

***Aspergillus* antigens: which are important?**

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Aspergillus fumigatus is a ubiquitous fungus that causes a variety of diseases in man and animals. A number of protein, carbohydrate, and glycoprotein antigens have been identified from *A. fumigatus*. The diseases are diverse, and therefore are the antigens and their roles in causing or modulating the diseases. The induction and binding of antibodies and the interaction of antigen and various immune cells are of immense significance in the diagnosis and prognosis of the disease. In recent years, over 20 genes encoding *A. fumigatus* antigens have been cloned and the proteins expressed. Among these allergens, Asp f 1, f 2, f 3, f 4, and f 6 showed strong but diverse IgE binding with sera from different groups of patients. Results currently available suggest that Asp f 2, f 3, and f 6 together reacted with IgE from more patients with asthma and allergic bronchopulmonary aspergillosis (ABPA), although they are only marginally effective in demonstrating specific IgE in patients with cystic fibrosis and ABPA. The molecular structure of allergens also plays a major role in the immunological response in the allergic patients. Antigens can be engineered with less or more binding with IgE, and such antigens may have significant roles as specific reagents or as immunomodulators.

Keywords allergic aspergillosis, *Aspergillus* allergens, ELISA, IgE, IgG antibody

Introduction

A large number of saprophytic fungi present in our environment can cause allergic diseases in humans [1–3]. Among the known allergenic fungi, members belonging to *Aspergillus*, particularly *A. fumigatus* are more frequently involved in human diseases [2]. *A. fumigatus* causes generalized invasive aspergillosis, and various forms of allergies in addition to saprophytic colonization in preexisting cavities of lungs. Allergies inflicted by *Aspergillus* include asthma, allergic bronchopulmonary aspergillosis (ABPA), allergic fungal sinusitis, and hypersensitivity pneumonitis. According to the classification of Gell and Coomb classification of hypersensitivity, allergic asthma is mediated by type 1 allergy with high serum IgE, while hypersensitivity pneumonitis is a type 4 immune response with no IgE antibody. ABPA, however, demonstrates a mixed

immune response representing type 1, 3, and 4. The present discussion will focus on antigens and immune responses in ABPA [4].

ABPA is an allergen-induced immunological disease of the lung characterized by increased IgE and IgG response, enhanced peripheral blood eosinophilia, and fleeting pulmonary infiltrates. Immune responses comprise both a humoral arm, mediated by the production of specific IgE and IgG antibody, and a cellular arm, mediated by T cells, T cell products, and other accessory and effector cells. Various cytokines and other modulators resulting from the interactions of the antigen and the cells of the immune system are responsible for the pathology of the disease [2,4].

Antigens of *Aspergillus*

The crude extracts from *Aspergillus* have been used for evaluating patients with possible ABPA. These antigens have inherent drawbacks because of the lack of reproducibility and consistency and frequent cross reactivity with other antigens [5,6]. Culture filtrate, cell sap, and cell wall antigens have been studied in the

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past for developing reliable reagents for immunodiagnosis of allergic diseases [7]. These extracts show considerable variation in their chemical composition, number of components, and in their reactivity with antibodies. These variations are primarily due to growth conditions, the nutrient/media used to grow them, and the genetic differences among the strains used for antigen preparation. The presence of various toxins, proteolytic enzymes, and non-antigenic materials also contribute significantly to the quality of the antigens.

Extracts from *A. fumigatus* have been further characterized as to their chemical nature and attempts have been made to purify them by conventional methods. Chemically these antigens can be classified broadly into proteins, polysaccharides, and glycoproteins. On interaction with the immune system these antigens produce antibodies and factors mediating the disease. In allergic diseases, particularly in type I allergy, it is IgE antibody and Th2 cytokines that modulate the disease process. In hypersensitivity pneumonitis, IgG antibody is consistently demonstrable, while the immune response is frequently of a Th1 type of cell-mediated immunity [8]. Over 100 allergens have been reported from fungi, including more than 20 antigens from *A. fumigatus*. Based on the physico chemical properties, these allergens have been classified as enzymes, such as proteases and enolases, transport proteins such as lipocalins, and regulatory proteins such as heat shock proteins. Other allergens possess diverse chemical nature and unknown biochemical functions and activities.

Crude antigen extracts

All the earlier investigations depended on crude mycelial and spore extracts from stationary or aerated cultures of *Aspergillus* grown in synthetic or complex media. Antigenic components in these preparations vary considerably. Similarly, metabolic antigens secreted outside the cells into the broth have also been isolated and used in various *in vitro* and *in vivo* assays for the diagnosis of *Aspergillus* allergies.

Native antigens purified from crude *Aspergillus* extracts

As the crude antigens contain a mixture of proteins, glycoproteins, carbohydrates, toxins, and inert components, attempts have been made to purify the active components. However, most of these conventional methods involve harsh physical and chemical treatments, which have adverse effect on the antigenicity. There has been considerable research into purifying

relevant antigens from *Aspergillus*. These include fractionation methods such as size exclusion chromatography, affinity chromatography using monoclonal antibodies, partition chromatography, electrophoresis, isoelectric focusing, and chromatofocusing [1,7,9,10]. None of these methods have yielded relevant allergens useful in the diagnosis of *Aspergillus*-induced allergies.

