aspergillus* species.² A comparison of the amino acid sequence of Asp f 1 with mitogillin from *A. restrictus* revealed more than 99% sequence similarity. A single amino acid in Asp f 1 molecule, aspartic acid (Asp) at position 115, is substituted by asparagine (Asn) in mitogillin. The CD spectroscopy showed similar secondary structure for both the proteins with 21% and 27% of alpha-helix and 69% and 73% random structure for Asp f 1 and mitogillin, respectively, with no beta-sheet structure. The pathogenesis of ABPA may be attributed to the dual role of Asp f 1, namely the direct cytotoxicity and the IgE mediated inflammatory reaction.

The recombinant Asp f 1 (rAsp f 1/a) expressed in *E. coli* as a functional molecule elicited immediate skin reaction; however, a tenfold functional difference was reported between the purified native ribotoxin

and rAsp f 1/a.⁶⁸ The enzymatically active recombinant protein cleaved the 28S rRNA within the universally conserved region resulted in cytotoxicity to Epstein-Barr virus (EBV)-immortalized or phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC). However, expression of a mutant restrictocin, H136L, had no effect on cell growth, suggesting that H136 may be at the active site of restrictocin. The secondary and tertiary structure of the wild type and mutant restrictocins are not known yet; therefore, it is not clear presently whether the altered activity is due to the active site changes or to conformational changes of the mutant protein.¹²

Asp f 2

The gene encoding the second major allergen, Asp f 2, is a 310 amino acid protein with a 42 amino acid-long signal peptide, a 268 amino acid open reading frame, two introns of 83 and 52 base pairs each, respectively, and four possible glycosylation sites. The gene bank sequence similarity searches revealed significant sequence homology of Asp f 2 to a protein from *A. nidulans*, Asp nd 1, and to a fibrinogen-binding protein and pH regulatory protein from *Candida albicans*. The sequence homology between Asp f 2 and fibrinogen-binding protein may explain its involvement in epithelial damage of host extracellular matrix that leads to the inflammatory reactions at the mucosal surfaces or at the site of fungal colonization.^{11, 15, 75, 85} The 37 kD *A. fumigatus* culture filtrate protein, purified using monoclonal antibody affinity column, exhibited N-terminal sequence homology to recombinant Asp f 2.⁵ Both the native and recombinant Asp f 2 have significantly high IgE binding with sera from patients with ABPA, specifically with sera from patients with central bronchiectasis.⁶ Another glycoprotein (gp55), a 55 kD allergen purified from water-soluble mycelial extract of *A. fumigatus*, exhibited complete N-terminal amino acid sequence homology to Asp f 2.⁹⁴ Although the N-terminal amino acid residues of both the proteins exhibit significant sequence homology, gp 55 has 11 more amino acids upstream of native Asp f 2 near the N-terminal region. The immunochemical characterization of Asp f 2 and gp 55 as well as the amino acid sequence data suggest that various antigens/allergens of *A. fumigatus* would have a common peptide part, and the differences in their molecular mass and reactivity with ABPA and other aspergillosis sera could be due to differential glycosylation and proteolytic processing of the antigens and the strain variability of antigen preparations. Moreover, the elevated level of Asp f 2-specific IgE in patients with ABPA-CB suggests its role in the latter stage of the disease and probably in the irreversible lung damages in patients with ABPA.

Asp f 6

Asp f 6 allergen, a 26.7 kD protein identified as the enzyme MnSOD, has been cloned and isolated from a cDNA library displayed on the surface of filamentous phage.²¹ The identified clone is reported to have 51.1% identity and 67.2% homology to the corresponding human en-

zyme. Both human and *A. fumigatus* MnSOD coding sequences were expressed in *E. coli* as histidine-tagged fusion proteins and purified by Ni²⁺-chelate affinity chromatography. These two recombinant proteins were both recognized by IgE antibodies from patients allergic to *A. fumigatus* and also elicited immediate-type allergic skin reaction in those individuals. Moreover, both recombinant human and *A. fumigatus* MnSOD induced proliferation in peripheral blood mononuclear cells of *A. fumigatus*-allergic patients who were positive for skin-prick test and IgE antibody to MnSOD. This proliferation implies the existence of *in vitro* and *in vivo* humoral and cell-mediated autoimmune reactivity to human MnSOD in patients allergic to *A. fumigatus*. It is still not clear whether this autoimmune reaction is caused by molecular mimicry between conserved T- and B-cell epitopes present on the fungal enzyme and structurally related human enzyme or through sensitization to fungal MnSOD. In a recent study, MnSODs from *A. fumigatus*, humans, yeast, *Drosophila melanogaster*, and *E. coli* showed about 50% identity and 70% similarity in the primary structures. All these recombinant MnSODs have been able to bind IgE in sera from individuals sensitized to the *A. fumigatus* MnSOD.⁶⁴

Asp f 12 (Hsp 90)

The 65 kD fusion protein (*Asp f 12*) cloned and expressed from the cDNA library of *A. fumigatus* belongs to the heat shock protein 90 (*Hsp 90*) family.⁴⁹ This fusion protein was recognized by IgE and IgG antibodies from patients with ABPA. Because of the extraordinary conservation of the heat shock proteins among phylogenetically distant organisms, and to their potential immunogenicity, these heat shock proteins are considered to be important for stress response during inflammation. The immune responses to the *Hsp 70* and *Hsp 90* family of proteins were reported to stimulate protective immunity against malaria in monkeys⁴²; however, no such information is available with the *A. fumigatus* *Hsp*.