Polysaccharide antigens

Polysaccharide fractions isolated from cell wall or cytoplasm of *Aspergillus* sp. were reported to demonstrate cross reactivity with other fungal antigens [7,11,12]. This limits their scope as diagnostic allergens for ABPA or other *Aspergillus*-induced diseases. Galactomannan antigen was isolated from mycelial extract using sequential chromatography on anion exchange and Con A affinity columns. Cell wall galactomannan of *A. flavus*, and *A. niger*. The demonstration of galactomannan in the serum of patients with invasive aspergillosis has been suggested to be a useful criterion for diagnosis. Recently, fungal extracellular polysaccharides (1- β 3)-glucan has been reported as a good marker to estimate overall levels of fungal concentration in dust samples of patients' residences [13].

Table 1 Allergens of *Aspergillus fumigatus*

Allergen*	kD	Nature of allergen	Binding of IgE†
Asp f 1	18	Ribotoxin	83‡ [42]
Asp f 2	37	Fibrinogen binding (?)	90 [42]
Asp f 3	19	Peroxisomal protein	94 [42]
Asp f 4	30	–	78 [42]
Asp f 5	40	Metalloproteinase	93 [28]
Asp f 6	26.5	Mn superoxide dismutase	56 [42]
Asp f 7	12	–	46 [28]
Asp f 8	11	Ribosomal protein-P2	–
Asp f 9	34	–	89 [2, 28]
Asp f 10	34	Aspartic protease	28 [28]
Asp f 11	24	Peptidyl-prolyl isomerase	–
Asp f 12	47	Heat shock protein-P90	–
Asp f 13	34	Alkaline serine proteinase	–
Asp f 15	16	–	–
Asp f 16	43	–	70 [2]
Asp f 17			
Asp f 18	34	Vacuolar serine proteinase	–
Asp f 22	46	Enolase	–
Asp f 23	44	L3 ribosomal protein	–

*Allergens of *Aspergillus fumigatus* approved by the International Union of Immunological Society, Allergen Nomenclature Committee, 2004.

†Patients allergic to *Aspergillus* and their binding to allergens.

‡Percentage of sera tested.

Protein allergens from *Aspergillus*

A number of proteins, particularly proteases, have been reported from *A. fumigatus*. Among these enzymes, proteinase, elastase, ribonuclease, chymotrypsin, catalase, and superoxide dismutase are the predominant ones [7,14]. Out of 22 antigens recognized from *Aspergillus*, 13 showed various enzyme activity and reacted with sera of ABPA patients [15]. Schonheyder *et al.* [16] recognized a 250 kDa enzyme, possibly a tetramer of catalase showing binding to IgE, from the sera of patients with cystic fibrosis (CF) and ABPA. Another 90 kDa catalase antigen was reported from *A. fumigatus* that detected specific antibodies in more than 90% of the sera of aspergilloma patients [17]. An antigenic component of Af with chymotrypsin-like activity was isolated by Tran-van Ky *et al.* [15] and found to be more specific to aspergilloma.

Glycoproteins from *Aspergillus*

A number of glycoprotein antigens from Af have been isolated using Con A affinity chromatography [18]. The high MW glycoprotein antigens from hyphae or culture filtrate preparations were purified using conventional methods and used to detect specific IgE in the sera of ABPA patients. An acidic glycoprotein (45 kDa) was isolated from cell sap, which contained four polypeptide fragments and showed strong reactivity with ABPA sera. Of the four glycoprotein antigens isolated using gel filtration, IEF, and affinity chromatography from crude Af extract, two – Ag7(150–200 kDa) and Ag13 (70 kDa) – bound to Con A and reacted with antibodies in the sera of ABPA and aspergilloma patients [19]. We have identified 18 antigen bands by crossed-immunoelectrophoresis, of which 12–14 bands showed Con A binding activity indicating their glycoprotein nature [20]. Using two-dimensional electrophoresis, antigenic fractions containing 20, 40, and 80 kDa size components with strong antibody binding to sera of ABPA patients have been isolated [21].

Using gel filtration and SDS-PAGE, a major allergenic protein (group 55) has been purified from water soluble extract of Af. This protein has N-glycosidically linked oligosaccharides and showed specific IgE binding on immunoblot with the sera of ABPA patients [22]. A 45 kDa glycoprotein allergen was purified and characterized, which on deglycosylation lost the IgE reactivity, but retained the IgG binding with patient sera [23]. A glycoprotein of 66 kDa was purified by ammonium sulfate precipitation, and immunoaffinity chromatography showed IgE reactivity when tested by crossed-radio-immunoelectrophoresis and ELISA

using sera of patients with ABPA and those with CF and ABPA [24].

Recombinant antigens

The search for reliable and pure antigens has continued in spite of the difficulties faced with conventional fractionation. In recent years, molecular biology techniques and gene cloning procedures have attracted attention for obtaining purified antigens. Currently, a number of allergens from *A. fumigatus* have been cloned and the relevant proteins expressed in suitable expression systems [25].

The cloning of allergens consists of the isolation of messenger RNA (mRNA) from 72 to 96 hour-old actively growing cultures. The total RNA from the mycelium of aerated cultures were extracted with guanidium isothiocyanate or using the newer commercial preparations available [26]. The mRNA was then purified using oligo (dT) cellulose chromatography and reverse transcribed to obtain cDNA [26,27]. A cDNA expression library was constructed for screening sera containing allergen-specific IgE antibody from patients [26–28]. The allergen encoding genes have been processed, and the proteins expressed with or without posttranslational modifications. Recently, phage-display libraries have been constructed to aid in the understanding of the immune responses of the allergens expressed [29].

Among the various vectors used for expression of fungal allergens, the pET (pET expression protocol, Novagen, Madison, WI) has been the favored prokaryotic expression system [30]. In this system, expression of the target protein is induced by a T7 RNA polymerase in the bacterial host, *Escherichia coli*. The allergen expressed can be purified using a histidine tag at the N- or C-terminal end of the protein. Most of these allergens of *A. fumigatus* expressed and purified showed a similar skin-test reactivity and *in vitro* IgE binding with native allergens. Since the IgE binding of these allergens is frequently directed to the protein parts of the molecules, posttranslational modification of the antigen may not impart any significant role in the IgE binding. However, with antigens demonstrating posttranslational modifications, such as glycosylation, it is possible to express the clones in eukaryotic systems. One of the expression systems that have been used for high-level expression of recombinant proteins is the yeast *Pichia pastoris*. The target gene in *Pichia* is under the control of an alcohol oxidase promoter. Several *A. fumigatus* proteins and glycoproteins have been expressed using this system [30].

Immunological response in allergic aspergillosis

The immune response of the host to invading microbes and their antigens results in clearance of the organism and in the induction of significant pathologic effects in the host. Continuous exposures to *Aspergillus* through either inhalation of conidia or fungal colonization in the bronchi result in late-phase asthmatic reactions [31,32]. The eosinophilic infiltration and mast cell degranulation in response to *Aspergillus* allergens and IgE interaction leads to release of additional proinflammatory mediators that play a major role in the pathogenesis of ABPA [32,33]. The degranulation products induce chemotaxis of eosinophils and other inflammatory cells such as activated CD4⁺ lymphocytes. In addition, anti-*Aspergillus* antibody enables soluble antigens and mycelial fragments to penetrate and deposit in the lung parenchyma [34,35]. One of the important immune responses of patients to *Aspergillus* antigens is immediate skin test reactivity, mediated mainly by IgE antibody. The late reaction (Arthus reaction) to *Aspergillus* allergens/antigens is the result of IgE-mediated mast cell activation or immune complex formation with *Aspergillus* antigens and antibody, which on binding to complement leads to cell lysis. Immune complexes of specific IgG and *A. fumigatus* antigens trigger the generation of leukotriene C4 from mast cells, which in turn promotes mucus production, bronchial constriction, hyperemia, and edema. However, the lack of vasculitis or deposition of complement and immunoglobulin in the vessel walls suggests ABPA as a non-immune complex-mediated disease, even though antibody responses are vigorous and there are circulating immune complexes in some of the ABPA patients [36]. Antibody-mediated cytotoxicity and delayed tuberculin-like allergic reaction in patients mediated by sensitized T-lymphocytes and lymphocyte-derived mediators are also reported in ABPA [37]. Granuloma formation in the lungs is not uncommon in ABPA [38].

Humoral immunity

Specific humoral responses differ considerably in patients with different clinical forms of aspergillosis. Patients with ABPA demonstrate elevated levels of antigen-specific circulating antibodies of IgG, IgE, and IgA isotypes in their sera [39]. The predominant isotypes represented are IgG₁ and IgG₂ antibodies in ABPA patients. Aspergilloma patients show markedly elevated levels of specific IgG and IgM against carbohydrate and glycoprotein antigens, but usually

show no IgE. Patients with *Aspergillus* skin test-positive asthma invariably show elevated IgE antibody production demonstrable *in vitro*. These differences in the *A. fumigatus*-specific antibodies in the serum samples of patients with ABPA, aspergilloma, and skin test-positive asthma suggest fundamental variations in the immune regulatory response to *Aspergillus* antigens in these disorders.

Cell-mediated immunity

The distinguishing characteristics of ABPA are the enhanced presence of IgE in the serum, Th2 cytokine expression, and proliferation and activation of eosinophils [4,34,40]. Other features reported in ABPA include *Aspergillus* antigen-specific *in vitro* proliferation of peripheral blood mononuclear cells (PBMCs) and active *in vitro* production of IgE by PBMC cultures [40,41]. B-cells of patients with ABPA and patients with ABPA and CF spontaneously secrete IgE. Moreover, CD4⁺ Th2 cells in ABPA patients secrete cytokines: interleukin-4 (IL-4), and IL-5. IL-4 enhances IgE synthesis of B-cells, while IL-5 induces the differentiation and recruitment of eosinophils [34]. Activated basophils also secrete cytokines and stimulate further activation of Th2 pathway, resulting in increased IgE and eosinophil production.