Other Recombinant Allergens

Other recombinant *A. fumigatus* allergens identified and characterized using the phage display method, as listed in Table 1, are proteins in the molecular weight range of 10 to 50 kD and have different enzymatic activities. These allergens exhibit extensive sequence homology with known proteins such as proteases from humans and other mammals. The reported metalloprotease (*Asp f 5*) and aspartic protease (*Asp f 9*) having allergenicity may also damage the airway epithelium and trigger allergic responses in patients.

ASPERGILLUS PROTEINS INVOLVED IN PATHOPHYSIOLOGY

Recently, a number of *A. fumigatus* genes encoding proteins with various functional and structural properties have been cloned, se-

quenced, and characterized. This list also includes various *Aspergillus* proteases, such as 42 kD elastinolytic metalloproteinase, 33 kD elastinolytic serine proteases from *A. fumigatus* and *A. flavus*, 39 kD aspartic proteinase, and 32 kD extracellular alkaline protease from *A. fumigatus* cloned and expressed in *E. coli*.^{40, 41, 46, 58, 62, 91} The *A. fumigatus* proteases play a role in the fungal penetration of the host barriers by catalyzing the hydrolysis of the major structural proteins of the basement membrane of lungs such as elastin, collagen, and laminin. Although these secreted proteases have not been reported to elicit an IgE response in patients with allergic aspergillosis, they undoubtedly play a role in the colonization and tissue invasion and may further facilitate the entry of *Aspergillus* antigens or allergens into submucosal tissues and exacerbate an existing allergic response in sensitized patients.⁷⁹

A hydrophobin rodlets gene (HYP 1) of *A. fumigatus*, which encodes an outercoat conidial protein, has been cloned and sequenced.^{74, 95} This 159 amino acid protein showed 85% homology to rodlet A gene of *A. nidulans*. These regularly arranged rodlets proteins are insoluble and may be responsible for conidial hydrophobicity of *Aspergillus* species.^{10, 18} The extreme resistance of rodlets to chemical degradation suggest their role in resistance of *A. fumigatus* to host phagocytic defenses.

A cDNA clone of *A. fumigatus* representing an 85 kD protein has been cloned recently in the yeast *Pichia pastoris*.⁹ The expressed glycoprotein is antigenic and possesses the characteristic properties of class IV dipeptidyl peptidases (DPPIV). This DPPIV from *A. fumigatus* displayed significant sequence homology to the surface differentiation marker CD26 present on T lymphocytes in mammals.³⁰ The peptidase activity of DPPIV and its binding to collagen may help the fungus in colonizing the proteinaceous matrix of the lung.³³

RECOMBINANT ALLERGENS FOR DIAGNOSIS OF ALLERGIC ASPERGILLOSIS

Fungal extracts contain a mixture of allergenic and nonallergenic components and are difficult to standardize. Total allergen extracts prepared from natural sources may sometimes lack certain components due to either poor extraction conditions or enzymatic degradation during isolation and purification of antigen preparations. Hence the currently used in vitro and in vivo diagnostic methods could be improved by using recombinant *Aspergillus* allergens. In view of the recent advances in molecular biology techniques, it is conceivable that a complete panel of recombinant allergens will be soon available for the diagnosis of allergic aspergillosis.

Among the recombinant *A. fumigatus* allergens, rAsp f 1 has been used extensively for diagnosis of *A. fumigatus*-mediated diseases such as ABPA and other respiratory diseases with complications of ABPA.^{69, 71} Asp f 1 showed specific IgE binding with sera from more than 75% ABPA patients without any significant binding with *A. fumigatus*-positive

allergic asthma and normal controls.⁶⁹ Recently, immuno CAP carrying immobilized rAsp f 1 (Pharmacia CAP system) and allergen-specific direct enzyme-linked immunosorbent assay (ELISA) were compared for IgE binding in various subject groups.^{23, 24} The results of these two detection systems correlated closely ($r=0.997$) and were in perfect agreement with skin-test results. Being a potent cytotoxin, extreme caution should be exercised in using Asp f 1 for in vivo skin testing. An amino acid residue, histidine, at position 136, is attributed to the cytotoxicity of Asp f 1; appropriate chemical modification can be made to render the protein noncytotoxic while still retaining antigenicity. The recombinant allergen Asp f 2 was reported to exhibit specific IgE binding with more than 90% of sera from ABPA patients without significant cross-reactivity with other aspergillosis and normal control subjects.^{5, 6} Recombinant Asp f 6 and Asp f 12 have also shown significant in vivo and in vitro IgE antibody binding and have shown considerable promise as standardizable allergens.