Aspergillus specific antibody in ABPA

Early diagnosis of ABPA is possible if sensitive *in vitro* methods and specific reagents are available to supplement the clinical features [42]. ABPA patients show a marked increase in IgG and IgE antibodies against the fungal extracts in their sera. The level of *A. fumigatus*-specific antibodies in the sera of patients with allergic asthma and ABPA vary and is used to differentiate these two diseases. Precipitating antibodies were demonstrable in up to 90% or more of patients with ABPA [2,4,5,31,43]. The reliability of laboratory diagnosis depends on the availability of reproducible antigen preparations. Antigen extracts currently available are hampered by the lack of purity, frequent cross reactivity with other fungi, and the presence of non-reactive components including toxins. Several semi-purified antigens have been prepared with claims of predictable reactivity [9,10]. Of the several immunoassays reported, agar gel double diffusion and ELISA are the most frequently used laboratory diagnostic tests for the diagnosis of ABPA [42]. Most laboratories all over the world use crude culture filtrate or mycelial antigens for detecting IgE and IgG antibodies in the sera of patients with ABPA.

Although a number of recombinant proteins having significant binding with IgE and IgG antibodies have been isolated, only very few studies have been carried out to evaluate these antigens. We have evaluated recombinant proteins from *A. fumigatus*, namely Asp f 1, f 2, f 3, f 4, and f 6, by ELISA using sera from Switzerland and the United States [42]. All the antigens and sera were evaluated by ELISA independently followed in the two laboratories. The results indicate that all five allergens reacted with IgE from ABPA, while Asp f 1 and Asp f 3 also showed widespread reactivity with sera from patients with allergic asthma. Recently, we have studied Asp f 1, 2, 3, 4, 6, 12, and 16 allergens against sera from ABPA skin test-positive asthmatics and normal controls [44]. Although significant antibody was detected in the serum of patients with ABPA, there was some overlap between the different groups.

Our group recently investigated Asp f 1, f 2, f 3, f 4, and f 6 from *A. fumigatus* for demonstrating IgE and IgG antibody in the sera of patients with CF, and compared the ELISA results with ImmunoCAP using the same sera (Fig. 1). Patients with CF and ABPA

showed strong reactivity with all allergens studied except Asp f 6 by ELISA. However, CF and asthma sera also reacted with Asp f 1, f 2, f 3, and f 4 by both procedures. Thus, it is evident that certain antigens have a better predictability than others. The results of our study indicate that both ELISA and ImmunoCAP results correlated closely with the clinical diagnosis. It was found that ABPA without CF can be differentiated from skin test-positive asthmatics and normal controls using ELISA or ImmunoCAP, using purified Af allergens, particularly Asp f 1 and Asp f 3 (Fig. 2). However, this differentiation is not feasible in patients with CF. The presence of IgE antibody to Asp f 12 and Asp f 16 in patients with ABPA and CF showed considerable variation in ABPA and ABPA with CF patients [44].

CF-ABPA patients with disease activity frequently showed strong IgE response against Asp f 2, f 3, and f 4 compared to the levels before exacerbation. Although enhanced IgG1, 2, and 4 levels against *Aspergillus* have been demonstrated in CF-ABPA, there was considerable overlap detected with CF with *Aspergillus* coloni-

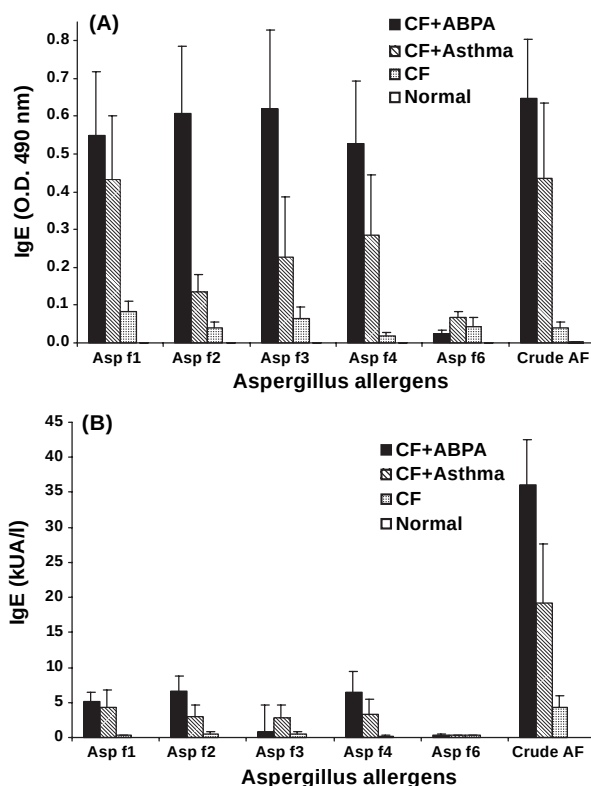


Fig. 1 Specific IgE antibody to *Aspergillus fumigatus* antigens in the sera. (A) ELISA using sera from patients with CF and ABPA, CF and *Aspergillus* skin test positive asthma, non-atopic CF, and normal controls. (B) Same as (A), except that ImmunoCAP was used to detect specific IgE antibody.

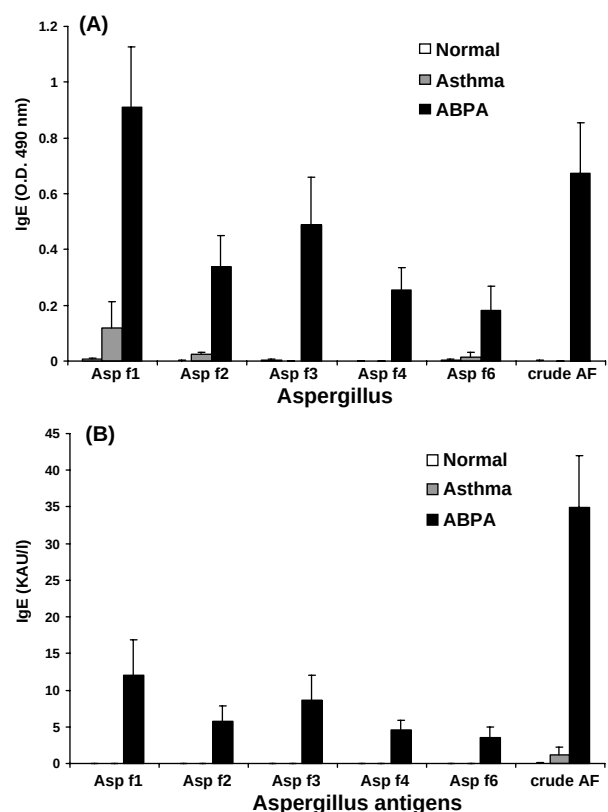


Fig. 2 IgE antibody in the sera against *Aspergillus fumigatus* antigens. (A) ELISA using sera from ABPA, asthma, and normal controls. (B) Same as A except that ImmunoCAP was used to detect specific IgE antibody.

zation, but no ABPA [45]. Other antibody isotypes such as IgA, IgG₂, and IgG₃ reacting to various *Aspergillus* antigens have been recognized in the sera of CF patients, but the titers were not significant when compared to non-ABPA and ABPA sera.

Engineered allergens

The immune response of the molecule depends on its ability to interact with immune cells. The antibody binding to epitopes may be based on the conformational or linear sequence of the amino acid residues of the allergens. Molecules can be engineered for enhancing or depleting the reactivity of allergens by the deletion of linear epitopes, alteration of the conformation (by constructing the proteins by selectively removing 'N,' 'C' or both 'N' and 'C' terminal ends), mutating the key amino acids in the epitope, and by altering posttranslational changes.

Deletion mutants were constructed from Asp f 2, f 3, and f 4 and the expressed proteins evaluated against antibody in the sera of ABPA, asthma and normal controls [46–49]. The N-terminal or C-terminal deletion mutants, whether glycosylated or not, showed weak reactions with sera compared to whole length proteins. Mutants with both 'C' and 'N' terminal deletions failed to react with IgE antibody from any of the ABPA patients studied [30]. Asp f 3 showed strong binding to IgE even when the 'C' terminal end was deleted, while deletion of other epitopes resulted in no IgE binding. Asp f 4 also retained IgE binding when one or two epitopes from the 'C' terminal end were deleted [47].

When Asp f 2 protein was expressed with cysteine residue mutated to alanine, the alteration in the sulfhydryl group and disulfide bonds results in marked changes in the IgE binding of the molecule. The mutation of C204C to 204A resulted in complete loss of IgE binding, and the results also indicate that C267 is responsible for strong IgE binding [49]. Asp f 4 also demonstrated the need for intact C-terminal cysteine residues for strong binding to IgE [47].

Cell-mediated immunity in patients has been demonstrated using several recombinant *Aspergillus* antigens. Most of the crude extracts of *A. fumigatus* failed to effect consistent stimulation of peripheral blood mononuclear cells (PBMC) from ABPA patients. This may be due to the toxic effects of some of the components or due to the presence of inert non-antigenic components in the antigens. It was found that Asp f 2 is capable of stimulating PBMC from patients with ABPA [50]. Using T cell cloning, a number of cell lines were generated and the epitopes were mapped. The epitopes

involved in T cell activation were mapped using overlapping synthetic peptides to stimulate the cell lines. Based on peptide-induced stimulation of T cell clones and IL-4 and IL-5 secretion, we found that epitopes consisting of amino acid residues from 54–74 were T cell stimulatory. On further analysis, two T cell epitopes, representing amino acids 54–68 and 60–67, were identified. These two epitopes have also been shown to be essential for the binding of IgE from patients with ABPA [50].

Antigens in immunotherapy and vaccination

Specific immunotherapy and vaccination are the probable means for controlling type I allergy [51–53]. In all the earlier studies, crude allergens have been used as immunotherapeutic agents. As a result of a clearer understanding of the pathogenesis of the disease in recent years, attention has been directed to reversing the Th2 type of response to a Th0 or a Th1 response in patients. Several major directions have been taken in immunotherapy of IgE-mediated allergy, which include peptide immunotherapy, immunotherapy using immunostimulatory sequences (ISS-ODN), and naked DNA vaccination [54]. The peptide immunotherapy utilizes synthetic peptides and engineered allergens.