EPITOPES OF *ASPERGILLUS* ALLERGENS

The sites on an allergen molecule that interact with cells of the immune system or antibodies are defined as *epitopes*. The allergen usually interacts with the immunoglobulin by its three-dimensional structure (B-cell epitope) or after ingestion and processing by antigen-presenting cell (APC) as peptide fragments linked to major histocompatibility (MHC) molecules by T cells (T-cell epitope). The allergen-specific immune responses start with the interaction of peptide MHC complexes on APC with T-cell receptors (TCR) on T cells. The T helper (Th) cells become activated to proliferate and secrete cytokines when co-stimulatory signals are provided by APC. In contrast, T-cell nonresponsiveness can be produced if co-stimulatory signals of APC are blocked or by administration of higher doses of antigenic peptides.^{19, 20, 73} Recent studies have revealed that T-cell epitopes of an allergen can also function as strong inducers of T-cell nonresponsiveness in humans.^{14, 37} The Asp f 1-specific T-cell lines established for ABPA have been characterized as CD4+ Th2-like in their cytokine synthesis pattern.⁴⁵ In addition, Asp f 1-specific T-cell clones have been reported to be interleukin (IL)-4 producing CD4+ T cells of Th2 phenotypes. The majority of these Asp f 1 specific T-cell clones isolated from peripheral blood of ABPA responded to two peptide fragments of Asp f 1 representing amino acid residues 46 to 65 and 106 to 125 and restricted by human leukocyte antigen (HLA)-DR2 and HLA-DR5 alleles. However, Asp f 1 peptides differentially inducing CD4+ Th2 and CD4+ Th1 cells in mice have recently been reported in a mouse model.^{16, 52} Therefore, attention needs to be focused on the delineation of the functional regions of *A. fumigatus* allergens with respect to their ability to stimulate T-cell proliferation in vitro.

Unlike T-cell epitopes, B-cell epitopes may be conformational or sequential.⁸⁶ The knowledge of IgE-binding epitopes of the major *A. fumigatus* allergens may be of great importance for increasing the sensi-

tivity of diagnostic tests by designing model allergens representing such epitopes. On the other hand, identification of IgE-binding haptens that bind IgE but are unable to cross-link IgE bound to the surface of effector cells might be useful for reducing the release of histamine and other vasoactive components. This hapten may also lead to saturation of IgE on the effector cells to prevent future activation by subsequent contact with complete allergens. Different approaches to the synthesis of T- and B-cell epitopes and methods for their immunologic evaluations are listed in Display Box 2.

Display Box 2. Characterization of Epitopes

Synthesis

1. Expression of cDNA fragments (*E. coli*, yeast, insect, mammalian cells)
2. PCR cloning and expression
3. Site-directed mutagenesis
4. Overlapping peptides (chemical synthesis)

Immunology

1. Antibody-binding assays (direct or inhibition RAST and ELISA, histamine release assay, skin test, other provocation tests)
2. T-cell proliferation assay
3. Cytokine expression

ALLERGEN-SPECIFIC THERAPEUTIC APPROACHES FOR ALLERGIC ASPERGILLOSIS

Allergen-specific therapy of allergic aspergillosis appears feasible in the future because of the availability of an increasing number of functional recombinant *Aspergillus* allergens. The precise determination of the patient's sensitization pattern (allergy profile/allergogram) and selection of a few model recombinant allergens that bind to specific IgE may be the new approach for safer immunotherapy.^{86, 97} Allergen-specific therapy may aim for the prophylaxis of atopy, the induction of tolerance, or to the modification of ongoing immune reaction.^{14, 28, 38, 65, 66, 99} Such attempts may be based on the application of T-cell epitopes as multiple peptides, pure recombinant allergens, or as IgE binding haptens. More recent approaches, such as modification of allergens by manipulating allergen-encoding cDNAs (i.e., site-directed mutagenesis) or gene immunotherapy using cDNA encoding relevant allergens (naked DNA therapy), may also find application in immune modulation and immune intervention of allergic aspergillosis.^{59, 92}

CONCLUSION

Despite the increasing knowledge of the molecular biologic properties of *Aspergillus* allergens, our understanding of the immunopathogenesis and pathophysiology of allergic aspergillosis is very much limited. Immunotherapeutic approaches using specific allergens need further evaluation. Taking into account the complexity of fungal antigen extract, selection of allergens for routine diagnosis may be carried out after multicenter studies with a panel of purified and standardized recombinant allergens. The future course of specific application of these allergens may depend on the clinical evaluation of synthetic peptides, recombinant allergens, degraded allergenic extracts, or modified allergens that can selectively induce tolerance.

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