Allergen-specific immunotherapy may aim for prophylaxis of atopy, induction of tolerance, or modification of ongoing immune responses [52,53]. Although attempts have been made with other allergens to induce T cell non-responsiveness in patients by selectively administering major T cell epitopes, no such studies have been carried out with *A. fumigatus* allergens. We studied Asp f 2, a major *A. fumigatus* allergen, and constructed a number of deletion and point mutants [30,49]. Some of these mutated proteins, while stimulating T cell proliferation with patients' cells, failed to bind to IgE in the sera of patients and mice immunized with the allergen. These candidate allergens for immunotherapy require further evaluation. Similarly, the potential role for the synthetic peptides as an immunotherapeutic agent in allergic aspergillosis based on the current findings needs further evaluation [53,55].

Another approach is the use of immunostimulatory CpG oligonucleotides (CpG-ODN) vaccines against IgE-mediated allergy [54]. Immunization with DNA-based vaccines, such as plasmid DNA of major *A. fumigatus* antigens or allergen and ISS-ODN co-administered, results in reversal of the Th2 response to that of a predominantly Th1 response. There was a marked shift to a Th1-type of response as evidenced by the threefold increase in specific serum IgG_{2a} in ISS-

treated BALB/c mice compared to mice treated with antigen alone [54].

Conclusions

Aspergillus-induced allergy comprises a major group of disease among ailments caused by *Aspergillus*. Until recently, the diagnosis of allergy was cumbersome owing to the lack of defined purified fungal antigens. The emergence of purified *Aspergillus* allergens through molecular cloning has enhanced the reliability of *in vitro* immunological tests as well as skin tests for diagnosis of the disease. The cross-reactivity among fungal allergens and lack of specificity of the tests has attracted attention, leading to more specific and reliable antigens by engineered molecules. With a group of selected *Aspergillus* allergens, the specific IgE against *A. fumigatus* could be demonstrated in the sera of patients with ABPA. However, the success of demonstrating *Aspergillus* recombinant allergen-specific IgE in the sera of patients with CF and ABPA is still unsatisfactory. Delineating the structure and function of the antigen molecules and their interaction with immune system may provide improved diagnosis, understanding of the immunopathogenesis, and will assist in the development of immunomodulatory therapy.

Acknowledgements

This study was supported by the V A Medical Research. The support of Drs. Kevin Kelly and Jordan Fink, the technical assistance of Nancy Elms and Laura Castillo, and the editorial assistance of Donna Schrubbe are gratefully acknowledged.

References

- Kurup VP. Fungal allergens. *Curr allergy Asthma Rep* 2003; **3**: 416–423.
- Kurup VP, Madan T, Sarma UP. Allergic bronchopulmonary aspergillosis: recent concepts and considerations. In: Domer JE, Kobayashi GS (eds). *The Mycota XII Human Fungal Pathogens*. Berlin-Heidelberg: Springer-Verlag, 2004: 225–241.
- Burge HA. Airborne-allergenic fungi. *Immunol Allergy Clin North Am* 1989; **9**: 307–319.
- Greenberger PA. Allergic bronchopulmonary aspergillosis. In: Adkins NF, Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER (eds). *Middletons Allergy, Principles and Practice*, 2nd edn. Philadelphia: C V Mosby, 2003: 1353–1371.
- Kurup VP, Apter AJ (eds). *Immunology and Allergy Clinics of North America*, vol. 18, Philadelphia: WB Saunders, 1998.
- Kurup VP, Cramer R. *Aspergillus* allergens. 2000. Available from: <http://www.aspergillus.man.ac.uk/secure/article/kurup/index.html>.
- Singh BP, Banerjee B, Kurup VP. *Aspergillus* antigens associated with allergic bronchopulmonary aspergillosis. *Frontiers Biosci* 2003; **8**: s102–109.
- Fink JN, Zacharisen MC. Hypersensitivity pneumonitis. In: Adkins NF, Jr, Yunginger JW, Busse WW, Bochner BS, Holgate ST, Simons FER (eds). *Middletons Allergy, Principles and Practice*, 2nd edn. Philadelphia: C V Mosby, 2003: 1373–1390.
- Longbottom JL. Antigens and allergens of *Aspergillus fumigatus*, II: their further identification and partial characterization of a major allergen (Ag3). *J Allergy Clin Immunol* 1986; **78**: 18–24.
- Hearn VM, Proctor AG, MacKenzie DWR. The preparation and partial characterization of antigenic fractions obtained from the mycelial walls of several *Aspergillus* species. *J Gen Microbiol* 1980; **119**: 41–49.
- Bennett JE, Bhattacharjee AK, Glaudemans DJ. Galactofuranosyl groups are immunodominant in *Aspergillus fumigatus* galactomannan. *Mol Immunol* 1985; **22**: 251–254.
- Azuma I, Kimura H, Hirao F, Tsubura E, Yamamura Y. Skin testing and precipitation antigens from *Aspergillus fumigatus* for diagnosis of aspergillosis. *Am Rev Resp Dis* 1967; **95**: 305–306.
- Chew GL, Douwes J, Doekes G, et al. Fungal extracellular polysaccharides, beta (1–>3) glucans and culturable fungi in repeated sampling of house dust. *Indoor Air* 2001; **11**: 171–178.
- Hearn VM. Antigenicity of *Aspergillus fumigatus*. *J Med Vet Mycol* 1992; **30**: 11–25.
- Tran-van Ky P, Torck C, Vaucelle T, Floc'h F. Comparative study on immunoelectrophoregrams of enzymes of the antigenic extract of *Aspergillus fumigatus* revealed by experimental serums and serums of patients with aspergillosis. *J Vet Med Mycol* 1969; **7**: 73–84.
- Schonheyder H, Jensen T, Laessoe IH. Serum antibodies to *Aspergillus fumigatus* catalase in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 1988; **7**: 40–44.
- Lopez-Medrano R, Ovejero MC, Calera JA, Peunte P, Leal F. An immunodominant 90 kilodalton *Aspergillus fumigatus* antigen is the subunit of catalase. *Infect Immun* 1995; **63**: 4774–4780.
- Piechura JE, Kurup VP, Daft LJ. Isolation and immunochemical characterization of fractions from membranes of *Aspergillus fumigatus* with protease activity. *Can J Microbiol* 1990; **36**: 33–41.
- Longbottom JL, Harvey C, Taylor ML, et al. Characterization of immunologically important antigens and allergens of *Aspergillus fumigatus*. *Int Arch Allergy Appl Immunol* 1989; **88**: 185–186.
- Kurup VP, Greenberger PA, Fink JN. Antibody response to low molecular weight antigens of *Aspergillus fumigatus* in allergic bronchopulmonary aspergillosis. *J Clin Microbiol* 1989; **27**: 1312–1316.
- Kurup VP, John KV, Resnick A, Fink JN. A partially purified glycoprotein antigen from *Aspergillus fumigatus*. *Int Arch Allergy Appl Immunol* 1986; **79**: 263–269.
- Sarma PU, Banerjee B, Bir N, Kurup VP. Immunodiagnosis of allergic bronchopulmonary aspergillosis. In: Kurup VP, Apter AJ (eds). *Immunology and Allergy Clinics of North America*, vol. 18. Philadelphia: WB Saunders, 1998: 525–547.
- Banerjee B, Madan T, Sharma GL, et al. Characterization of glycoprotein antigen (45 kD) of *Aspergillus fumigatus*. *Serodiag Immunother Infect Dis* 1995; **7**: 147–152.
- Little SA, Warner JO. Improved diagnosis of allergic bronchopulmonary aspergillosis with group 66 formerly antigen 7 of *Aspergillus fumigatus* for specific IgE detection. *J Allergy Clin Immunol* 1996; **98**: 55–63.
- Cramer R. pJuFo: a phage surface display system for cloning genes based on protein-ligand interaction. In: Schaefer BC (ed.). *Gene Cloning and Analysis. Current Innovations*. Whymondham: Horizon Scientific Press, 1997: 29–42.

- 26 Chirgwin JM, Przbyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. *Biochem* 1979; **18**: 5294–5299.
- 27 Banerjee B, Kurup VP, Phadnis S, *et al.* Molecular cloning and expression of a recombinant *Aspergillus fumigatus* protein Asp f II with significant immunoglobulin E reactivity in allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 1996; **127**: 253–262.
- 28 Crameri R. Recombinant *Aspergillus fumigatus* allergens: from the nucleotide sequences to clinical applications. *Int Arch Allergy Immunol* 1998; **115**: 99–114.
- 29 Crameri R, Jaussi R, Menz G, Blaser K. Display of expression products of cDNA libraries on phage surfaces: a versatile screening system for selective isolation of genes by specific gene product/ligand interaction. *Eur J Biochem* 1994; **226**: 53–58.
- 30 Tang B, Banerjee B, Greenberger PA, *et al.* Antibody binding of deletion mutants of Asp f 2, the major *Aspergillus fumigatus* allergen. *Biochem Biophys Res Comm* 2000; **270**: 1128–1135.
- 31 Kurup VP. Immunology of allergic bronchopulmonary aspergillosis. *Indian J Chest Dis Allied Sci* 2000; **42**: 225–237.
- 32 Banerjee B, Greenberger PA, Fink JN, Kurup VP. Molecular characterization of *Aspergillus fumigatus* allergens. *Indian J Chest Dis Allied Sci* 2000; **42**: 239–248.
- 33 Kauffman HF, Tomee JFC, van de Riet MA, Timmerman AJB, Borger P. Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol* 2000; **105**: 1185–1193.
- 34 Knutsen AP, Chauhan B, Slavin RG. Cell-mediated immunity in allergic bronchopulmonary aspergillosis. In: Kurup VP, Apter AJ (eds). *Immunology and Allergy Clinics of North America*, vol. 18. Philadelphia: WB Saunders, 1998: 575–599.
- 35 Kurup VP, Grunig G, Knutsen AP, Murali PS. Cytokines in allergic bronchopulmonary aspergillosis. *73rd Forum in Immunology* 1998; 466–477.
- 36 Madan T, Banerjee B, Bhatnagar PK, Shah A, Sarma PU. Identification of 45 kDa antigen in immune complexes of patients of allergic bronchopulmonary aspergillosis. *Mol Cell Biochem* 1997; **166**: 111–116.
- 37 Kurup VP, Nair MPN, Schwartz SA, Fink JN. Serum antibodies and their role in antibody-dependent cell-mediated cytotoxicity in aspergillosis. *Immunobiol* 1985; **169**: 362–371.
- 38 Myers JL. Pathology of allergic bronchopulmonary aspergillosis. In: Patterson R, Greenberger PA, Roberts ML (eds). *Allergic Bronchopulmonary Aspergillosis*. Providence: Ocean Side Publications, 1995: 39–46.
- 39 Kurup VP, Resnick A, Kalbfleish J, Fink JN. Antibody isotype responses in *Aspergillus*-induced diseases. *J Lab Clin Med* 1990; **115**: 298–303.
- 40 Murali PS, Kurup VP, Greenberger PA, Fink JN. Concanavalin A-nonbinding *Aspergillus fumigatus* antigen: a major immunogen in allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 1992; **119**: 377–384.
- 41 Murali PS, Pathial K, Saff RH, *et al.* Immune responses to *Aspergillus fumigatus* and *Pseudomonas aeruginosa* antigens in cystic fibrosis and allergic bronchopulmonary aspergillosis. *Chest* 1994; **106**: 513–519.
- 42 Kurup VP, Banerjee B, Hemmann S, *et al.* Selected recombinant *Aspergillus fumigatus* allergens bind specifically to IgE in ABPA. *Clin Exp Allergy* 2000; **30**: 988–993.
- 43 Kurup VP. Fungal Allergy. *Handbook of Fungal Biotechnology*. New York: Marcel Dekker, 2004: 515–525.
- 44 Knutsen AP, Hutcheson PS, Slavin RG, Kurup VP. IgE antibody to *Aspergillus fumigatus* recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Allergy* 2004; **59**: 198–203.
- 45 Skov M, Pressler T, Jensen HE, Hoiby N, Koch C. Specific IgG subclass antibody pattern to *Aspergillus fumigatus* in patients with cystic fibrosis with allergic bronchopulmonary aspergillosis (ABPA). *Thorax* 1999; **54**: 44–50.
- 46 Ramachandran H, Jayaraman V, Banerjee B, *et al.* IgE binding conformational epitopes of Asp f 3, a major allergen of *Aspergillus fumigatus*. *Clin Immunol* 2002; **103**: 324–333.
- 47 Ramachandran H, Banerjee B, Greenberger PA, *et al.* Role of C-terminal cysteine residues of *Aspergillus fumigatus* allergen Asp f 4 in immunoglobulin E binding. *Clin Diag Lab Immunol* 2004; **11**: 261–265.
- 48 Banerjee B, Greenberger PA, Fink JN, Kurup VP. Conformational and linear B-cell epitopes of Asp f 2, a major allergen of *Aspergillus fumigatus*, bind differently to immunoglobulin E antibody in the sera of allergic bronchopulmonary aspergillosis patients. *Infect Immun* 1999; **67**: 2284–2291.
- 49 Banerjee B, Kurup VP, Greenberger PA, Kelly KJ, Fink JN. C-terminal cysteine residues determine the IgE binding of *Aspergillus fumigatus* allergen Asp f 2. *J Immunol* 2002; **169**: 5137–5144.
- 50 Rathore VB, Johnson B, Fink JN, *et al.* T cell proliferation and cytokine secretion to T cell epitopes of Asp f 2 in ABPA patients. *Clin Immunol* 2001; **100**: 228–235.
- 51 Hoyne CF, Callow MG, Kuo MC, Thomas WR. Inhibition of T-cell responses by feeding peptides containing major and cryptic epitopes: studies with Der allergen. *Immunology* 1994; **83**: 190–195.
- 52 Sarma PVGK, Purkayastha S, Madan T, Sarma PU. Expression of an epitopic region of Asp f 1, an allergen/antigen/cytotoxin of *Aspergillus fumigatus*. *Immunol Lett* 1999; **170**: 151–155.
- 53 Svirshchevskaya E, Frolova E, Alekseeva L, Kotzareva O, Kurup VP. Intravenous injection of major and cryptic peptide epitopes of ribotoxin, Asp f 1 inhibits T cell response induced by crude *Aspergillus fumigatus* antigens in mice. *Peptides* 2000; **21**: 1–8.
- 54 Banerjee B, Xia J-Q, Rickaby DA, *et al.* Modulation of airway inflammatory CpG oligodeoxynucleotide in a murine model of aspergillosis. *J Allergy Clin Immunol* 2001; **107**: S216. [abstract]
- 55 Kurup VP, Hari V, Guo J, *et al.* *Aspergillus fumigatus* peptides differentially express Th1 and Th2 cytokines. *Peptides* 1996; **17**: 183–190.